

Theoretical considerations about carbon isotope distribution in glucose of C₃ plants

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Abstract. The origin of the non-statistical intramolecular distribution of ¹³C in glucose of C₃ plants is examined, including the role of the aldolisation of triose phosphates as proposed by Gleixner and Schmidt (1997). A modelling approach is taken in order to investigate the relationships between the intramolecular distribution of ¹³C in hexoses and the reactions of primary carbon metabolism. The model takes into account C–C bond-breaking reactions of the Calvin cycle and leads to a mathematical expression for the isotope ratios in hexoses in the steady state. In order to best fit the experimentally-observed intramolecular distribution, the values given by the model indicate that (i), the transketolase reaction fractionates against ¹³C by 4–7‰ and (ii), depending on the photorespiration rate used for estimations, the aldolase reaction discriminates in favour of ¹³C by 6‰ during fructose-1,6-bisphosphate production; an isotope discrimination by 2‰ against ¹³C is obtained when the photorespiration rate is high. Additionally, the estimated fractionations are sensitive to the flux of starch synthesis. Fructose produced from starch breakdown is suggested to be isotopically heavier than sucrose produced in the light, and so the balance between these two sources affects the average intramolecular distribution of glucose derived from stored carbohydrates. The model is also used to estimate photorespiratory and day respiratory fractionations that appear to both depend only weakly on the rate of ribulose-1,5-bisphosphate oxygenation.

Keywords: Calvin cycle, isotope effects, photorespiration, respiration, starch.

Introduction

It is well established that photosynthesis discriminates against ¹³C during CO₂ diffusion and fixation by ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) (for a review, see Brugnoli and Farquhar 2000). Consequently, C₃ plants are depleted in ¹³C. For example, the δ¹³C value of glucose (Glc) from C₃ plants is around –25‰ (Rossmann *et al.* 1991) while the δ¹³C of atmospheric CO₂ is around –8‰. In addition, Glc from sugar beet syrup and maize flour has a reproducible non-statistical ¹³C pattern showing relative enrichment in positions C-3 and C-4 and depletion in positions C-1 and C-6 (Rossmann *et al.* 1991; Gleixner *et al.* 1998; Fig. 1). ‘Thermodynamic order’ (free carbon atoms exchange through equilibria) does not satisfactorily explain the intramolecular δ¹³C in glucose (Schmidt 2003),

and the aldolase reaction, which catalyses the condensation of triose phosphates to fructose-1,6-bisphosphate (FBP) has been proposed to explain this pattern and particularly the ¹³C enrichment in the C-3 and C-4 positions (Gleixner and Schmidt 1997; Schmidt 2003). The isotopic effects during other reactions of the Calvin cycle that could also contribute to the carbon isotope distribution in hexoses as well as in the photorespiratory carbon oxidation cycle are unknown. However, the natural intramolecular distribution of stable carbon isotopes in carbohydrates is a critical point, since the isotopic composition of all the various compounds in plants inherits that observed in the primary products of photosynthesis. For example, it has been suggested that the isotope distribution in Glc is mainly responsible for the ¹³C enrichment of the CO₂ respired by *Phaseolus vulgaris* L.

Abbreviations used: DHAP, dihydroxyacetone-phosphate; E4P, erythrose-4-phosphate; FBP, fructose-1,6-bisphosphate; Glc, glucose; G3P, glyceraldehyde-3-phosphate; PGA, phosphoglyceric acid; R5P, ribose-5-phosphate; RuBP, ribulose-1,5-bisphosphate; X5P, xylulose-5-phosphate.

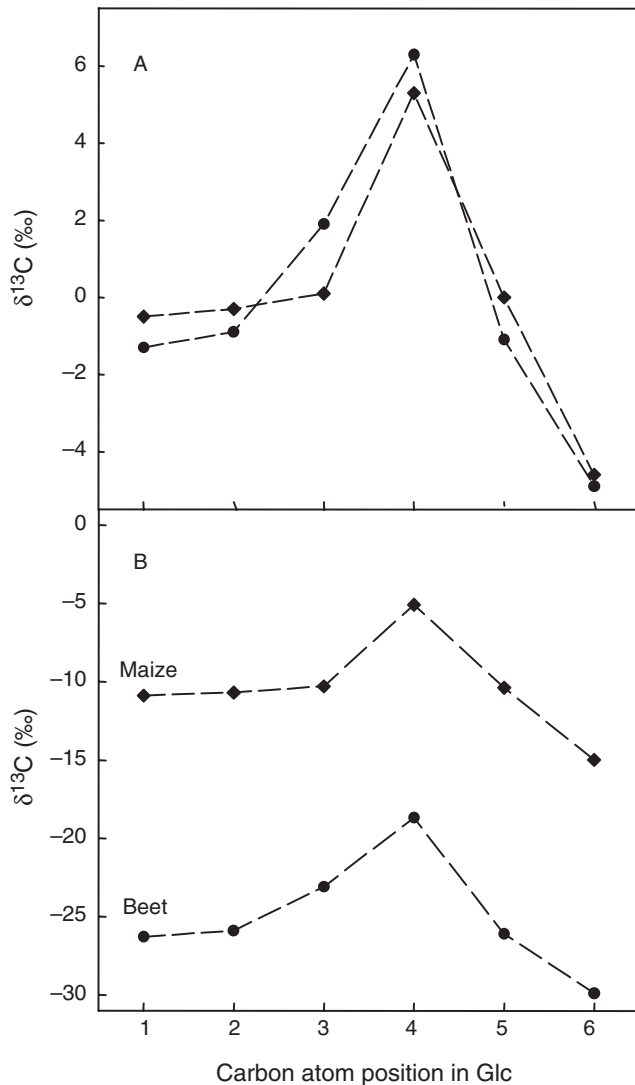


Fig. 1. Carbon isotope deviation from mean isotope composition ($\Delta\delta^{13}\text{C}$, A) and carbon isotope composition ($\delta^{13}\text{C}$, B) in glucose isolated from sugar beet syrup (●) and hydrolysed maize flour (◆) by Rossmann *et al.* (1991).

leaves in the dark (Duranceau *et al.* 2001; Tcherkez *et al.* 2003). Another consequence of the carbon isotope distribution in carbohydrates and acetyl-CoA, which is polymerised to fatty acids, is the strong ^{13}C depletion of lipids and oil (DeNiro and Epstein 1977). Nevertheless, direct experimental investigation of the metabolic origin of the carbon isotope distribution in carbohydrates is complicated, at least, by the three following reasons: (i) obtaining sufficient amounts of Calvin-cycle intermediates for isotopic analysis is difficult, (ii) the isolation of chloroplasts for *in vitro* studies of the Calvin cycle often requires some organic reagents that can adulterate the isotope composition of metabolites and (iii) enzymes do not behave rigorously in the same way *in vitro* and *in vivo*. Thus, in this paper a theoretical method for investigating the origin of the intramolecular

isotope distribution in Glc and carbohydrates produced by photosynthesis, and the effect of the fractionating reactions of the Calvin cycle, photorespiration, day respiration and starch synthesis is proposed. The model that is developed here suggests that the aldolase and transketolase reactions of the Calvin cycle are fractionating steps. In addition, sucrose produced in the night from starch breakdown is suggested to be isotopically heavier than sucrose produced in the light, and this effect could explain the variability of the sucrose isotopic signature in the literature. The model is also used to estimate the respiratory and photorespiratory fractionations in the light that appear to depend only weakly on the photorespiratory activity. Such a model, which takes into account the mechanisms of the main reactions involved in the carbon primary metabolism, could be a useful theoretical background for studying leaf photosynthetic discrimination and metabolism in the future.

Methods

Assumptions and methods

The model presented here focuses on the main mechanisms of primary processes of carbon metabolism: carbon dioxide fixation through the Calvin cycle, starch synthesis, photorespiration and dark respiration. It ignores any discrimination during the reactions that do not involve modifications of C–C bonds. In the case of strictly unidirectional processes, no fractionation occurs because the fluxes for each isotopomer adjust in the steady state. When there are some branching points in the reaction scheme, a fractionation can emerge since a reaction can consume the remaining molecules that were not used by the other fractionating reactions. Nevertheless, we recognise that, in practice, fractionation can also occur when reactions are composed of reversible steps in which the equilibrium thermodynamic constant is not the same for the different isotopomers. The model takes into account a set of reactions with a net flux directed in a given direction, i.e. towards the regeneration of ribulose-1,5-bisphosphate (RuBP) for the Calvin cycle so that the reactions can be considered as irreversible steps.

Reactions considered in the model

The model takes into account the main chemical reactions of the Calvin cycle (Scheme 1, Appendix), and it is supposed that the discriminating enzymes are those involved in modifying C–C bonds: Rubisco, aldolase, transketolase, and glycine decarboxylase. As described in Scheme 1 (Appendix), the modelled Calvin cycle is composed of one reaction of carboxylation (ribulose-1,5-bisphosphate + $\text{CO}_2 \rightarrow 2$ glyceraldehyde-3-phosphate), one reaction of isomerisation (glyceraldehyde-3-phosphate \rightarrow dihydroxyacetone-phosphate), two (trans) aldolase-catalysed reactions (dihydroxyacetone-phosphate + glyceraldehyde-3-phosphate \rightarrow fructose-1,6-bisphosphate and dihydroxyacetone-phosphate + erythrose-4-phosphate \rightarrow sedoheptulose-7-phosphate) and two transketolase reactions (glyceraldehyde-3-phosphate + fructose-1,6-bisphosphate \rightarrow erythrose-4-phosphate + xylulose-5-phosphate and glyceraldehyde-3-phosphate + sedoheptulose-7-phosphate \rightarrow ribose-5-phosphate + xylulose-5-phosphate). Ribose-5-phosphate and xylulose-5-phosphate are converted to ribulose-1,5-bisphosphate. The photorespiratory pathway is modelled as follows: oxygenation (ribulose-1,5-bisphosphate + $\text{O}_2 \rightarrow$ Gly), condensation of two Gly molecules to Ser ($2 \text{ Gly} \rightarrow \text{Ser} + \text{CO}_2$), and conversion of Ser to glyceraldehyde-3-phosphate (G3P). The triose phosphates exported from the chloroplast (with a flux E) are directed to aldolisation and also other purposes like glycolysis. The latter is assumed to consume one third of the export flux

(= $E/3$) and so the molar flux of hexoses to Suc synthesis is also $E/3$. Isotope discriminations during reactions that do not make or break C–C bond, such as the $\text{HCO}_3^-/\text{CO}_2$ equilibration can occur, but again it is assumed in this model that the discriminating enzymes are only those modifying bonds between carbon atoms.

Synthesis of starch and sucrose

In the light, part of the assimilated carbon is converted to transitory starch from FBP and another part is exported from the chloroplast, involving the triose-phosphate/phosphate translocator (Flügge 2000). We assume that dihydroxyacetone-phosphate (DHAP) is the major molecule exported from the chloroplast (Mohr and Schopfer 1994). DHAP is converted to FBP and then to Suc in the cytoplasm (Scheme 1, Appendix). During the night, transitory starch is hydrolysed to glucose and maltose, which are exported from the chloroplast into the cytoplasm (Lu and Sharkey 2004; Weise *et al.* 2004). Maltose is subsequently converted to glucose and glucose molecules are then used for Suc synthesis (Trethewey and Smith 2000). Thus, the carbohydrates accumulated in storage organs may come from both transitory starch degradation and light export of Suc, and the proportions of each depend on the species and growth conditions. Glc molecules from sugar beet (syrup) and maize (hydrolysed flour) analysed by Rossmann *et al.* (1991) also probably originate from both night- and light-derived Suc. The model takes into account this aspect by partitioning the flux of assimilated carbon to light export of DHAP from the chloroplast and synthesis of transitory starch from FBP (the molar flux of hexose to starch synthesis is denoted as T times the molar rate of RuBP carboxylation). For isotope effects calculations, the proportion of carbohydrates in storage organs derived from light-produced Suc is denoted as L . The Suc export flux to phloem is $E/3$ (see Scheme 1 and previous paragraph); thus $L = E/(E + 3T)$.

Isotope effects

The variables used in the model are the isotope ratios $R = {}^{13}\text{C}/{}^{12}\text{C}$ and the inverse isotope effects. The ‘isotope effect’ of a given reaction is the isotope ratio of the source compound divided by that of the product. Then we have: $R_{\text{product}} = R_{\text{substrate}}/\alpha = \alpha^{-1}R_{\text{substrate}}$ where α is the isotope effect. This relationship can be rearranged with the ‘discrimination’ that is defined by $\Delta = \alpha - 1$. Then we have $\Delta = (R_{\text{substrate}} - R_{\text{product}})/R_{\text{product}}$. The carbon isotope composition is defined as $\delta^{13}\text{C} = (R_{\text{compound}} - R_{\text{standard}})/R_{\text{standard}}$ (the standard is the international reference material Pee Dee Belemnite), and then we have: $\Delta = (\delta^{13}\text{C}_{\text{substrate}} - \delta^{13}\text{C}_{\text{product}})/(\delta^{13}\text{C}_{\text{product}} + 1)$ that is, $\delta^{13}\text{C}_{\text{product}} + \Delta \approx \delta^{13}\text{C}_{\text{substrate}}$.

As noted above, in the main model we only consider enzymes involved in breaking C–C bonds. Thus, five isotope effects are assumed to occur by the action of (trans)aldolase and transketolase. The aldolase reaction may discriminate at the C-3 and C-4 positions of FBP as suggested by Gleixner and Schmidt (1997). An isotope effect in the C-2 position of FBP could occur because the enzyme links DHAP to G3P at this position and makes a Schiff base during the reaction with DHAP. We denote the aldolase inverse isotope effects (in the direction of FBP production) on C-2, C-3 and C-4 positions of FBP as a_2 , a_3 and a_4 , respectively. The transketolase reaction, which requires thiamine pyrophosphate as a cofactor, may discriminate between carbon atoms at positions involved in C–C bonds, that is, C-2 and C-3 positions of xylulose-5-phosphate (X5P) and C-1 of erythrose-4-phosphate (E4P) and ribose-5-phosphate (R5P). The inverse isotope effects, in the direction of production of FBP, are t_2 , t_3 and t_1 , respectively. The isotope composition ($\delta^{13}\text{C}$) of carbon dioxide that is fixed to RuBP by Rubisco is assumed to be equal to that of CO_2 in air ($\delta_a = -7.8\%$) minus the photosynthetic discrimination of typically close to 20‰ in C₃ plants (i.e. -28%). The corresponding ${}^{13}\text{C}/{}^{12}\text{C}$ ratio of CO_2 which

is fixed to RuBP is denoted R^* . By this we mean that the isotope ratio of the C-3 position of the PGA molecule that incorporated the CO_2 fixed by RuBP carboxylase is R^* . Photorespiratory decarboxylation of Gly fractionates between carbon isotopes and the associated carbon isotope discrimination is denoted as g with respect to the C-1 of Gly.

We also recognise that Rubisco fractionates against ${}^{13}\text{C}$ in the C-3 position of RuBP during carboxylation by approximately 8‰ (Roeske and O’Leary 1984). Nevertheless, in the steady state, there is no branching at this point, so the RuBP pool becomes enriched in C-3 by 8‰, and there is no further effect. While it is true that the RuBP can be used for oxygenation as well as carboxylation, the C-3 from RuBP ends up in the C-1 of GAP each time. For that reason the isotope ratio in C-1 of GAP does not depend on the fractionation at RuBP C-3, and so the latter is not taken into account in the present model.

Experimental data used for comparisons

Isotope effects of aldolase isolated from rabbit muscles have been investigated *in vitro* by Gleixner and Schmidt (1997). Kinetic isotope effects (ratios of the rate constants of the isotopomers: k_{12}/k_{13}) during FBP breakdown ($\text{FBP} \rightarrow \text{DHAP} + \text{G3P}$) have been found to be 1.0159 ± 0.0066 and 0.9968 ± 0.0090 in C-3 and C-4 positions of FBP, respectively, and equilibrium isotope effects have been found to be 1.0036 ± 0.0002 and 1.0049 ± 0.0001 , respectively. That is, during the breakdown of FBP when the products DHAP and G3P are kept at extremely low concentrations, the C-1 position of DHAP is 15.9‰ depleted compared with the C-3 position of FBP, and the C-1 position of G3P is enriched compared with the C-4 position in FBP by 3.2‰. A negative kinetic isotope effect is unusual and its origin remains unclear. When the aldolase products are at equilibrium, the C-1 position of DHAP is 3.6‰ depleted compared with the C-3 position of FBP and the C-1 position of G3P is depleted compared with the C-4 position of FBP by 4.9‰. This means that the kinetic isotope discriminations during the formation of FBP are $15.9 - 3.6 = 12.3\%$ in the C-3 position and $-3.2 - -4.9 = -8.1\%$ in the C-4 position. The measured kinetic isotope effect in C-3 was greater than that found in C-4 during FBP breakdown, and these non-symmetric values suggest that the C–C bond fission between dihydroxyacetone-phosphate and glyceraldehyde-3-phosphate is not solely responsible for this isotope effect. The simultaneous ${}^{13}\text{C}$ enrichments in C-3 and C-4 positions in Glc support the assumption that the equilibrium isotope effects that favour the ${}^{13}\text{C}$ isotopomers during FBP formation are mainly responsible for the Glc isotopic pattern. The equilibrium isotope effects in C-3 and C-4 positions during FBP formation calculated from Gleixner and Schmidt (1997) are $1/a_3 = 1/1.0036 = 0.9964$ (the discrimination value is $\Delta = -3.6\%$) and $1/a_4 = 1/1.0049 = 0.9951$ (the discrimination value is $\Delta = -4.9\%$).

Mathematical background of the model

The positional isotope ratio of one molecule at the n th Calvin cycle round is described by a recurrence equation deduced from the mass balance equation. For a given chemical reaction with isotope effect α , the isotope ratio ${}^{13}\text{C}/{}^{12}\text{C}$ in the product is $\alpha^{-1}R_{\text{substrate}}$ (see above) and consequently the proportion of ${}^{13}\text{C}$ in the product of a reaction is $\alpha^{-1}R_{\text{substrate}}/(1 + \alpha^{-1}R_{\text{substrate}})$. The quantity of ${}^{13}\text{C}$ in a given position of a particular molecule is expressed at the $n + 1$ th round of the Calvin cycle with the ${}^{13}\text{C}$ quantity in the same position at the n th round, plus the ${}^{13}\text{C}$ quantities gained by the producing fluxes, minus those lost through the consuming fluxes. For example, consider the case of the ${}^{13}\text{C}$ amount in C-1 position of G3P. This amount is denoted as $[\text{G3P-C1}]_{n+1}$ at the $n + 1$ th round of the Calvin cycle. Carbon in C-1 of G3P comes from the C-3 position of RuBP and from carbon dioxide. It is consumed by some reactions that are here simplified to one, with an isotope effect α . If there is no photorespiration and s (mol of C) is the

flux of carbon through reactions for a given time interval, $[G3P-C1]_{n+1}^{13}$ is given by:

$$[G3P-C1]_{n+1}^{13} = [G3P-C1]_n^{13} + \frac{s}{2} \left(\frac{[RuBP-C3]_n^{13}}{[RuBP-C3]_n^{13} + [RuBP-C3]_n^{12}} + \frac{[CO_2]^{13}}{[CO_2]^{13} + [CO_2]^{12}} \right) - \frac{1}{\alpha} \frac{R(G3P-C1; n)}{1 + \frac{1}{\alpha} R(G3P-C1; n)},$$

where $R(G3P-C1; n)$ and $[G3P-C1]_n^{13}$ are the isotope ratio ($R = {}^{13}C / {}^{12}C$) and the amount of ${}^{13}C$ in C-1 of G3P at the n th round of the Calvin cycle, respectively, and α is the isotope effect at C-1 of the reaction that consumes G3P.

In the steady state $[G3P-C1]_{n+1}^{13} = [G3P-C1]_n^{13}$ and so s drops out as a factor. Because natural isotope ratios (R) are normally much smaller than 1, we ignore terms in R^2 and therefore make the approximation (detailed in the Appendix):

$$\frac{R(G3P-C1; n)}{1 + \frac{1}{\alpha} R(G3P-C1; n)} \approx R(G3P-C1; n).$$

So the stable isotope ratio in the steady state is given by:

$$R(G3P-C1) \frac{1}{\alpha} = \left(\frac{R(RuBP-C3) + R(CO_2)}{2} \right).$$

Note that with the delta values ($\delta^{13}C$) that are the R values scaled to the R value of the Pee Dee Belemnite international standard (that is, $\delta^{13}C = [R - R_{\text{standard}}] / R_{\text{standard}}$), the expression rearranges as follows (again neglecting second order terms):

$$\delta^{13}C_{G3P-C1} - \Delta_{\alpha} = \left(\frac{\delta^{13}C_{RuBP-C3} + \delta^{13}C_{CO_2}}{2} \right),$$

where $\Delta_{\alpha} = \alpha - 1$, and is the discrimination at C-1 of the 'reaction' consuming G3P.

The equations for all the carbon atom positions in the molecules of the Calvin cycle are written in the same way (see the Appendix).

General procedure followed in this study

Two procedures are then possible to use this model: choosing arbitrary isotope effects and thereby calculating isotope ratios obtained in the steady state ($n = +\infty$), or with given isotope ratios to calculate isotope effects. Since positional isotope ratios have already been measured in Glc from sugar beet syrup (Rossmann *et al.* 1991) we choose the latter route. That is, the model is used to get putative isotope effects ('reverse' modelling). The calculated inverse isotope effects of aldolase are denoted a_2 , a_3 and a_4 and those of transketolase are denoted t_1 and t_2 . The mathematical expressions obtained for positional isotope ratios in hexoses do not depend on t_3 , which thus, cannot be estimated (see Appendix).

The study in this paper is composed of three steps. First, the isotope effects of aldolase and transketolase are calculated under standard conditions of starch synthesis, photorespiratory activity, and glycine decarboxylase discrimination. 'Standard conditions' here mean physiological conditions that are usually found in C_3 plant leaves that is, 40% of the assimilated carbon is directed to starch synthesis, RuBP oxygenation activity relative to carboxylation is approximately 0.3, and isotope discrimination during glycine decarboxylation is +20%. Briefly, the latter comes from published results of gas exchange experiments, suggesting that the fractionation approaches the value of 20%. Second, the starch synthesis flux and photorespiratory activity are changed in order to investigate the sensitivity of the calculated isotope effects to these parameters. Third, setting the isotope effects to the values obtained in the standard

conditions, we investigate the effect of the photorespiratory activity on the photorespiratory and respiratory discrimination f and e , respectively, introduced by Farquhar *et al.* (1982) as 'forward modelling'. Eventually, the isotope effects of aldolase are discussed in relation to the composition of carbohydrates and (photo) respiratory CO_2 and the difference of isotope composition between starch and sucrose produced in the light is proposed to be linked to the aldolase isotope effects.

Results

Isotope effects of aldolase and transketolase in standard conditions

Aldolase is thought to be largely responsible for the isotopic pattern observed in hexoses by Rossmann *et al.* (1991; Gleixner and Schmidt 1997). However, this enzyme may not be the unique origin of heterogeneous carbon isotope distribution. There is a difference between C-1 and C-3 isotope ratios in natural Glc (Fig. 1) and yet the carbon atoms in C-1 and C-3 positions in FBP are continuously exchanged through the Calvin cycle by transketolase. In other words, the isotopic ratios in C-1 and C-3 of chloroplastic hexoses are identical if transketolase does not discriminate at all whatever the values of aldolase isotope effects are. That is why we assume in this model that transketolase fractionates with the discriminations $\Delta(t_3)$, $\Delta(t_2)$ and $\Delta(t_1)$ in positions C-3 and C-2 of X5P and C-1 of E4P or R5P, respectively.

The enzyme isotope discriminations were first calculated with the model assuming that the storage of starch is around 40% of the assimilated carbon (i.e. the quantity in moles of FBP consumed by starch synthesis relative to moles of assimilated CO_2 is $T \sim 0.056$, see below) and the photorespiratory oxygenation relative rate ($\Phi = v_o / v_c$) is 0.3. In such T conditions of carbon partitioning to starch, light-produced Suc and Suc from transitory starch contribute similarly to the accumulation of carbohydrates in storage organs (i.e. the contribution of light-produced Suc is approximately $L = 0.5$). That is, the carbon isotope ratios found by Rossmann *et al.* (1991) are supposed to result from an equimolar mixture of light- and night-produced Suc. The fitted discrimination values are shown in Table 1 for $\Phi = 0.3$ and 0.5. The isotope effects given in Table 1 are calculated with the isotopic ratios in positions C-1 to C-5 in sugar beet Glc measured by Rossmann *et al.* (1991). The isotopic ratio in C-6 position is not used in Table 1 because the modelled isotope ratios in C-1 and C-6 are equal. If the isotope ratio in C-6 is then used instead of that in C-1, the calculated discrimination values of aldolase are +6.8% for C-2, +20.2% for C-3 and +4.5% for C-4 and the calculated discrimination values of transketolase are +6.5 and -7.4% for C-1 and C-2 of R5P (E4P) and X5P, respectively.

Taking photorespiratory oxygenation as 0.3 ($=\Phi = v_o / v_c$) with $g = 20\%$ (g was set to that value for reasons that are explained below) the modelled isotope

Table 1. Observed and calculated values of carbon isotope discrimination by aldolase and transketolase

Calculated values were obtained with the model assuming that the oxygenation rate is 0.3 or 0.5 with a discrimination value during glycine decarboxylation of 20‰. The FBP flux to starch synthesis, T , is 0.056. The contribution of light-produced Suc to storage carbohydrates from which the Rossmann *et al.* (1991) data were obtained is $E/(E + 3T)$ where E is the export flux from the chloroplast

Reaction name	Equation	Values in literature	Values estimated with this model	Values calculated with β factors (equilibrium)
Aldolisation	DHAP + G3P \rightarrow FBP and	Equilibrium / kinetic	with $\Phi = 0.3/0.5$	
	DHAP + E4P \rightarrow S7P	$\Delta(a_3) - 3.6/+12.3\text{‰}^A$	$\Delta(a_2) - 1.2/-0.4\text{‰}$	-1.09‰
		$\Delta(a_4) - 4.9/-8.3\text{‰}^A$	$\Delta(a_3) - 5.8/+2.6\text{‰}$	-18.24‰
			$\Delta(a_4) - 16.1/-7.8\text{‰}$	-7.33‰
Transketolisation	G3P + FBP \rightarrow E4P + X5P and	-	$\Delta(t_1) + 7.6/+4.7\text{‰}$	+8.03‰
	G3P + S7P \rightarrow R5P + X5P	-	$\Delta(t_2) = -0.8/0.0\text{‰}$	0
		-	$\Delta(t_3)$ not determined	-8.09‰
Acetoin synthesis	2 pyruvate (CH ₃ -CO-COOH) \rightarrow	Δ approximately -8‰ in C-2 ^B	-	-
	acetoin (CH ₃ -CO-CHOH-CH ₃) + 2CO ₂	Δ approximately +2‰ in C-3 ^B	-	-
Triose phosphate isomerisation	G3P \leftrightarrow DHAP	in C-1	-	+10.07‰
		in C-2	-	-9.81‰
		in C-3	-	-0.77‰

^AGleixner and Schmidt (1997).

^BRinaldi *et al.* (1974).

discriminations of aldolase on C-3 and C-4 positions of FBP are -5.8 and -16.1‰, respectively (Table 1). The calculated discrimination value in the C-2 position is smaller (-1.2‰). The modelled discrimination value of transketolase in the C-1 position of E4P or R5P is +7.6‰ and that in the C-2 position of X5P is -0.8‰.

Sensitivity of the isotope effects to starch synthesis and compartmentation

Isotope effects (see above) were calculated with a 'usual' rate of starch synthesis. It means that the flux of starch synthesis is approximately 40% of the carbon that is assimilated (Sharkey *et al.* 1985; Trethewey and Smith 2000), which corresponds to $T = 0.056$ (T is the rate of hexose diversion to starch, calculated with $0.4*(1 - \Phi/2)/6$ with $\Phi = 0.3$; the coefficient 6 comes from the fact that there are six carbon atoms in FBP). Obviously, the calculated isotope effects depend on T and Fig. 2A shows the calculated discrimination values (at $\Phi = 0.3$ with $g = 20\text{‰}$) of the aldolase and transketolase reactions as a function of the starch synthesis rate T . The maximal value of T is fixed by the constraint $E > 0$, so that T_{\max} is $(1 - \Phi/2)/6$. It should be noted that changing the value of T modifies the value of L , which is the proportion of light-produced Suc in stored carbohydrates. Although the transketolase discrimination in C-2 of X5P varies only slightly with changed assumptions regarding the value of T , the calculated transketolase discrimination in C-1 of X5P diverges when T tends towards zero. The calculated discrimination values of aldolase also rapidly diverge except for $\Delta(a_4)$ when the assumed value of T tends towards T_{\max} , but do not vary greatly for the T values comprised between 0.05 and 0.07. The

divergence of the calculated discriminations when T tends towards T_{\max} is the consequence of the competition between the export and the starch synthesis fluxes in the model: when starch synthesis increases, export decreases, and aldolase fractionation becomes less effective. Thus, to explain the observed data, the reverse model requires that the aldolase isotope effect increase as T tends towards T_{\max} .

Assuming that the aldolase reaction favours the ¹³C-isotopomers in C-3 and C-4 of FBP, the remaining exported DHAP molecules are ¹³C-depleted in C-1, so that cytoplasmic FBP is not ¹³C-enriched in modelled C-3 and C-4 positions. As a consequence, the cytoplasmic FBP and hexoses in transitory starch do not have the same isotope ratios and this aspect is taken into account in the model when calculating the isotope effects by assuming that the Glc molecules derived from light-produced (cytoplasmic) Suc represent the proportion L of the Glc analysed by Rossmann *et al.* (1991). For given values of Φ and T , L is determined by the relationship $L = E/(E + 3T)$ in Fig. 2A, where it is assumed that the accumulation of transitory starch reflects its contribution to storage of carbohydrates. Nevertheless, we recognise that the accumulation of transitory starch and its contribution to reserves in storage organs may be uncoupled because the conversion of exported Suc to stored carbohydrates may not be the same for night- and light-produced Suc. Indeed, in the model, L reflects exactly the contribution of light sucrose and starch in storage organs. However, it is possible that during the light, Suc exported by leaves is not converted to stored carbohydrates with the same efficiency as night-exported sucrose (for a discussion, see Trethewey and Smith 2000). The effect of L with fixed

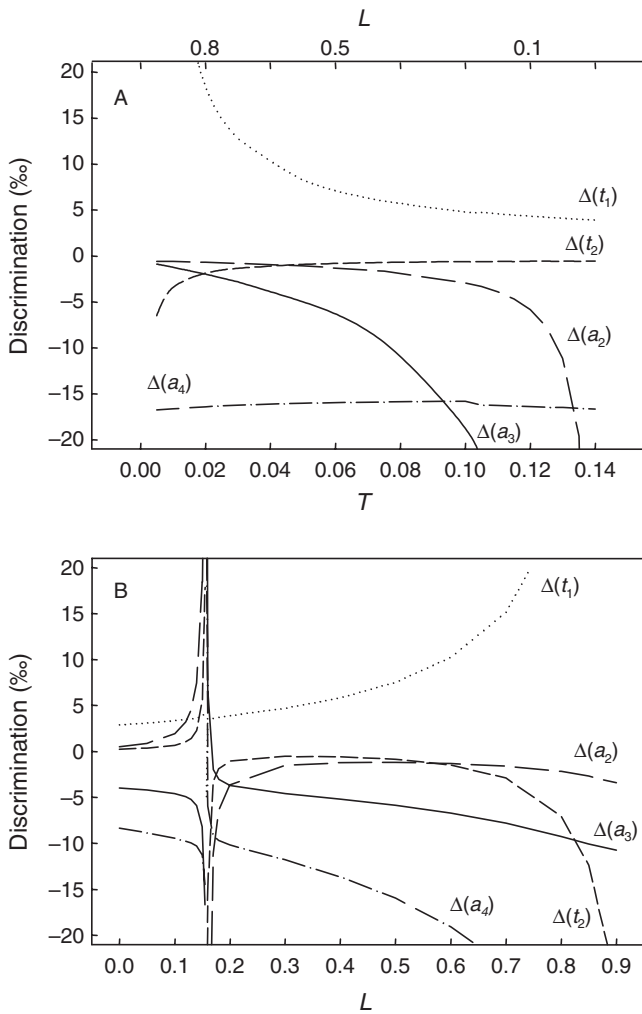


Fig. 2. Modelled effects of (A) starch synthesis and (B) contribution of light-produced carbohydrates on the discrimination values of the aldolase and transketolase reactions calculated with the model. (A) The hexose flux to starch synthesis is denoted as T which represents the quantity in moles of FBP consumed by starch synthesis relative to moles of CO_2 fixed. The contribution of light-produced Suc to storage carbohydrates is $L = E / (E + 3T)$ where E is the DHAP export flux from the chloroplast and is indicated in the upper axis. (B) The contribution of light-produced Suc to storage carbohydrates is denoted as L . The FBP flux to starch synthesis is imposed constant at 0.056. For both A and B, $\Delta(a_i)$ is the discrimination corresponding to the aldolase inverse isotope effect a_i and $\Delta(t_i)$ is the discrimination corresponding to the transketolase inverse isotope effect t_i . Discriminations are calculated as follows: $\Delta(a_i) = 1/a_i - 1$. The oxygenation rate Φ is 0.3 with $g = 20\%$.

values of T ($= 0.056$) and Φ ($= 0.3$) on calculated isotope discriminations is shown in Fig. 2B. The discrimination values are not very sensitive to L values around 0.5 except for $\Delta(a_4)$ and $\Delta(t_1)$ which vary between -13 and -19% and 5 and 10% , respectively, for $L = 0.4-0.6$. For a particular value of L , the discrimination values diverge because the denominators of some expressions tend towards zero.

Sensitivity of the isotope effects to the photorespiratory activity

Photorespiration was modelled with three steps: production of Gly by oxygenation of RuBP, condensation of two Gly molecules to Ser, and conversion of Ser to G3P. Any recycling of photorespiratory (or day respired CO_2) is taken into account in R^* . The photorespiratory activity is half the rate of RuBP oxygenation, and the latter (RuBP oxygenation in ratio to carboxylation rate) is Φ and the decarboxylation of Gly fractionates with a discrimination value g with respect to C-1 atom position in Gly. That said, the sensitivity of the calculated discriminations to Φ was investigated (Fig. 3A), assuming that $g = 20\%$.

With higher rates of photorespiration than assumed in standard conditions, that is $\Phi = 0.5$, the model gives aldolase isotope effects at C-2, C-3 and C-4 of FBP of -0.4 , $+2.6$ and -7.8% , respectively (Table 1). The calculated discriminations of aldolase are negative when $\Phi = 0.3$, which means that they favour the ^{13}C isotopomers, while those of transketolase are positive, discriminating against the ^{13}C isotopomers. This induces a ^{13}C enrichment in the remaining substrate (FBP), which, in turn, is the substrate for starch formation. The isotope discriminations values calculated with the isotope ratios found in maize Glc by Rossmann *et al.* (1991) assuming zero photorespiration and an R^* value corresponding to -12% (maize is a C_4 plant) and the same value for T ($T = 0.056$) are 0.2 , -9.3 and -19.1% in C-2, C-3 and C-4, respectively, for aldolase, and 4.8 and -1.0% in C-1 and C-2 for transketolase. With a value of T that more likely occurs in maize, that is corresponding to 15% of the assimilated carbon (Evans 1993) that is, $T = 0.025$, the results are -0.1 , -5.5 and -19.5% in C-2, C-3 and C-4, respectively, for aldolase, and $+9.3$ and -1.7% in C-1 and C-2 for transketolase. Interestingly, the isotope effect values obtained with isotope compositions in sugar beet Glc with $\Phi = 0.3$ and $t = 0.056$ are similar to those obtained with the isotope composition in maize with zero photorespiration and $t = 0.025$.

The (photo) respiratory fractionations e and f

The effects of photorespiration on the ^{13}C distribution in FBP and on the (photo) respiratory fractionation was investigated with fixed aldolase and transketolase isotope effects — those obtained in the ‘standard’ conditions (Fig. 3B).

When $g = 0$, the photorespiratory activity induces a slight ^{13}C -depletion of the FBP molecule (Fig. 3B, ----) because (i) one C-2 carbon atom of RuBP is lost through Gly decarboxylation and substituted by a C-1 carbon atom of RuBP in the C-2 position of G3P and (ii) the C-2 carbon atom of RuBP is more enriched in ^{13}C than that at the C-1 position. For example, the modelled mean isotope composition ($\delta^{13}\text{C}$) of FBP is around -24.8% when photorespiratory activity is as high as 50% of carboxylation and around -24.4% when

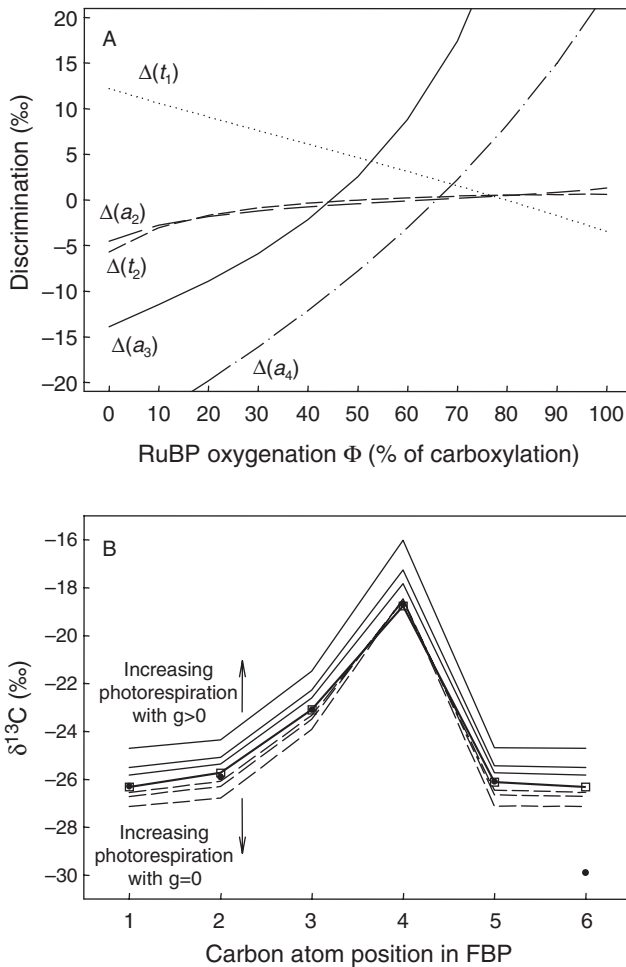


Fig. 3. Modelled effects of photorespiration on (A) estimated discriminations of aldolase and transketolase and on (B) carbon isotope distribution in FBP with fixed isotope effects. (A) The oxygenation of RuBP is denoted as Φ and expressed as a fraction of carboxylation. The flux of starch synthesis is fixed at 0.056 and the discrimination during Gly decarboxylation, g , is fixed at 20‰. $\Delta(a_i)$ is the discrimination corresponding to the aldolase inverse isotope effect a_i and $\Delta(t_i)$ is the discrimination corresponding to the transketolase inverse isotope effect t_i . Discriminations are calculated as follows: $\Delta(a_i) = 1/a_i - 1$. (B) The distribution without photorespiration is indicated with a thick line and squares. Oxygenation is equal to 30, 50, and 100% of carboxylation with (---) or without (—) isotope discrimination by Gly decarboxylase reaction (g). Data from Rossmann *et al.* (1991) are also indicated (●).

photorespiration is zero. Measurements of photorespiratory discrimination with gas-exchange measurements have shown a discrimination value of f around 7‰ (Rooney 1988) and 9‰ (Lanigan *et al.* 2003). That is, the photorespiratory decarboxylation discriminates against the ^{13}C isotopomers of Gly. Such values indicate that the discrimination in C-1 of Gly during its decarboxylation (g) is around 20‰, that is, around twice f . This originates from the fact that only one half of the Gly pool is involved in the decarboxylation (the other half becoming the C-2 and C-3 of

Ser) (see also below and Appendix). When photorespiratory RuBP oxygenation is equal to 50% of carboxylation and $g = 20$ ‰, photorespiration induces a ^{13}C enrichment in the chloroplastic FBP (Fig. 3B, —), with a modelled $\delta^{13}\text{C}$ value around -21.4 ‰. The isotopic composition of photorespired CO_2 is then -34.6 ‰, which explains the slight ^{13}C enrichment of FBP because of mass balance. In brief, the effect of photorespiration on isotope ratios in FBP is of variable importance depending on the oxygenation rate (Φ) and the discrimination during the decarboxylation of Gly (g), the ^{13}C -depletion induced by the photorespiration (the carbon in C-1 of RuBP going to the C-2 position of G3P) being partly balanced by the ^{13}C -enrichment in Gly induced by g . In the present study, R^* is assumed to be constant (the corresponding isotope composition is -28 ‰). Nevertheless, in physiological conditions that accompany the increase of photorespiration, such as stomatal closure, R^* would change to higher values (more enriched in ^{13}C), which could exaggerate the ^{13}C enrichment in hexoses. Alternatively, in a physiological experiment where oxygen concentration is increased, one would expect to see both more photorespiration and a depletion of ^{13}C .

From a theoretical viewpoint, the photorespiratory discrimination was defined by Farquhar *et al.* (1982) for gas-exchange measurements (denoted as f) as:

$$f = \frac{R_{\text{fixed carbon}}}{R_{\text{CO}_2}} - 1,$$

where R_{CO_2} is the isotopic ratio of photorespiratory CO_2 . The ‘fixed carbon’ is the net result of carboxylation and decarboxylations in the light that is, photorespiration and day respiration (see Appendix for calculations details). The value of f is not equal to g but it is around half the value of g (see Appendix for the mathematical evidence). With fixed isotope effects for aldolase and transketolase (the values obtained in *Isotope effects of aldolase and transketolase in standard conditions*) and the assumed value of $g = 20$ ‰, the predicted value of f is $+9.2$ ‰ (photorespiration discriminates against the ^{13}C isotopomer by 9.2‰). The sensitivity of f to the oxygenation rate Φ is shown in Fig. 4 and, clearly, f is not very sensitive to Φ . Similarly, there is a sensitivity of e and f to the rate of day respiration, but again it is very small (see Fig. 4).

Similarly, the effect of day respiration (involving glycolysis and the Krebs cycle) during the light period on photosynthetic discrimination has been modelled by Farquhar *et al.* (1982) with a mathematical term that is a function of respiratory discrimination denoted as e and defined as:

$$e = \frac{R_{\text{fixed carbon}}}{R_{\text{CO}_2}} - 1,$$

where R_{CO_2} is the isotopic ratio of respired CO_2 . Assuming that the triose phosphates molecules that enter glycolysis are completely degraded to CO_2 through respiration, the

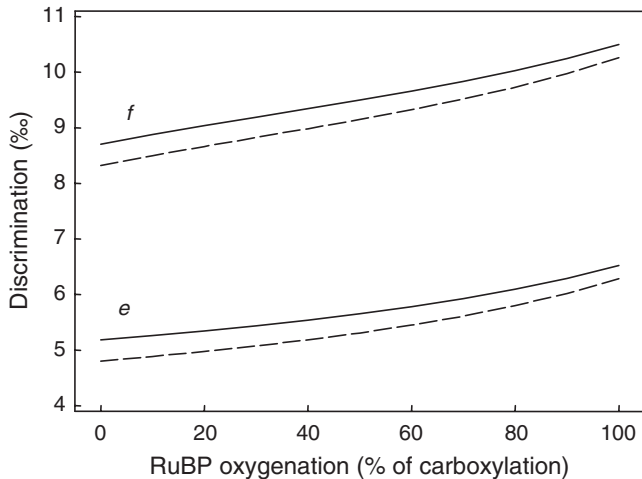


Fig. 4. Modelled effect of the oxygenation rate (Φ) on the estimated photorespiration discrimination f and day respiratory discrimination e . The starch synthesis flux is fixed at 0.056 and the discrimination during Gly decarboxylation g is fixed at 20%. Day respiration is supposed to be maximal ($E/3$, —) or minimal (converging to zero, ----).

predicted value of e is +5.5‰. Like f , e depends on photorespiration, but it is not very sensitive to Φ as shown in Fig. 4.

Discussion

Aldolase and transketolase reactions do discriminate between carbon isotopes

The carbon isotope composition in Glc in the data of Rossmann *et al.* (1991) is not homogeneous and bears at least two remarkable features: the asymmetric ^{13}C enrichment of the C-3 and C-4 positions and the strong ^{13}C depletion of the C-6 position. It has been proposed that the aldolase reaction is responsible for the ^{13}C enrichment of the C-3 and C-4 positions. However, because the C-1 and C-3 carbon atoms of FBP are exchanged through the Calvin cycle by transketolase (see Scheme 1), the (trans)aldolase reaction may not be the sole fractionating enzyme of the cycle and, presumably, transketolase could also discriminate between carbon isotopes. As it is difficult to directly measure the *in vivo* isotope effects, the main reactions involving C–C bond modifications were modelled and isotope effects were calculated with the data of Rossmann *et al.* (1991). The model is limited and considers only reactions in which C–C bonds are broken. It also considers that all reactions are irreversible. Nevertheless, we recognise that reactions that do not make or break C–C bonds can fractionate between isotopes, such as the triose phosphates isomerase, which catalyses the DHAP/G3P interconversion through simple exchanges of H between catalytic amino-acids and triose phosphate molecules. Although it has not yet been shown, it is possible that this enzyme fractionates as hypothesised by Hobbie and Werner (2004).

For standard values of photorespiration and starch accumulation, the calculated carbon isotope discriminations of aldolase in C-2, C-3 and C-4 of FBP were equal to -1.2 , -5.8 and -16.1 ‰, respectively (Table 1). These values for aldolase can be compared with the values obtained *in vitro* by Gleixner and Schmidt (1997) for isotope equilibrium effects of aldolase where equilibrium discrimination values were around -3.6 and -4.9 ‰ in C-3 and C-4 positions of FBP. The values can also be compared with the isotope effects predicted with the β factors of Galimov (1985). Importantly, the predictions obtained with this latter method also favour the ^{13}C isotopomers, although with values different from those obtained with the present model (Table 1).

Again, it should be noted that the isotope effects predicted by the present model are calculated with the positional isotope ratios in Glc and could be seen as ‘apparent’ isotope effects, i.e. isotope effects that are inferred from observed isotope ratios found in Glc. Of course, apparent isotope effects depend on the model that has been used for calculations. In particular, it should be highlighted that only the positions C-1 to C-5 in sugar beet Glc measured by Rossmann *et al.* (1991) were used; the isotopic ratio in C-6 position is not taken into account because the modelled isotope ratios in C-1 and C-6 are equal. This comes from the isomerisation of triose phosphate in the chloroplast and from the absence of (secondary) isotope effects on C-1 or C-6 of FBP in the model (see below).

The results of the model also suggest that transketolase discriminates in C-1 against ^{13}C when transferring the two-carbon unit on G3P, with discrimination values in C-1 of R5P (and E4P) and in C-2 of X5P around $+7.6$ and -0.9 ‰, respectively. Again, those values can be compared with the discrimination values calculated with the β factors procedure (Galimov 1985) and interestingly are very close to them; the discrimination in C-1 of R5P (E4P) is also against the ^{13}C isotopomers with a value of 8.03‰, and a discrimination value in C-2 of X5P of zero (Table 1). Accordingly, previous observations showed that the carbon atom of the carbonyl group in acetoine is ^{13}C enriched (Rinaldi *et al.* 1974, see Table 1) and that there is an isotope effect when transferring active acetaldehyde to benzaldehyde (H-L Schmidt personal communication). Thus, linking one two-carbon unit may give rise to an isotope effect on the carbon atom position decorated with an aldehyde function ($-\text{CHO}$), and this effect may occur in the Calvin cycle at the transketolase level.

The modelled isotope effects depend on the photorespiratory activity and starch synthesis flux

The discrimination values obtained with the model depend on three ‘key’ parameters used for ‘reverse modelling’: the photorespiratory oxygenation rate (Φ), the hexose flux to transitory starch synthesis (T), and the proportion of light-produced carbohydrates that contributed to the synthesis of the Glc analysed by Rossmann *et al.* (1991) (L). The value

assumed for photorespiratory activity has a strong effect on the calculated value of aldolase discrimination in C-3 and C-4 (Fig. 3A). The calculated aldolase discrimination in C-3 changes in sign when Φ exceeds approximately 0.45 (Fig. 3A), a value that can occur in C₃ plants. For such values of Φ , the aldolase isotope effects are similar to the kinetic isotope effects measured by Gleixner and Schmidt (1997), who showed that they favour ¹²C in C-3 and ¹³C in C-4. This observation addresses the question of the kinetic properties of the aldolase reaction. Although it has been postulated by Gleixner and Schmidt (1997) that equilibrium isotope effects are predominant during FBP production, some experimental data suggest that the aldolase reaction is not at equilibrium in the chloroplast (Gerhardt *et al.* 1987). In that case, the isotope effects of aldolase may be partly kinetic, i.e. between full equilibrium and kinetic values.

Increase of the starch synthesis value (T) used for calculations results in a strong divergence of the isotope effects of aldolase in C-2 and C-3 and of the transketolase effect in C-1 (Fig. 2A). Hence, the choice of the T and Φ parameters is critical. When both parameters increase, the ¹³C enrichment induced in Ser and then in triose phosphates by photorespiration is balanced by the ¹³C depletion induced by the aldolase reaction when export is diminished by starch synthesis. The value of T chosen here as a 'normal' condition ($T = 0.056$) appears to be reasonable in that it corresponds to 40% of the assimilation flux being directed to starch synthesis, and such a value has been observed (Sharkey *et al.* 1985; Lin *et al.* 1992). From a theoretical point of view, the relative contribution of light- v. night-produced Suc (L) to storage carbohydrates also has an effect on the calculated enzymatic discriminations (Fig. 2B), but the numerical study shows that the latter are not very sensitive to L when it is around 0.5, which is the value obtained with $T = 0.056$.

The origin of the ¹³C depletion in C-6 position of glucose

The origin of the ¹³C depletion of the C-6 carbon atom in Glc is unclear. The C-6 carbon atom follows the prediction 'low β factor coupled to low $\delta^{13}\text{C}$ ' first stated by Galimov in 1985. However, no reversible reaction can promote the 'thermodynamic order' in this position *in vivo*; in contrast, photosynthetic carbon assimilation includes some irreversible reactions (Schmidt 2003). So the C-6 depletion is not yet clearly explained. In the framework of the present model, this feature could not be simply explained within the Calvin cycle because of the isomerisation between G3P and DHAP. This reaction is responsible for the equilibration between the C-1 and C-6 carbon atoms in Glc. NMR studies with ¹³C labelling have shown that this equilibration occurs *in vivo* (Keeling *et al.* 1988). So, it is highly probable that some particular reactions are involved in the ¹³C depletion of the C-6 in Glc, such as the cyclisation (that is, the equilibrium between the cyclic and the open forms of hexoses) or the isomerisation itself, as suggested by Hobbie

and Werner (2004). However, β factors calculations suggest that the isomerisation equilibrium does not discriminate at the C-3 position of G3P (Table 1). The ¹³C enrichment of the C-1 in glucose as compared with C-6 could also be related to starch synthesis (the C1-C4 hexose-hexose bond during polymerisation could favour the ¹³C in C-1) or the pentose phosphate pathway, which occurs in storage organs. Indeed, as has been shown for some enzymatic decarboxylations (O'Leary 1976, 1980, 1988), the decarboxylation of the C-1 of Glc in the pentose phosphates pathway probably involves discrimination against ¹³C, thus, contributing to a ¹³C enrichment in the remaining Glc molecules. However, the isotope ratio in C-6 of Glc determined by Rossmann *et al.* (1991) gives rise to aldolase isotope effects which are not in accordance with those found by Gleixner and Schmidt (1997) even when R^* (the isotope ratio of fixed CO₂) is set to other values (data not shown). In addition, experiments with ¹⁴C (reviewed by Rabinowitch 1956) indicated a similar labelling level in C-1, C-2, C-5 and C-6 in Glc, strongly suggesting that the reactions of the Calvin cycle *per se* lead to an isotopic signature in C-6 similar to that in C-1, and that other reactions are responsible for the ¹³C depletion of the C-6 carbon atom in Glc.

Physiological isotopic consequences of the isotope effects

Spatial and temporal compartmentation has an effect on the carbon isotope signature of the cytoplasmic hexoses. Indeed, because aldolase favours ¹³C, the remaining DHAP molecules that are exported from the chloroplast are ¹³C depleted. Thus the hexoses and Suc produced in the cytoplasm during the light period are ¹³C depleted or much less ¹³C enriched than those in the chloroplast (Table 2). If the exchange of triose phosphates between the chloroplast and the cytoplasm were easy and rapid, that is, the frontier between the two compartments virtually disappeared, the aldolase reactions in the chloroplast and the cytoplasm would use the same pool of triose phosphates. As a consequence, chloroplastic and cytoplasmic FBP (starch and light-produced Suc) would have the same isotopic signature. On the one hand, some data in the literature show a difference between starch and light-produced Suc suggesting that the compartmentation is tight (Table 2). On the other hand, Gleixner *et al.* (1998) showed that the carbon atoms in C-3 and C-4 in soluble sugars are approximately 5‰ enriched in ¹³C compared with the mean isotope composition of the molecules, suggesting that chloroplastic and cytoplasmic aldolases produce FBP from the same triose phosphates pool. Nevertheless, in the work of Gleixner *et al.* (1998), the total-leaf extract was used for purification so that the cytoplasmic and chloroplastic hexoses were mixed. It is likely that the compartmentation of triose phosphates is in between the two extreme cases, resulting in depleted light-produced soluble sugars in which the C-3 and C-4 are somewhat ¹³C-enriched. Further experimental work is now needed to check

Table 2. Calculated and observed values of carbon isotope composition of carbohydrates and CO₂ in leaf cells

Predicted values are calculated assuming that the hexose flux to transitory starch synthesis is 0.056 and that oxygenation activity is $\Phi = 0.3$ with a discrimination value during Gly decarboxylation (g) of 20‰. The isotope effects of aldolase and transketolase are those calculated previously (see Results). The values in brackets indicate isotopic difference (‰) of compounds compared with transitory starch

		This model	<i>Phaseolus</i> ^A	<i>Nicotiana</i> ^B	<i>Helianthus</i> ^C	<i>Solanum</i> ^D	<i>Beta</i> ^E
Light	Fru	-26.5 (-4.4)	-	-	-	-27.3 (-2.3)	-28 (-3.7)
	Suc	-26.5 (-4.4)	-	-	-28.8 (-0.7)	-28 (-3)	-28 (-3.7)
	Glc	-26.5 (-4.4)	-	-	-	-27.3 (-2.3)	-28 (-3.7)
	Day respired CO ₂	-31.6	-	-	-	-	-
	Photorespired CO ₂	-35.2	-	-	-	-	-
	Transitory starch	-22.2	-24.7	-27	-28.1	-25	-24.3
Night	Fru	-22.2 (0)	-25.3 (-0.6)	-27.8 (-0.8)	-	-	-
	Suc	≥ -22.2 (≥ 0)	-23.7 (+1)	-26.5 (+0.5)	-28.2 (-0.1)	-	-
	Glc	≥ -22.2 (≥ 0)	-25.9 (-1.2)	-28 (-1)	-	-	-
	Dark respired CO ₂	≥ -26.0 (≥ -3.8)	-21 (+3.7)	-22.8 (+4.2)	-	-	-
			This model	<i>Glycine</i> ^F	<i>Bromus</i> ^F	<i>Picea</i> ^F	<i>Populus</i> ^G
Light	Fru	-26.5 (-4.4)	-32.2 (-5.9)	-28.4 (-2.1)	-28.7 (-1.5)	-27 (-1.9)	-24 (-0.5)
	Suc	-26.5 (-4.4)	-32.2 (-5.9)	-28.4 (-2.1)	-28.7 (-1.5)	-27 (-1.9)	-24 (-0.5)
	Glc	-26.5 (-4.4)	-32.2 (-5.9)	-28.4 (-2.1)	-28.7 (-1.5)	-27 (-1.9)	-24 (-0.5)
	Day respired CO ₂	-31.6	-	-	-	-	-
	Photorespired CO ₂	-35.2	-	-	-	-	-
	Transitory starch	-22.2	-26.3	-26.3	-27.2	-25.1	-23.5
Night	Fru	-22.2 (0)	-	-	-	-	-
	Suc	≥ -22.2 (≥ 0)	-	-	-	-	-
	Glc	≥ -22.2 (≥ 0)	-	-	-	-	-
	Dark respired CO ₂	≥ -26.0 (≥ -3.8)	-	-	-	-	-

^ATcherkez *et al.* (2003); ^BDuranceau *et al.* (2001); ^CGhashghaie *et al.* (2001); ^DGleixner *et al.* (1993); ^EGleixner *et al.* (1998); ^FWanek *et al.* (2001); ^GBrugnoli *et al.* (1988).

this assumption, such as the analysis of the ¹³C content in phloem sap Suc during a light/dark cycle under controlled conditions.

The present model has been first used to calculate isotope effects but can also be used, with given isotope effects, to investigate the effect of changing particular metabolic fluxes or to estimate variables that are difficult to measure *in vivo*, like the (photo) respiratory discrimination ('forwards

modelling'). Photorespiration has an effect on the calculated carbon isotope composition in hexoses (Fig. 3B). With fixed isotope effects, increasing photorespiration with $g > 0$ leads to a greater difference of the ¹³C/¹²C ratio between the C-4 and C-1 positions (Fig. 3B). It is noteworthy that the difference in isotope composition between those two positions in Glc is greater in beet than in maize (Fig. 1A), perhaps reflecting lower photorespiration in maize (a C₄

plant) than in beet (a C₃ plant). Further work is needed to test experimentally the effect of photorespiration on the C-1 and C-4 isotope ratios in glucose, such as the analysis of the positional ¹³C content in hexoses from C₃ plants grown in low- and high-photorespiratory conditions.

The discriminations associated with photorespiration (f) and day respiration (e) depend on isotope discrimination during Gly decarboxylation reaction (denoted as g). The g value obtained *in vitro* with isolated mitochondria is highly variable, and depends on the species and the cofactors in the reaction medium (Ivlev *et al.* 1996). Nevertheless, independent experiments on leaves with gas-exchange systems found f values around 8 and 9‰ (Rooney 1988; Gillon and Griffiths 1997; Lanigan *et al.* 2003). With $g = 20$ ‰ and $\Phi = 0.3$, f is close to +9.2‰ which is in this range of values (see Appendix for calculations). f is a function of the photorespiratory oxygenation flux Φ , but is not very sensitive to it (Fig. 4). The calculated value of e is +5.5‰ and, like photorespiration, it discriminates against the ¹³C isotopomer. The predicted value of e in Fig. 4 was obtained assuming equal fluxes between the aldolase reaction and other reactions (e.g. the glycolytic flux is $E/3$), without pyruvate dehydrogenase discrimination. However, the glycolytic flux may be lower than the aldolase flux *in vivo*. In addition, it has been shown that purified pyruvate dehydrogenase discriminates against ¹³C *in vitro* (Melzer and Schmidt 1987). So e could be greater than 5.5‰ depending on (i) the magnitude of the flux devoted to respiration (ii) the isotope effects of the respiratory enzymes. By contrast, the value of e is not very sensitive to Φ or to day respiration rate, so that e and f can be considered as Φ - and R_d -independent parameters, as already supposed by Farquhar *et al.* (1982).

In darkness, the CO₂ produced by respiration originates partly from oxidation of hexoses that are produced by depolymerisation of transitory starch. The glycolytic degradation of hexoses begins with the aldolase reaction in the cytoplasm and thus depletes the resulting triose phosphates in ¹³C so that the respired CO₂ obtained by the present model is ¹³C depleted (Table 2). However, the dark-respired CO₂ is constantly enriched in *Phaseolus vulgaris* compared with soluble sugars (Duranceau *et al.* 1999), as well as in *Nicotiana sylvestris* (Ghashghaie *et al.* 2001). It was proposed that this ¹³C-enrichment originates from the C-3 and C-4 ¹³C enrichment in Glc (Tcherkez *et al.* 2003). In that case, it is assumed that aldolase (and also enzymes involved in the respiration pathway such as pyruvate dehydrogenase) does not discriminate so that those carbon atom positions in Glc remain ¹³C enriched. We propose here that FBP formation in the dark is a committed step of the glycolytic pathway in the dark; all the FBP molecules produced by fructose-6-phosphate phosphorylation are directed to triose-phosphate production through the aldolase reaction and trioses are immediately oxidised to pyruvate or used for biosynthetic processes. With this commitment, aldolase would not be able to fractionate. Interestingly, it has been

shown in carrot roots that there is a physical interaction between aldolase and the ATP- and pyrophosphate-dependent phosphofructokinases, that could facilitate the channelling of fructose-6-phosphate to triose phosphates (Moorhead and Plaxton 1992).

Concluding remarks

The present model points out that the CO₂ produced by (photo) respiration is not only affected by isotope effects of individual reactions, but also by the positional isotope distribution in molecules. More generally, a difference of isotope composition between a source and a product of a given biological reaction can originate from the fragmentation of molecules with heterogeneous isotope distribution and not (only) from a kinetic- or equilibrium-isotope effect. This kind of fractionation that is typically occurring during the production of dark-respired CO₂, deserves a name and we propose the term 'fragmentation fractionation'.

In order to limit the number of parameters that have to be studied, the present model does not take into account any reversibility of the reactions. However, isotope effects depend on the kinetic state of the reaction (close-to, or far-from equilibrium) and so carbon isotope distribution in molecules may be influenced by reverse reactions. For example, the aldolase isotope effects obtained (with $\Phi = 0.3$) with the present model are larger than those found *in vitro*. This discrepancy can originate from the fact that in the model the chloroplastic and cytosolic aldolases were assumed to have the same positional discriminations (that assumption was made so that the number of variables did not exceed the number of equations that allow their numerical determination). However, it is likely that the chloroplastic and cytosolic aldolase reactions do not have the same kinetic properties (Gerhardt *et al.* 1987; Kutoba and Ashihara 1990) so that they do not have similar isotope effects. In addition, the fact that the present model gives, as a fit to the Rossmann values, negative discriminations at both C-3 and C-4 for aldolase (when photorespiration is low) implies that these must be reversible reactions. Our present model gives little attention to reversible aspects and this aspect will be addressed in a subsequent work.

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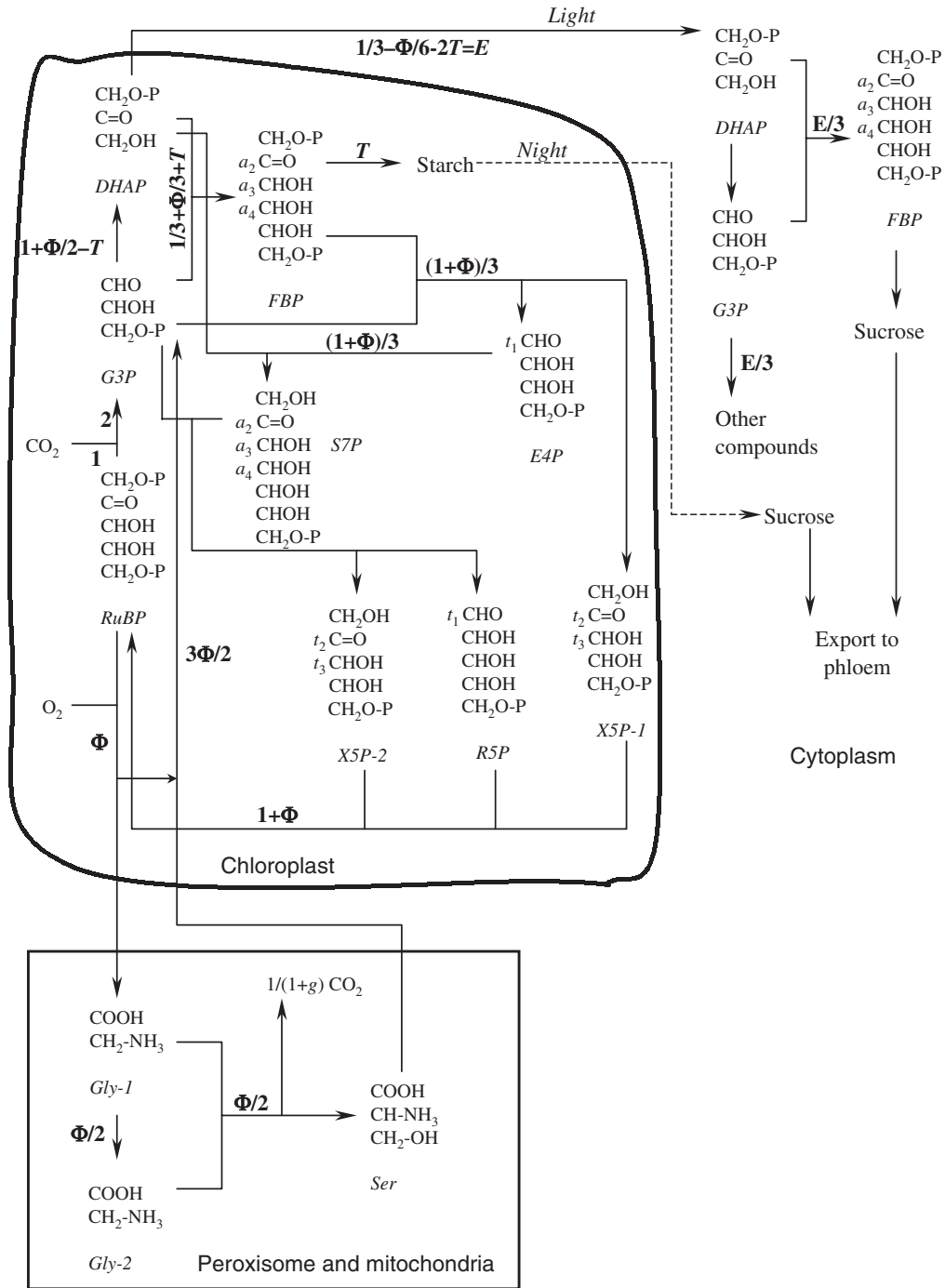
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Appendix Model description

The modelled Calvin cycle is described in the Scheme 1. The flux of ribulose-1,5-bisphosphate (RuBP) carboxylation is v_c and it is supposed that $v_c = 1$ and the flux of photorespiratory RuBP oxygenation is $v_o = \Phi v_c$. Assuming $v_c = 1$, $v_o = \Phi$. The flux entering the glyceraldehyde-3-phosphate (G3P) is then $2 + 3\Phi/2$. The isomerisation flux to dihydroxyacetone-phosphate (DHAP) is $1 + \Phi/2$ and the export flux is $1 - \Phi/2$ so that all the other fluxes are equal to $(1 + \Phi)/3$ because of mass balance.



Scheme 1.

The compounds are abbreviated as follows:

Compound name	Abbreviation
Glyceraldehyde-3-phosphate	G3P
Dihydroxyacetone-phosphate	DHAP
Fructose-1,6-bisphosphate	FBP
Erythrose-4-phosphate	E4P
Sedoheptulose-7-phosphate	S7P
Xylulose-5-phosphate	X5P
Ribose-5-phosphate	R5P
Ribulose-1,5-bisphosphate	RuBP
Glycine	Gly
Serine	Ser
Photorespiratory CO ₂	C

As pointed out in *Assumptions and methods*, the variables used at first in the model are isotope ratios and inverse isotope effects. Inverse isotope effects are simpler to use through numerical calculations because they are simply multiplied by isotope ratios. The main parameters used in the model are listed below.

Parameter	Description
Φ	RuBP-oxygenation flux as a proportion of carboxylation
T	Flux of FBP to transitory starch synthesis
E	Export flux of DHAP from the chloroplast
L	Proportion of light-derived Suc in storage carbohydrates
R^*	Isotope ratio of CO ₂ fixed to RuBP by Rubisco
R_A	Isotope ratio of CO ₂ assimilated (<i>net</i> assimilation)
R_R	Isotope ratio of day respired CO ₂
a_2	Inverse isotope effect of aldolase in C-2 of FBP
a_3	Inverse isotope effect of aldolase in C-3 of FBP
a_4	Inverse isotope effect of aldolase in C-4 of FBP
t_1	Inverse isotope effect of transketolase in C-1 of E4P or R5P
t_2	Inverse isotope effect of transketolase in C-2 of X5P
t_3	Inverse isotope effect of transketolase in C-3 of X5P

General procedure

The procedure used for stable isotope ratios is detailed assuming that the exported molecule is DHAP and, initially, that there is no starch synthesis ($T = 0$). The isotope ratio $^{13}\text{C} / ^{12}\text{C}$ of a given molecule M at the n th round of the Calvin cycle is denoted as $[M]_n$ and $[M]$ in the steady state. The recurrence equations are derived from the procedure in *Assumption and methods*. For example, for G3P-C1, the amount of ^{13}C in G3P in position C1 is denoted as $[\text{G3P-C1}]^{13}$ and has the following general expression:

$$[\text{G3P-C1}]_{n+1}^{13} = [\text{G3P-C1}]_n^{13} + \frac{s}{2} \left(\frac{[\text{RuBP-C3}]_n^{13}}{[\text{RuBP-C3}]_n^{13} + [\text{RuBP-C3}]_n^{12}} + \frac{[\text{CO}_2]^{13}}{[\text{CO}_2]^{13} + [\text{CO}_2]^{12}} \right) - s \frac{1}{\alpha} \frac{[\text{G3P-C1}]_n}{1 + \frac{1}{\alpha} [\text{G3P-C1}]_n},$$

where s (mol of C) is the flux of carbon through reactions for a given time interval. We divide by the carbon pool size S (which comprises ^{13}C and ^{12}C isotopomers) and then we have:

$$\frac{[\text{G3P-C1}]_{n+1}^{13}}{S} = \frac{[\text{G3P-C1}]_n^{13}}{S} + \frac{s}{S} \frac{1}{2} \left(\frac{[\text{RuBP-C3}]_n^{13}}{[\text{RuBP-C3}]_n^{13} + [\text{RuBP-C3}]_n^{12}} + \frac{[\text{CO}_2]^{13}}{[\text{CO}_2]^{13} + [\text{CO}_2]^{12}} \right) - \frac{s}{S} \frac{1}{\alpha} \frac{[\text{G3P-C1}]_n}{1 + \frac{1}{\alpha} [\text{G3P-C1}]_n}.$$

That is, neglecting the ratios, compared with 1:

$$[\text{G3P-C1}]_{n+1} = [\text{G3P-C1}]_n + \frac{s}{S} \left(\frac{[\text{RuBP-C3}]_n + [\text{CO}_2]}{2} \right) - \frac{s}{S} \frac{1}{\alpha} [\text{G3P-C1}]_n.$$

In the steady state, we have the relationship:

$$\frac{1}{\alpha} [\text{G3P-C1}] = \left(\frac{[\text{RuBP-C3}] + [\text{CO}_2]}{2} \right),$$

which does not depend on the amount S . It should be noted that the relationship with isotope compositions ($\delta^{13}\text{C}$) can then be derived from this equation. If R_{st} is the isotope ratio in the standard material, the previous equation is equivalent to:

$$\frac{1}{\alpha} R_{\text{st}} (\delta^{13}\text{C}_{\text{G3P-C1}} + 1) = \left(\frac{(\delta^{13}\text{C}_{\text{RuBP-C3}} + 1) (\delta^{13}\text{C}_{\text{CO}_2} + 1)}{2} \right) R_{\text{st}},$$

that is,

$$\frac{1}{\alpha} \delta^{13}\text{C}_{\text{G3P-C1}} + \left(\frac{1}{\alpha} - 1 \right) = \left(\frac{(\delta^{13}\text{C}_{\text{RuBP-C3}} + \delta^{13}\text{C}_{\text{CO}_2})}{2} \right).$$

If the discrimination in the ‘reaction’ consuming G3P-C1 is denoted as $\Delta(\alpha) = \alpha - 1$, and neglecting the second order terms, then we have:

$$\delta^{13}\text{C}_{\text{G3P-C1}} - \Delta(\alpha) = \left(\frac{\delta^{13}\text{C}_{\text{RuBP-C3}} + \delta^{13}\text{C}_{\text{CO}_2}}{2} \right).$$

That said, we can write the equations in the steady state for the other compounds, including the effects of photorespiration. Then we have:

$$\left(\frac{1 + \Phi}{3} (a_4 + 2t_3) + \left(1 + \frac{\Phi}{2} \right) \right) [\text{G3P-C1}] = 2 \frac{[\text{RuBP-C3}] + R^*}{2} + \Phi [\text{RuBP-C3}] + \frac{\Phi}{2} [\text{Ser-C3}]$$

$$\left(2 + \frac{3}{2} \Phi \right) [\text{G3P-C2}] = 2 \frac{[\text{RuBP-C2}] + [\text{RuBP-C4}]}{2} + \Phi [\text{RuBP-C4}] + \frac{\Phi}{2} [\text{Ser-C2}]$$

$$\left(2 + \frac{3}{2} \Phi \right) [\text{G3P-C3}] = 2 \frac{[\text{RuBP-C1}] + [\text{RuBP-C5}]}{2} + \Phi [\text{RuBP-C5}] + \frac{\Phi}{2} [\text{Ser-C1}]$$

$$\left(2a_3 \frac{1 + \Phi}{3} + \frac{1 - \Phi/2}{3} \right) [\text{DHAP-C1}] = \left(1 + \frac{\Phi}{2} \right) [\text{G3P-C1}]$$

$$\left(2a_2 \frac{1 + \Phi}{3} + \frac{1 - \Phi/2}{3} \right) [\text{DHAP-C2}] = \left(1 + \frac{\Phi}{2} \right) [\text{G3P-C2}]$$

$$\left(1 + \frac{\Phi}{2} \right) [\text{DHAP-C3}] = \left(1 + \frac{\Phi}{2} \right) [\text{G3P-C3}]$$

$$\frac{1 + \Phi}{3} [\text{FBP-C1}] = \frac{1 + \Phi}{3} [\text{DHAP-C3}]$$

$$t_2 \frac{1 + \Phi}{3} [\text{FBP-C2}] = a_2 \frac{1 + \Phi}{3} [\text{DHAP-C2}]$$

$$t_1 \frac{1 + \Phi}{3} [\text{FBP-C3}] = a_3 \frac{1 + \Phi}{3} [\text{DHAP-C1}]$$

$$\frac{1 + \Phi}{3} [\text{FBP-C4}] = a_4 \frac{1 + \Phi}{3} [\text{G3P-C1}]$$

$$\frac{1 + \Phi}{3}[\text{FBP-C5}] = \frac{1 + \Phi}{3}[\text{G3P-C2}]$$

$$\frac{1 + \Phi}{3}[\text{FBP-C6}] = \frac{1 + \Phi}{3}[\text{G3P-C3}]$$

$$a_4 \frac{1 + \Phi}{3}[\text{E4P-C1}] = t_1 \frac{1 + \Phi}{3}[\text{FBP-C3}]$$

$$\frac{1 + \Phi}{3}[\text{E4P-C2}] = \frac{1 + \Phi}{3}[\text{FBP-C4}]$$

$$\frac{1 + \Phi}{3}[\text{E4P-C3}] = \frac{1 + \Phi}{3}[\text{FBP-C5}]$$

$$\frac{1 + \Phi}{3}[\text{E4P-C4}] = \frac{1 + \Phi}{3}[\text{FBP-C6}]$$

$$\frac{1 + \Phi}{3}[\text{S7P-C1}] = \frac{1 + \Phi}{3}[\text{DHAP-C3}]$$

$$t_2 \frac{1 + \Phi}{3}[\text{S7P-C2}] = a_2 \frac{1 + \Phi}{3}[\text{DHAP-C2}]$$

$$t_1 \frac{1 + \Phi}{3}[\text{S7P-C3}] = a_3 \frac{1 + \Phi}{3}[\text{DHAP-C1}]$$

$$\frac{1 + \Phi}{3}[\text{S7P-C4}] = a_4 \frac{1 + \Phi}{3}[\text{E4P-C1}]$$

$$\frac{1 + \Phi}{3}[\text{S7P-C5}] = \frac{1 + \Phi}{3}[\text{E4P-C2}]$$

$$\frac{1 + \Phi}{3}[\text{S7P-C6}] = \frac{1 + \Phi}{3}[\text{E4P-C3}]$$

$$\frac{1 + \Phi}{3}[\text{S7P-C7}] = \frac{1 + \Phi}{3}[\text{E4P-C4}]$$

$$\frac{1 + \Phi}{3}[\text{X5P1-C1}] = \frac{1 + \Phi}{3}[\text{FBP-C1}]$$

$$\frac{1 + \Phi}{3}[\text{X5P1-C2}] = t_2 \frac{1 + \Phi}{3}[\text{FBP-C2}]$$

$$\frac{1 + \Phi}{3}[\text{X5P1-C3}] = t_3 \frac{1 + \Phi}{3}[\text{G3P-C1}]$$

$$\frac{1 + \Phi}{3}[\text{X5P1-C4}] = \frac{1 + \Phi}{3}[\text{G3P-C2}]$$

$$\frac{1 + \Phi}{3}[\text{X5P1-C5}] = \frac{1 + \Phi}{3}[\text{G3P-C3}]$$

$$\frac{1 + \Phi}{3}[\text{X5P2-C1}] = \frac{1 + \Phi}{3}[\text{S7P-C1}]$$

$$\frac{1 + \Phi}{3}[\text{X5P2-C2}] = t_2 \frac{1 + \Phi}{3}[\text{S7P-C2}]$$

$$\frac{1 + \Phi}{3}[\text{X5P2-C3}] = t_3 \frac{1 + \Phi}{3}[\text{G3P-C1}]$$

$$\frac{1 + \Phi}{3}[\text{X5P2-C4}] = \frac{1 + \Phi}{3}[\text{G3P-C2}]$$

$$\frac{1 + \Phi}{3}[\text{X5P2-C5}] = \frac{1 + \Phi}{3}[\text{G3P-C3}]$$

$$\frac{1 + \Phi}{3}[\text{R5P-C1}] = t_1 \frac{1 + \Phi}{3}[\text{S7P-C3}]$$

$$\frac{1 + \Phi}{3}[\text{R5P-C2}] = \frac{1 + \Phi}{3}[\text{S7P-C4}]$$

$$\frac{1 + \Phi}{3}[\text{R5P-C3}] = \frac{1 + \Phi}{3}[\text{S7P-C5}]$$

$$\frac{1 + \Phi}{3}[\text{R5P-C4}] = \frac{1 + \Phi}{3}[\text{S7P-C6}]$$

$$\frac{1 + \Phi}{3}[\text{R5P-C5}] = \frac{1 + \Phi}{3}[\text{S7P-C7}]$$

$$(1 + \Phi)[\text{RuBP-C1}] = \frac{1 + \Phi}{3}([\text{X5P1-C1}] + [\text{X5P2-C1}] + [\text{R5P-C1}])$$

$$(1 + \Phi)[\text{RuBP-C2}] = \frac{1 + \Phi}{3}([\text{X5P1-C2}] + [\text{X5P2-C2}] + [\text{R5P-C2}])$$

$$(1 + \Phi)[\text{RuBP-C3}] = \frac{1 + \Phi}{3}([\text{X5P1-C3}] + [\text{X5P2-C3}] + [\text{R5P-C3}])$$

$$(1 + \Phi)[\text{RuBP-C4}] = \frac{1 + \Phi}{3}([\text{X5P1-C4}] + [\text{X5P2-C4}] + [\text{R5P-C4}])$$

$$(1 + \Phi)[\text{RuBP-C5}] = \frac{1 + \Phi}{3}([\text{X5P1-C5}] + [\text{X5P2-C5}] + [\text{R5P-C5}])$$

$$\Phi[\text{Gly1-C1}] = \Phi[\text{RuBP-C1}]$$

$$\frac{\Phi}{2} \left(\frac{1}{1 + g} + 1 \right) [\text{Gly1-C2}] = \Phi[\text{RuBP-C2}]$$

$$\frac{\Phi}{2}[\text{Gly2-C1}] = \frac{\Phi}{2}[\text{Gly1-C1}]$$

$$\frac{\Phi}{2}[\text{Gly2-C2}] = \frac{\Phi}{2}[\text{Gly1-C2}]$$

$$\frac{\Phi}{2}[\text{Ser-C1}] = \frac{\Phi}{2}[\text{Gly1-C1}]$$

$$\frac{\Phi}{2}[\text{Ser-C2}] = \frac{\Phi}{2}[\text{Gly2-C1}]$$

$$\frac{\Phi}{2}[\text{Ser-C3}] = \frac{\Phi}{2}[\text{Gly2-C2}]$$

$$\frac{\Phi}{2}[\text{C}] = \frac{1}{1+g} \frac{\Phi}{2}[\text{Gly1-C2}].$$

Using a substitution procedure the following relationships can be deduced:

$$[\text{DHAP-C1}] = [\text{G3P-C1}] \frac{3(1 + \Phi/2)}{(2a_3 + 1) + \Phi(2a_3 - 1/2)} \stackrel{\text{def}}{=} [\text{G3P-C1}]\tilde{a}_3.$$

where the notation *def* means that this relationship defines \tilde{a}_3 . Similarly,

$$[\text{DHAP-C2}] = [\text{G3P-C2}] \frac{3(1 + \Phi/2)}{(2a_2 + 1) + \Phi(2a_2 - 1/2)} \stackrel{\text{def}}{=} [\text{G3P-C2}]\tilde{a}_2$$

$$[\text{DHAP-C3}] = [\text{G3P-C3}].$$

When using \tilde{a}_2 and \tilde{a}_3 we have:

$$[\text{RuBP-C1}] = \frac{1}{3}(a_3\tilde{a}_3[\text{G3P-C1}] + 2[\text{G3P-C3}])$$

$$[\text{RuBP-C2}] = \frac{1}{3}(2a_2\tilde{a}_2[\text{G3P-C2}] + a_3\tilde{a}_3[\text{G3P-C1}])$$

$$[\text{RuBP-C3}] = \frac{1}{3}(a_4 + 2t_3)[\text{G3P-C1}]$$

$$[\text{RuBP-C4}] = [\text{G3P-C2}]$$

$$[\text{RuBP-C5}] = [\text{G3P-C3}].$$

And for photorespiratory CO_2 :

$$[\text{C}] = \frac{2}{2+g}[\text{RuBP-C2}] = \frac{2}{2+g} \cdot \frac{1}{3}(2a_2\tilde{a}_2[\text{G3P-C2}] + a_3\tilde{a}_3[\text{G3P-C1}]).$$

Eventually, substituting RuBP ratios into G3P equations and rearranging gives:

$$[\text{G3P-C1}] = \frac{R^*}{1 + \frac{\Phi}{2} - \frac{\Phi}{3} \frac{1+g}{2+g} (\epsilon + 2a_2\tilde{a}_2\epsilon')}, \quad \text{where } \epsilon \text{ and } \epsilon' \text{ are defined by :}$$

$$[\text{G3P-C2}] = \frac{(1 + 3\Phi/2)a_3\tilde{a}_3[\text{G3P-C1}]}{3(1 + \frac{\Phi}{2} - \frac{2a_2\tilde{a}_2}{3})} \stackrel{\text{def}}{=} \epsilon'[\text{G3P-C1}]$$

$$[\text{G3P-C3}] = a_3\tilde{a}_3[\text{G3P-C1}] \stackrel{\text{def}}{=} \epsilon[\text{G3P-C1}].$$

The isotopic ratios in FBP when expressed as a function of $[\text{G3P-C1}]$ are:

$$[\text{FBP-C1}] = \epsilon[\text{G3P-C1}]$$

$$[\text{FBP-C2}] = \frac{a_2\tilde{a}_2}{t_2}\epsilon'[\text{G3P-C1}]$$

$$[\text{FBP-C3}] = \frac{\epsilon}{t_1}[\text{G3P-C1}]$$

$$[\text{FBP-C4}] = a_4[\text{G3P-C1}]$$

$$[E5] = \varepsilon'[\text{G3P-C1}]$$

$$[\text{FBP-C6}] = \varepsilon[\text{G3P-C1}].$$

It should be noted that these isotopic ratios are not dependent on t_3 , which then cannot be expressed as a function of FBP isotopic ratios. Moreover, we have the relationship: $[\text{FBP-C1}] = [\text{FBP-C6}]$, which is a consequence of isomerisation by triose-phosphate isomerase and the absence of secondary isotope effects on C-3 of trioses in the model. However, this equality does not occur in natural Glc (Rossmann *et al.* 1991) and the isotope ratios in C-1 to C-5 positions only are used for calculations of inverse isotope effects.

Introducing starch synthesis

The same procedure can be used assuming that there is a net flux of FBP for transitory starch synthesis in the chloroplast (T), and that there is a trade-off between DHAP export and starch synthesis. In this case, the DHAP export flux is $(1 - (\Phi/2)/3) - 2T$, the isomerisation flux is $(1 + (\Phi/2) - T)$ and the FBP synthetic flux is $(1 + (\Phi)/3) + T$. The maximum value of T can be calculated with the constraint $(1 - (\Phi/2)/3) - 2T \geq 0$, which gives $T \leq (1 - (\Phi/2)/6)$. We denote this maximum value as T_{\max} . Relationships giving isotopic ratios are very similar to those in section *a*, giving for FBP:

$$[\text{FBP-C1}] = \varepsilon \cdot [\text{G3P-C1}]$$

$$[\text{FBP-C2}] = \frac{a_2 \tilde{a}_2 \tilde{t}_2}{t_2} \varepsilon' \cdot [\text{G3P-C1}]$$

$$[\text{FBP-C3}] = \frac{\varepsilon \tilde{t}_1}{t_1} \cdot [\text{G3P-C1}]$$

$$[\text{FBP-C4}] = a_4 \cdot [\text{G3P-C1}]$$

$$[\text{FBP-C5}] = \varepsilon' \cdot [\text{G3P-C1}]$$

$$[\text{FBP-C6}] = \varepsilon \cdot [\text{G3P-C1}],$$

with:

$$[\text{G3P-C1}] = \frac{R^*}{1 + \Phi \left(\frac{1}{2} - \frac{1}{3} \frac{1+g}{2+g} (\varepsilon + 2a_2 \tilde{a}_2 \varepsilon') \right) + T(a_4 - 1)},$$

where:

$$\tilde{a}_i = \frac{1 + \Phi/2 - T}{\frac{2a_i+1}{3} + \Phi \frac{2a_i-1/2}{3} + T(a_i - 2)} \quad \text{and} \quad \tilde{t}_i = \frac{1 + 3T}{t_i + 3T} t_i \quad \text{for } i = 1, 2 \text{ or } 3, \text{ and}$$

$$\varepsilon' = \frac{(\tilde{t}_1 + 3\Phi/2)a_3 \tilde{a}_3}{3(1 + \Phi/2 - (1 + \tilde{t}_2)a_2 \tilde{a}_2/3)}.$$

Cytoplasmic FBP

Carbohydrates from storage organs may come from those supplied by leaves through light export of Suc or night degradation of transitory starch. Suc produced in light is synthesised in the cytoplasm from DHAP exported from the chloroplast (Scheme 1). The export flux of DHAP from the chloroplast is $E = 1/3 - \Phi/6 - 2T$. The DHAP molecules in the cytoplasm are isomerised to G3P and FBP is produced by aldolase. One part of the G3P is diverted to other metabolic purposes (like respiration) and the flux of Suc synthesis in the cytoplasm is $E/3$. Thus the isotopic ratios in cytoplasmic FBP are as follows:

$$[\text{DHAP-C3}]$$

$$\frac{3a_2}{2 + a_2} [\text{DHAP-C2}]$$

$$\frac{3a_3}{2 + a_3}[\text{DHAP-C1}]$$

$$\frac{2a_4}{1 + a_4} \frac{3}{2 + a_3}[\text{DHAP-C1}]$$

$$\frac{3}{a_2 + 2}[\text{DHAP-C2}]$$

$$[\text{DHAP-C3}]$$

where [DHAP-Ci] are the isotopic ratios of DHAP *in the chloroplast*.

Calculation of isotope effects

Glc from which Rossmann *et al.* (1991) measured isotope ratios result from storage (root storage in beet, grain storage in maize) and so are derived from both light-produced (cytosolic) and night-produced Suc (transitory starch). The proportion of Glc that comes from light-produced Suc in storage Glc is denoted as L . From the relationships given before, it is deduced that the isotope ratios in the Glc analysed by Rossmann *et al.* (1991) are the following:

$$R_1 = \varepsilon \cdot [\text{G3P-C1}]$$

$$R_2 = \left(L \frac{3a_2}{2 + a_2} \tilde{a}_2 + (1 - L) \frac{a_2 \tilde{a}_2 \tilde{t}_2}{t_2} \right) \cdot \varepsilon' [\text{G3P-C1}]$$

$$R_3 = \left(L \frac{3a_3}{2 + a_3} \tilde{a}_3 + (1 - L) \varepsilon \frac{\tilde{t}_1}{t_1} \right) \cdot [\text{G3P-C1}]$$

$$R_4 = \left(L \frac{2a_4}{1 + a_4} \frac{3}{2 + a_3} \tilde{a}_3 + (1 - L) a_4 \right) \cdot [\text{G3P-C1}]$$

$$R_5 = \left(L \frac{3}{2 + a_2} \tilde{a}_2 + 1 - L \right) \varepsilon' \cdot [\text{G3P-C1}]$$

$$R_6 = \varepsilon \cdot [\text{G3P-C1}],$$

where [G3P-C1] is the isotope ratio of G3P in the chloroplast and is given by the relationship shown in *Introducing starch synthesis*. Those expressions do not allow a direct resolution and a linearisation is more convenient. The inverse isotope effects are written as $a_i = 1 + o(a_i)$ so that the isotope discrimination is then $\Delta(a_i) = 1 / a_i - 1$ that is, $-o(a_i)$. If the second order terms in the previous equations are neglected, the discriminations are given by:

$$\Delta(a_3) = - \frac{\frac{R_1}{R^*} K_0 - K_1}{K_2}$$

$$\Delta(a_2) = - \frac{\frac{R_5}{R_1} - 1 - K_3 \left(\frac{R_2}{R_5} + \frac{R_3}{R_1} - 2 \right) + \frac{LK_3}{3} \Delta(a_3)}{K_4}$$

$$\Delta(a_4) = \frac{\frac{R_4}{R_1} - 1 - \left(L \left(\frac{1}{3} + \lambda \right) + 1 - \lambda \right) \Delta(a_3)}{1 - \frac{L}{2}}$$

$$\Delta(t_1) = \frac{\frac{R_3}{R_1} - 1 - \frac{L}{3} \Delta(a_3)}{(1 - L)(1 + \gamma)}$$

$$\Delta(t_2) = \frac{\frac{R_2}{R_5} - 1 + (L + (1 - L)(1 - \lambda)) \Delta(a_2)}{(1 - L)(1 + \gamma)},$$

with the relationships:

$$K_0 = 1 + \frac{\Phi}{4}g$$

$$K_1 = 1 + \frac{1}{K_0} \left(\frac{2\pi}{3} \left(\frac{R_5}{R_1} - 1 \right) + \frac{T \left(1 - \frac{R_4}{R_1} \right)}{1 - \frac{L}{2}} \right) + \frac{\frac{R_5}{R_1} - 1 - K_3}{K_4}$$

$$K_2 = \frac{-LK_3}{3K_4} \left(\left(1 - \frac{\pi}{K_0} \right) (1 - \lambda) - T \frac{1 - \lambda + L \left(\lambda + \frac{1}{3} \right)}{K_0 \left(1 - \frac{L}{2} \right)} + \frac{2\pi}{3K_0} \left(1 - \lambda + \frac{R_5}{R_2} L \left(\lambda + \frac{1}{3} \right) \right) \right)$$

$$K_3 = \frac{\gamma}{\left(1 + \frac{3}{2}\Phi \right) (1 - L)(1 + \gamma)}$$

$$K_4 = \frac{2(1 - \lambda)}{1 + \frac{3}{2}\Phi} - L \left(\lambda + \frac{1}{3} \right) \frac{R_5}{R_2} - K_3 (L + (1 - L)(1 - \lambda))$$

and

$$\pi = \frac{\Phi}{2} \left(1 + \frac{g}{2} \right) \quad \gamma = \frac{1}{1 + 3T} \quad \lambda = \frac{\frac{2}{3}(1 + \Phi) + T}{1 + \frac{\Phi}{2} - T}.$$

Photorespiratory discrimination

Isotope discrimination occurring during photorespiration in on-line gas-exchange systems is defined using net assimilated carbon as a reference material (Farquhar *et al.* 1982) and is equal to $f \stackrel{\text{def}}{=} \frac{R_A - C}{C}$, where R_A is the isotope ratio of the net assimilated carbon. This ratio can be simply derived from the assimilation equation:

$$R_A A = R^* v_c - C \frac{v_o}{2} - R_R R_d,$$

where A is the net assimilation rate, R_R the carbon isotope ratio of day-respired CO₂ and R_d the rate of day respiration. C is the isotope ratio in photorespired CO₂ (see above). Rearranging, gives:

$$R_A = \frac{R^* - \Phi C/2 - R_R R_d}{1 - \Phi/2 - R_d}.$$

Assuming that G3P molecules entering glycolysis are completely degraded through respiration, R_R is the mean isotope ratio in cytoplasmic G3P. The value of R_d is positive and its maximal value is $E/3$ (Scheme 1). With the relationship $C = 2[\text{RuBP-C2}]/(2 + g)$ (see above) and neglecting second order terms, we get the approximation ($f \sim \xi + (1 - R_d)/1 - \Phi/2 - R_d)(g/2)$ where ξ is a term of the same order as f (per mil). That is, f is linearly related to $g/2$. The day respiratory discrimination is calculated with the relationship $e \stackrel{\text{def}}{=} (R_A - R_R)/R_R$.

Scheme 1 Representation of the carbon fluxes taken into account in the model with the associated positional inverse isotope effects. Oxygenation is expressed as Φ compared with carboxylation. The flux of starch synthesis is T . See the text for the abbreviations.