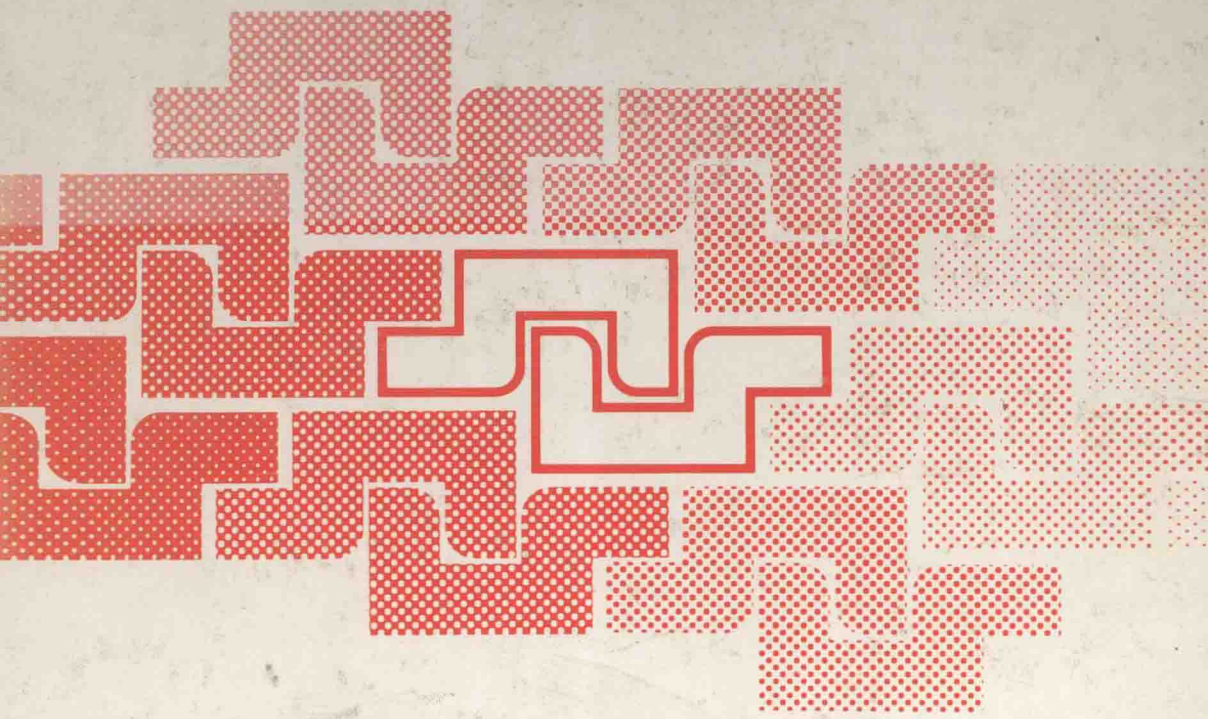

Plant Molecular Biology

Edited by

Diter von Wettstein and

Nam-Hai Chua

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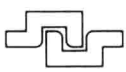

Series A: Life Sciences

PREFACE

The present volume contains the invited lectures and abstracts of the posters presented at the NATO Advanced Study Institute on Plant Molecular Biology held at the Carlsberg Laboratory in Copenhagen, June 10 to 19.

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Without this generous support it would not have been possible to bring together so many outstanding plant molecular biologists for exchange of experience and knowledge and for animated as well as fruitful discussions on future experiments which can advance the understanding of plant biology. Also discussed were the possibilities to apply this knowledge to crop improvement.

Diter von Wettstein

Nam-Hai Chua

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THREE-DIMENSIONAL STRUCTURE OF RIBULOSE-1,5-BISPHOSPHATE CARBOXYLASE/ OXYGENASE FROM RHODOSPIRILLUM RUBRUM

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INTRODUCTION

Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) is the key enzyme in photosynthetic carbon dioxide fixation. Due to its central role in the carbon metabolism of plants, Rubisco has been intensively studied (for a review see (1)). The enzyme catalyses the initial step in photosynthetic carbon dioxide fixation, the carboxylation of ribulose-1,5-bisphosphate, yielding two molecules of phosphoglycerate. This reaction of the enzyme results in the annual fixation of 10^{11} tons CO_2. The same enzyme also catalyses the oxygenation of ribulose-1,5-bisphosphate, the first step in photorespiration. This reaction substantially reduces the overall rate of photosynthesis and consequently plant productivity. The dual function of Rubisco makes it a challenging target in attempts to improve the efficiency of photosynthesis and thus increase crop productivity with the help of modern DNA-techniques.

Rubisco from higher plants, algae and most photosynthetic micro-organisms is a complex multisubunit protein. The enzyme consists of eight large (MW 56000 d) and eight small (MW 15000 d) subunits, building up a L_8S_8 type enzyme. The catalytic function resides on the large subunit; the function of the small subunits is still unknown. The primary structures for the large subunits of higher plant and algal carboxylases are very similar. The amino acid sequence homology amongst the carboxylases of L_8S_8 type is in the range of 70 - 80 % (1).

In contrast to these L_8S_8 type carboxylases, the enzyme from the photosynthetic bacterium Rhodospirillum rubrum differs considerably in amino acid and subunit composition. This carboxylase is only a dimer of large subunits and lacks the small subunits. The overall amino acid homology to higher plant type large subunits is 25 % (2,3). However,

despite this low overall sequence homology, some peptide regions are highly conserved amongst all the carboxylases. Three of these peptide regions have been identified as parts of the active site (4,5,6). This indicates, that the topology of the active site and the overall tertiary structure for the large subunit are very similar in the Rh. rubrum and the higher plant enzymes.

The gene for the Rh. rubrum Rubisco has been cloned and expressed in E. coli. The gene product, obtained by Somerville and Somerville (7), is a fusion peptide containing an additional 24 amino acid peptide from β -galactosidase as the N-terminus. The kinetic parameters of the wild - type enzyme and this recombinant Rubisco are however indistinguishable. Recently, Larimer et al. (8) obtained an authentic, wild-type Rubisco without the 24 additional amino acids from β-galactosidase at the N-terminus.

A number of site-directed mutagenesis experiments have been carried out without the knowledge of the three-dimensional structure of the enzyme, probing the active site (9 - 13). The crystal structure of Rubisco from Rh. rubrum has recently been determined to 2.9 Å resolution (14). In the following, we summarize the structure determination of Rubisco and describe its three-dimensional structure.

CRYSTALLISATION

Rubisco from Rh. rubrum, both the authentic wild-type and the recombinant enzyme, has been crystallized under a number of different conditions (15 - 18). Four different crystal forms have been obtained so far (Table I). Under identical conditions, both the wild-type and the recombinant Rubisco, containing the 24 additional amino acide peptide, crystallize in the same spacegroup with identical cell dimensions (17).

Table 1. Crystal forms of Rubisco from Rhodospirillum rubrum

Source of Rubisco	pH	Spacegroup	Cellparameters (Å)	Reference
native	8.0	$P4_12_12$	82. 82. 290.9	(16)
native and recombinant	7.8-8.2	$P4_12_12$	82. 82. 290	(17)
recombinant	6.5-6.8	$P4_12_12$	82.4 82.4 324.	(15)
recombinant	5.6	$P2_1$	65.5 70.6 104.1 β =92.1°	(18)
recombinant	5.6	$P2_12_12_1$	70.9 100.1 131.	(18)

Structure Determination

The monoclinic crystal form (spacegroup $P2_1$) was used for the structure determination. Recombinant Rubisco from Rh. rubrum, containing the 24 additional amino acid peptide as the N-terminus, was used for all X-ray work. The monoclinic crystal form contains the whole dimer in

the crystal asymmetric unit and has a local, non-crystallographic twofold rotation axis, relating the two subunits of the of the molecule. Intensity data for the native enzyme and two heavy metal derivatives were collected to 2.9 Å resolution. X-ray data for four additional heavy metal derivatives were collected to lower resolution. The local non-crystallographic axis was used to refine the initial MIR-phases, calculated from the heavy metal positions. An initial model was built from the averaged electron density map. A more detailed account of the structure determination can be found in reference (14).

The crystallographic refinement of our model of Rubisco from Rh. rubrum is in progress. At present, the crystallographic R-factor is 26.5 % at 2.9 Å resolution.

The enzyme subunit

The enzyme subunit consists of two domains. The N-terminal domain comprises amino acid residues 1 - 137. The larger C-terminal domain consists of residues 138 - 466. Figure 1 shows a schematic view of the subunit.

The N-terminal domain is built up of a central, mixed five-stranded β-sheet. Two α-helices are found on one side and one α-helix on the other side of the β-sheet. The connection to the C-terminal domain is a short α-helix, followed by a piece of extended chain.

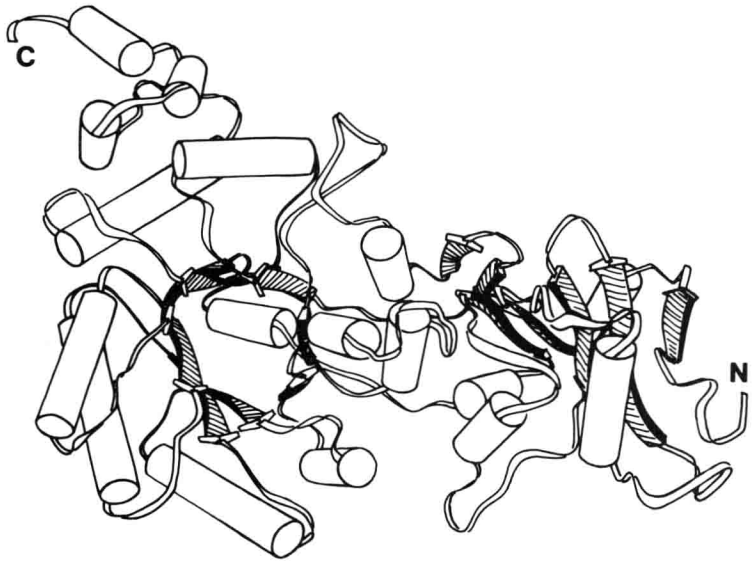

Figure 1: Schematic view of the subunit of Rubisco from Rhodospirillum rubrum. Cylinders represent α-helices and arrows represent β-strands.

The C-terminal domain has an α/β barrel structure, as found in triose phosphate isomerase (19) and other functionally non-related proteins. This fold consists of eight α/β units, which are joined adjacent to each other sequentially along the polypeptide chain. The eight parallel β-strands form the core of the barrel, with the eight helices on the outside.

This domain starts with an α-helix, which does not belong to the α/β barrel motif. This helix is located at the bottom of the α/β barrel and closes off the barrel from this side. After this helix, the chain enters strand no 1 of the α/β barrel. Additional secondary structural elements are found in this domain. After helix no 6., the polypeptide chain forms two additional antiparallel β-strands, before the chain enters strand no 7 of the barrel (figure 1). These two antiparallel β-strands are involved in domain-domain interactions. A small additional α-helix is found in the loop between strand no 8 and helix no 8 of the α/β barrel. At the C-terminal end of the polyopeptide chain, we find three consecutive α-helices.

At the present state of resolution and refinement, two loop regions of the subunit are not defined in our electron density maps. One such loop region is found in the N-terminal domain and comprises amino acid residues 54 - 63. The second loop region is found in the C-terminal domain and comprises residues 325 - 333. These residues are located in loop no 6 of the α/β barrel. We do not observe electron density for the additional 24 amino acid residues from β-galactosidase at the N-terminus. These residues are probably disordered in this crystal form.

The dimeric molecule

Figure 2 shows a picture of the C_α backbone atoms for the Rubisco dimer from Rh. rubrum. The molecule has approximate dimensions 50 x 72 x 105 Å.

It can be seen from figure 2, that the subunit-subunit interactions are tight and extensive. Two main interface areas are found. One such area is between the C-terminal domains of the two subunits, which built up the core of the molecule. The second contact area is beween the C-terminal domain of one subunit and the N-terminal domain of the second subunit. Loop regions between the β-strands and the α-helices of the α/β barrel from the C-terminal domain are involved in subunit-subunit interactions at both interface regions. Amino acid residues from the loops no 1,2 and 3 interact with the N-terminal domain of the second subunit. These loop regions are at the carboxy end of the β-strands, which built up the α/β barrel. Loop no 1 and 2 exhibit extensive amino acid sequence homology between the bacterial and higher plant enzymes. Parts of these conserved peptide regions are involved in subunit-subunit interactions. This is strong evidence that some of these interactions, observed in the Rh. rubrum enzyme, are preserved in the higher plant enzymes.

Subunit contacts are also formed through homologous interactions of residues from loops no 3, 4 and 5 across the local twofold axis with corresponding residues of the C-terminal domain of the second subunit. No obvious amino acid sequence homology is found for these regions. As a consequence, the dimer interactions in the Rh. rubrum enzyme might not be identical with those in the plant enzymes.

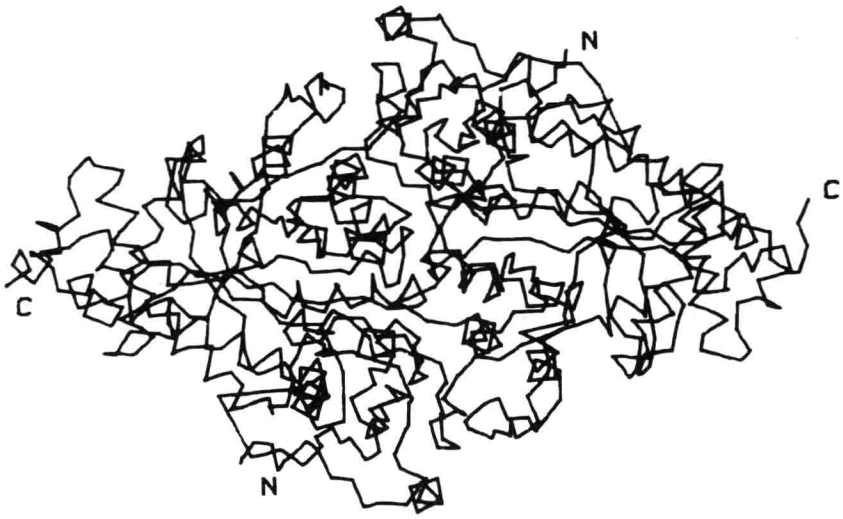

Figure 2: View of the C α backbone of the dimer of Rubisco from Rhodospirillum rubrum. The N- and C-termini of the two subunits are indicated.

The active site

The active site of Rubisco is found at the carboxy end of the eight parallel β-strands of the C-terminal domain, at one end of the barrel. Active site residues are located at the carboxy end of the β-strands or in the loops between the strands and the helices of the barrel.

This side of the barrel is partly covered by the N-terminal domain of the second subunit. Amongst those residues of this domain which are close to the active site, is glu 48, which is part of a highly conserved region in the amino acid sequence. In our model, which represents the decarbamylated enzyme, these residues are too far away to participate directly in catalysis. However it is conceivable, that a conformational change upon activation or substrate binding might decrease the distance for some of these residues to the active site.

Three conserved lysine residues, 166, 191 and 329 have been suggested to be involved in either catalysis or the activation process (4,5,20). It has been shown (4), that lys 191 is the site of carbamylation. Recently, it has been suggested, that lys 166 is the base, which initiates catalysis by abstracting the C-3 proton from ribulose-1,5-bisphosphate (13,21): lys 329 has been identified as an active site residue by chemical modification. All three lysine residues are found at the carboxy end of β-strands or in loops between the carboxy end of β-strands and α-helices of the α/β barrel. Lys 166 is located in the loop after strand no 1, lys 191 is the last residue in strand no 2 and lys 329 is found in loop no 6.

The function of lys 191 is best understood. This residue is involved in the activation process of the enzyme, a process common to all carboxylases. During activation, a carbamate is formed between the ε-NH_2 group of lys 191 and an activator CO_2 molecule. This carbamate is

then stabilized by an Mg ion. Figure 3 shows the surroundings of lys 191 in the deactivated enzyme. Two acidic residues, asp 193 and glu 194, are found in close proximity of lys 191. These two residues are conserved in all the carboxylases. It is very likely, that these two residues are part of the Mg^{2+} binding site in the activated ternary complex. In close proximity of this site is the sidechain of his 287. From our present model, it is possible that this sidechain is also involved in the binding of the metal ion in the ternary complex. This would be in agreement with EPR-measurements of the activated ternary complex, where Mg^{2+} had been replaced by Cu^{2+} (22,23). From these studies, a nitrogen ligand has been proposed to be part of the coordinationssphere of the metal ion. Upon formation of the quaternary complex, this nitrogen ligand is displaced, presumably by oxygen atoms from the substrate (22,23,24).

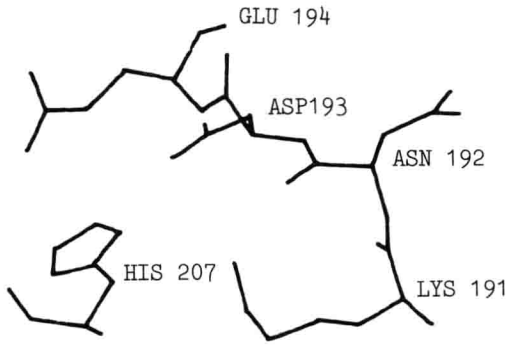

Figure 3: Picture of the Mg^{2+} binding site in Rubisco. Amino acid residues in the immediate surrounding of the activator lysine (lys 191) are shown.

Site-directed mutagenesis

Site-directed mutagenesis experiments have been carried out to probe the function of certain conserved amino acid residues (9 - 13). Two such mutatagenesis experiments have focussed on residues, involved in activation and assumed to participate in metal binding (9,10).

The activator lysine 191 has been replaced by a glutamic acid, such preserving the negative charge at this residue as in the carbamylated enzyme. The obtained mutant is inactive, probably due to the fact that the mutant enzyme is unable to bind Mg^{2+} (10). Modelbuilding experiments of the ternary complex, assuming coordination of the metal ion to asp 193, glu 194 and the carbamate show, that the replacement of the carbamylated lysine sidechain by the shorter glu sidechain destroys the metal binding site. The oxygen atoms of the carboxyl group of glu 191 are too far away from the metal for direct coordination. Consequently, the proper surrounding of the Mg binding site is not maintained and the ability of the enzyme can no longer bind Mg in its proper place.