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PREVIEW

**Rejda, Joan Marie**

**CHEMICAL MODIFICATION AND ENZYMIC CHARACTERIZATION OF HIGHER  
PLANT RIBULOSE 1,5-BISPHOSPHATE CARBOXYLASE/OXYGENASE**

*The University of Nebraska - Lincoln*

Ph.D. 1983

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PREVIEW

CHEMICAL MODIFICATION AND ENZYMIC CHARACTERIZATION  
OF HIGHER PLANT RIBULOSE 1,5-BISPHOSPHATE  
CARBOXYLASE/OXYGENASE

by

Joan M. Rejda

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For the Degree of Doctor of Philosophy

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(Biochemistry)

Under the Supervision of Professor Raymond Chollet

Lincoln, Nebraska

May, 1983

**TITLE**

Chemical Modification and Enzymic Characterization of Higher Plant

Ribulose 1,5-Bisphosphate Carboxylase/Oxygenase

**BY**

Joan M. Rejda

**APPROVED**

**DATE**

<u>Raymond Chollet</u>	<u>April 8, 1983</u>
<u>Fred W. Wagner</u>	<u>April 8, 1983</u>
<u>Herman W. Knoche</u>	<u>April 8, 1983</u>
<u>Sheldon M. Schuster</u>	<u>April 8, 1983</u>
<u>Thomas R. Thompson</u>	<u>April 8, 1983</u>
<u> </u>	<u> </u>
<u> </u>	<u> </u>

**SUPERVISORY COMMITTEE**

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Abbreviations

Bicine	N,N-bis(2-hydroxyethyl)glycine
BSA	bovine serum albumin
DEP	diethylpyrocarbonate
DTNB	5,5'-dithiobis-(2-nitrobenzoic acid)
DTT	dithiothreitol
Ellman's reagent	see DTNB
Hepes	N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid
MES	2-(N-morpholino)ethanesulfonic acid
MOPS	3-(N-morpholino)propanesulfonic acid
NaDodSO <sub>4</sub>	sodium dodecyl sulfate
Na <sub>2</sub> EDTA	disodium ethylenediaminetetraacetic acid
NADPH	β-nicotinamide adenine dinucleotide phosphate
2-PG	2-phosphoglycolic acid
3-PGA	3-phosphoglyceric acid
PLP	pyridoxal 5'-phosphate
PMB	p-mercuribenzoate
Rubisco	ribulosebisphosphate carboxylase/oxygenase
RuBP	ribulose 1,5-bisphosphate
SuBP	sedoheptulose 1,7-bisphosphate
TNBS	trinitrobenzenesulfonic acid
Tricine	N-tris(hydroxymethyl)methylglycine
Tris	N-tris(hydroxymethyl)aminomethane

## GENERAL INTRODUCTION

Ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco; EC 4.1.1.39), the major soluble leaf protein in plants (Ellis, 1979), has two catalytic activities (Jensen & Bahr, 1977; Lorimer, 1981a):

1. Carboxylase: the initial reaction in the C<sub>3</sub> photosynthetic carbon reduction cycle in which CO<sub>2</sub> reacts with RuBP to form 2 molecules of 3-phosphoglycerate
2. Internal Monooxygenase: the initial reaction in the C<sub>2</sub> photorespiratory carbon oxidation cycle where O<sub>2</sub> reacts with RuBP to form 1 molecule of 3-phosphoglycerate and 1 molecule of 2-phosphoglycolate, the immediate precursor of the photorespiratory substrate, glycolic acid

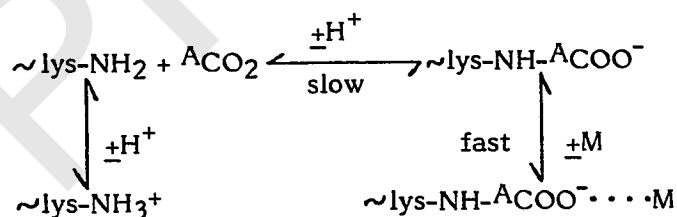
and therefore provides a common link between photosynthetic CO<sub>2</sub> fixation and photorespiration in plants (Chollet, 1977; Lorimer, 1981a; Somerville & Ogren, 1982). The enzyme from all higher plants and most microorganisms is a hexadecamer ( $M_r$ ,  $5.5 \times 10^5$ ) composed of 8 large, catalytic subunits (56 kDa) and 8 small, non-catalytic subunits (13 kDa), arranged in a 422 molecular symmetry (Akazawa, 1979; Jensen & Bahr, 1977). Rubisco from Rhodospirillum rubrum is unique in that it is a dimer of large subunits (Tabita & McFadden, 1974).

Most major crop plants except corn, sorghum, sugarcane, and millet have relatively high rates of photorespiration. O<sub>2</sub> and CO<sub>2</sub> act as competitive inhibitor and substrate, respectively, in the carboxylase reaction and, conversely, as substrate and competitive inhibitor in the oxygenase reaction. This competition between O<sub>2</sub> and CO<sub>2</sub> determines the relative rates of photorespiration and photosynthesis (Chollet, 1977; Chollet & Ogren, 1975). If



photorespiration and O<sub>2</sub> inhibition of carboxylation could be reduced, a net increase in photosynthesis and crop productivity might result (Chollet & Ogren, 1975; Lorimer, 1981a; Ogren, 1977). Attempts made to alter the photorespiratory cycle per se have resulted in deleterious effects on net photosynthesis (Kumarasinghe *et al.*, 1977; Servaites & Ogren, 1977; Somerville & Ogren, 1982). Since Rubisco is isolated fairly easily from various sources and provides a common link between the two cycles, it may be possible to exploit this enzyme for the improvement of crop productivity. However, more information concerning its reaction mechanism is necessary. Excellent reviews have recently appeared which discuss all of the latest experimental data in terms of the catalytic mechanism (Lorimer, 1981a; Lorimer & Miziorko, 1981).

Overall catalysis can be divided arbitrarily into two parts: activation and catalysis. Preincubation of Rubisco with CO<sub>2</sub> and divalent metal ion (usually Mg<sup>2+</sup>) results in increased carboxylase and oxygenase activity (Badger & Lorimer, 1976; Lorimer *et al.*, 1976, 1977). This CO<sub>2</sub>/Mg<sup>2+</sup>-activation is readily reversible upon removal of CO<sub>2</sub> and Mg<sup>2+</sup>. From kinetic data, the following order of reaction was proposed (Lorimer *et al.*, 1976; where ACO<sub>2</sub> represents activator CO<sub>2</sub> and M represents a divalent metal ion):



Further evidence for the reaction of activator CO<sub>2</sub> with the ε-amino group of Lys-201 of the large subunit to form a carbamate has been provided (Lorimer, 1981b; Lorimer & Miziorko, 1980; O'Leary *et al.*, 1979). This activation by Mg<sup>2+</sup> and low concentrations of CO<sub>2</sub> can further be enhanced by certain

photosynthetic chloroplast metabolites (such as low concentrations of NADPH, 6-phosphogluconate, fructose 1,6-bisphosphate, and inorganic phosphate) (Chu & Bassham, 1974; Chollet & Anderson, 1976; Hatch & Jensen, 1980; McCurry et al., 1981). Activation by these compounds is not observed when the preincubation involves high levels of CO<sub>2</sub>. This effector enhancement of activation at low CO<sub>2</sub> is markedly pH dependent, decreasing sharply with increasing pH. It is possible that the effectors act to stabilize the activated form of the enzyme by stabilizing the binding of <sup>A</sup>CO<sub>2</sub> and Mg<sup>2+</sup> (McCurry et al., 1981). However, since the effector molecules appear to occupy the RuBP binding site, an enzyme molecule cannot be simultaneously effector-activated and catalytically competent.

The suggested overall mechanism for catalysis of both reactions is shown in Figure 1 as well as the fate of the various atoms (Lorimer, 1981a). The proposed steps involved in the carboxylase reaction are shown in Figure 2. Coordination of Mg<sup>2+</sup> in the ternary complex with the O atom of carbon-3 of RuBP presumably facilitates the deprotonation of this carbon to form an enediol. This reversible step is likely rate limiting for overall catalysis. Upon loss of a proton, a carbanion is formed at carbon-2 thus making it nucleophilic. The electrophilic carbon of substrate CO<sub>2</sub> then attacks carbon-2 of RuBP to form the 6-carbon intermediate, 3-keto-2-carboxyarabinitol 1,5-bisphosphate which is then hydrolytically cleaved to form 2 molecules of 3-phosphoglycerate. Several reviews of the catalytic mechanism have appeared (Akazawa, 1979; Jensen & Bahr, 1977; Lorimer, 1981a).

Figure 1. Reactions catalyzed by Rubisco [adapted from Lorimer (1981a)] showing the origin and destination of the various atoms ( $^{14}\text{C}$ , substrate  $\text{CO}_2$ ; 2-PG, 2-phosphoglycolate; 3-PGA, 3-phosphoglycerate; RuBP, ribulose 1,5-bisphosphate).

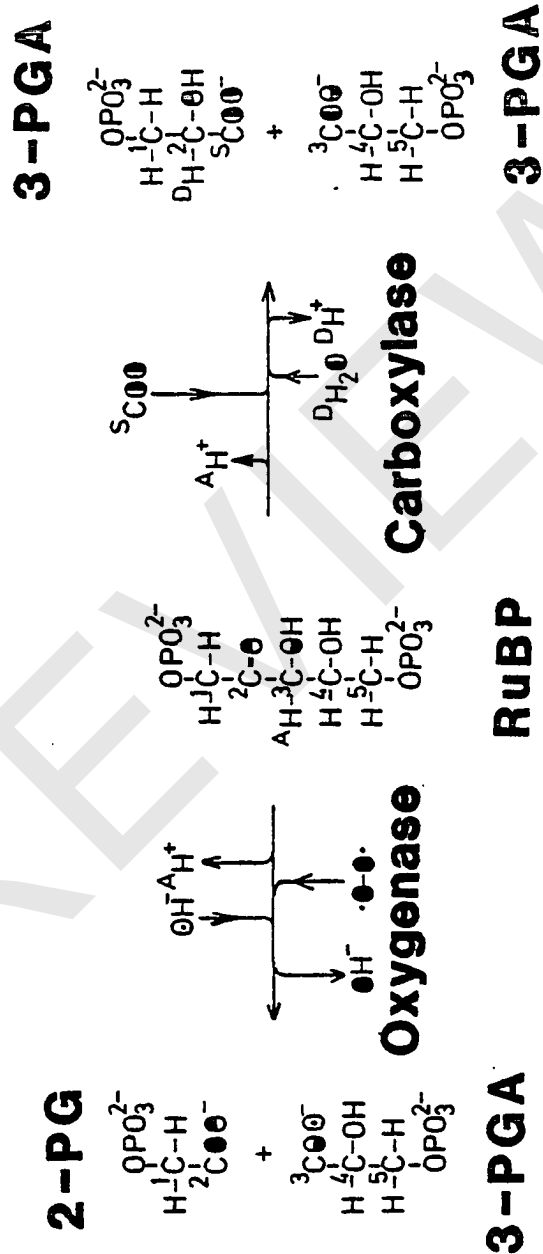


Figure 2. Proposed mechanism of RuBP carboxylation [adapted from Lorimer (1981a)]:  $S\text{CO}_2$ , substrate  $\text{CO}_2$ ;  $A\text{CO}_2$ , activator  $\text{CO}_2$ ;  $M^{2+}$ , divalent metal ion (usually  $\text{Mg}^{2+}$ ); B, base (possibly Lys-175, Cys, His or Tyr)].

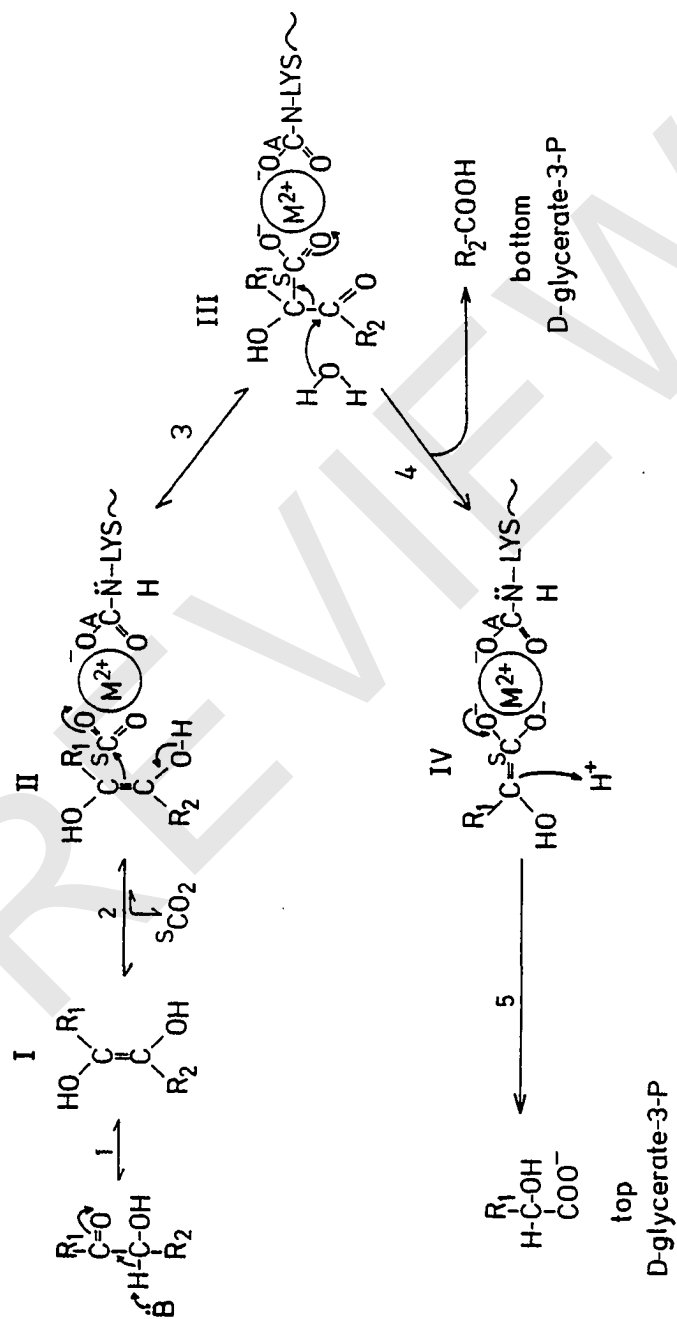
Step 1: Enediol formation

Step 2: Binding of substrate  $\text{CO}_2$

Step 3: Formation of transition state complex

Step 4: Expulsion of "bottom" 3-phosphoglycerate

Step 5: Release of "top" 3-phosphoglycerate.



top  
D-glycerate-3-P

bottom  
D-glycerate-3-P

### Materials

Buffers, crystalline BSA, sodium salts of RuBP, SuBP and periodate, Ellman's reagent, fluorescamine, methylene blue,  $\text{NH}_2\text{OH}\cdot\text{HCl}$ , PLP, PMB, and TNBS were obtained from Sigma Chemical Co.  $[1\text{-}^{14}\text{C}]$  Ethanol (4.7 Ci/mol) for the synthesis of  $[^{14}\text{C}]\text{DEP}$  and  $\text{NaH}^{14}\text{CO}_3$  were purchased from New England Nuclear. DEP and rose bengal were supplied by Aldrich Chemical Co. Sephadex G-75 and G-100, Sepharose 6B and prepacked columns of Sephadex G-25M (9.1 ml bed volume) were obtained from Pharmacia Fine Chemicals and  $\text{NaDodSO}_4$ , Bio-Rad protein assay reagent and Bio-Gel P-6DG desalting gel from Bio-Rad Labs. Ultrapure  $(\text{NH}_4)_2\text{SO}_4$  and sucrose were supplied by Schwarz/Mann and polyethylene glycol-6000 by Fisher Scientific Co. All other chemicals were of reagent-grade quality.

Plant material. Spinach for Rubisco isolations was obtained at a local supermarket (Brown *et al.*, 1980). *Nicotiana tabacum* L. cv. Xanthi was grown in a controlled environment room ( $250 \mu\text{einsteins m}^{-2}\text{s}^{-1}$ , 16-h photoperiod,  $20^\circ$  day/ $16^\circ$  night) and freshly harvested, fully expanded leaves were used for obtaining crystalline Rubisco (Kung *et al.*, 1981). Seeds and tillers of the diploid (cvs. Gremie, Perma and 64038-50-308) and tetraploid (cvs. Reveille, Barlatra and 64038-1-312) perennial ryegrass cultivars [see Garrett (1978)] were kindly supplied by Dr. M. K. Garrett, the Queen's University of Belfast. The plants were grown in the controlled environment room described above and ploidy level was verified by chromosome counts using root tip smears (Ahloowalia, 1965). The tetraploid isogenic cultivar (64038-1-312) was not used in our study as it was found to be very unstable and reverted completely back to the diploid state under our growth conditions.

## Chapter 1

### Histidyl Modification of Higher Plant Rubisco

(Submitted, in part, as a manuscript entitled "Critical Examination of Histidyl Modification in Ribulosebiphosphate Carboxylase by Diethylpyrocarbonate and Rose Bengal" by J. M. Rejda and R. Chollet to Archives of Biochemistry and Biophysics)

PREVIEW