THE AUSTRALIAN NATIONAL UNIVERSITY

# THE RELATIONSHIP BETWEEN SIDE REACTIONS AND SLOW INHIBITION OF RIBULOSE-BISPHOSPHATE CARBOXYLASE REVEALED BY A LOOP 6 MUTANT OF THE TOBACCO ENZYME.


## GRANT PEARCE

**PHD SUPERVISOR:**
Prof John Andrews,
Dr Spencer Whitney

**LOCATION:**
Research School of
Biological Sciences



**JOURNAL REFERENCE:**
Pearce, FG and Andrews, TJ.
*Journal of Biological Chemistry*
(2003) **278:** 32526-36


JOURNAL OF BIOLOGICAL CHEMISTRY

The first directed mutant of a higher plant ribulose-bisphosphate carboxylase/oxygenase (Rubisco), constructed by chloroplast transformation, is catalytically impaired but still able to support the plant's photosynthesis and growth (Whitney, S. M., etal (1999) *Plant Physiol.* 121, 579-588).

This mutant enzyme has a Leu to Val substitution at residue 335 in the flexible loop 6 of the large subunit, which closes over the substrate during catalysis. Its active site was intact, as judged by its barely impaired competency in the initial enolization step of the reaction sequence, and its ability to bind tightly the intermediate analog, 2'-carboxy-D-arabinitol-1,5-bisphosphate. Prompted by observations that the mutant enzyme displayed much less slow inhibition during catalysis in vitro than the wild type, its tendency to catalyze side reactions and its response to the slow inhibitor D-xylulose-1,5-bisphosphate were studied. The lessening in slow inhibition was not caused by reduced production of inhibitory side products. Except for pyruvate production, these reactions were strongly enhanced by the mutation, as was the ability to catalyze the carboxylation of D-xylulose-1,5-bisphosphate. Rather, reduced inhibition was the result of lessened sensitivity to these inhibitors. The slow isomerization phase that characterizes inhibition of the wild-type enzyme by D-xylulose-1,5-bisphosphate was completely eliminated by the mutation, and the mutant was more adept than the wild type in catalyzing the benzylic acid-type rearrangement of D-glycero-2,3-pentodiulose-1,5-bisphosphate (produced by oxidation of the substrate, D-ribulose-1,5-bisphosphate). These observations are consistent with increased flexibility of loop 6 induced by the mutation, and they reveal the underlying mechanisms by which the side reactions cause slow inhibition.