Rapid Communication J. Biochem. 120, 1064-1066 (1996)

Crystallization and Preliminary Crystallographic Studies of Ribulose 1,5-Bisphosphate Carboxylase/Oxygenase from a Red Alga, *Galdieria partita*, with a High Specificity Factor

Naoki Shibata,* Hiroki Yamamoto,* Tsuyoshi Inoue,* Koichi Uemura,†,2 Akiho Yokota,†,3 and Yasushi Kai*,1

*Department of Applied Chemistry, Faculty of Engineering, Osaka University, Suita, Osaka 565; and †Plant Molecular Physiology Laboratory, Research Institute of Innovative Technology for the Earth, Soraku-gun, Kyoto 619-02

Received for publication, September 10, 1996

Ribulose 1,5-bisphosphate carboxylase/oxygenase (RuBisCO) from a red alga, *Galdieria partita*, has been crystallized by the hanging drop vapor diffusion method. Two forms (Forms I and II) of crystals were obtained under distinct conditions. The Form I crystal belongs to monoclinic space group $C2$ with cell dimensions of $a=190.2$, $b=140.0$, $c=189.0$ Å, and $\beta=102.6°$, and diffracts up to 3.0 Å resolution. Diffraction from the Form II crystal was too weak to determine crystal data.

Key words: crystallization, high specificity factor, red alga, ribulose 1,5-bisphosphate carboxylase/oxygenase, X-ray crystallography.

Ribulose 1,5-bisphosphate carboxylase/oxygenase (RuBisCO: EC 4.1.1.39) is an enzyme that catalyzes primary reactions in photosynthesis as well as in photorespiration (*1*). In the photosynthetic process, the enzyme catalyzes the carboxylation of ribulose 1,5-bisphosphate (RuBP), yielding 2 mol of 3-phosphoglycerate. The competing oxygenation reaction produces 1 mol of 3-phosphoglycerate and 1 mol of phosphoglycolate from RuBP. The oxygenation reaction reduces the rate of photosynthesis and severely limits the crop yield. Consequently, the enhancement of the carboxylation/oxygenation ratio is one of the most important factors for creating RuBisCOs with more useful properties for current global problems.

Most RuBisCOs have a hexadecameric structure composed of eight large (L, 50-55 kDa) and eight small (S, 12-18 kDa) subunits. The crystal structure of this L_8S_8 type RuBisCO was reported for two higher plants, tobacco (*2-5*) and spinach (*6-10*), and a cyanobacterium, *Synechococcus* (*11, 12*). The rate of carboxylation and the affinity to substrates are quite different between the higher plants and cyanobacterium (*1*). Nevertheless no significant structural difference was found at the active site of RuBisCO (*12*), probably due to the conservation of the amino acid sequence around the active site.

Red algal RuBisCOs have higher substrate specificity factors ($V_{CO_2}K_{O_2}/V_{O_2}K_{CO_2}$) (*13, 14*) than higher plants, green algae, and photosynthetic bacteria (*1, 14*). The gene sequences of red algal RuBisCOs (*15-17*) indicate that the amino acid sequences around the active site are rather different from those of RuBisCOs obtained from other species. In order to determine the reason for the high specificity factor, structural studies, especially at the active site, are essential. Here we report the crystallization and preliminary crystallographic studies of RuBisCO obtained from a red alga, *Galdieria partita*.

Purification of the *Galdieria* RuBisCO was carried out by the same method as that for the red alga, *Porphyra yezoensis*, described by Uemura *et al.* (*14*). A quaternary complex of RuBisCO and 2-carboxy-arabinitol 1,5-bisphosphate (CABP) was prepared as reported previously (*18*), and concentrated to 10 mg/ml by ultracentrifugation. *Galdieria* RuBisCO was crystallized by the hanging drop vapor diffusion technique. Three microliters of a protein solution comprising 20 mM $MgCl_2$, 20 mM $NaHCO_3$, and 2 mM CABP was mixed with the same volume of the precipitant solution on a cover slide glass, which was placed over a reservoir well containing the precipitant solution. The droplet and the reservoir well were stored at 298 K. The crystallization conditions are listed in Table I.

X-ray diffraction studies were carried out at room temperature with a Rigaku R-AXIS IIC imaging plate detector system mounted on a Rigaku RU-300 rotating-anode X-ray generator with a graphite monochromator (fine-focused Cu$K\alpha$, operating at 40 kV and 100 mA). The crystal-to-detector distance was set at 160 mm and the exposure time for each frame was 1 h. Each crystal was sealed in a thin-walled glass capillary. Cell constants were determined by means of the auto-indexing routine of the program, *DENZO* (*19*). Data processing was carried out with the programs *DENZO* and *SCALEPACK*. V_m was calculated as described by Matthews (*20*).

The crystallographic data are summarized in Table I. Two crystal forms were obtained: Form I crystals grew as prismatic rods, and Form II ones as square plates (Fig. 1). Both forms of crystals were grown using polyethylene glycol 8000 as the precipitant. Form I crystals appeared after two months under low ion strength conditions, while

[1] To whom correspondence should be addressed. E-mail: kai@chem.eng.osaka-u.ac.jp
[2] Present address: R&D Center, Yunichika Co., Ltd., Uji, Kyoto 611.
[3] Permanent address: Graduate School of Biological Sciences, Nara Institute of Science and Technology, Ikoma, Nara 630-01.

TABLE I. **Crystallization conditions and crystal data for crystals of *Galdieria* RuBisCO.**

Crystal form	Shape	Crystallization conditions	Space group	Cell constants	Z	V_m (Å³·Da⁻¹) (Solvent content, %)
Form I	Prismatic rod	10% PEG8000 8% Ethylene glycol 100 mM Hepes, pH 7.5	$C2$	$a=190.2$ Å $b=140.0$ Å $c=189.0$ Å $\beta=102.6°$	4	2.23 (44.8)
Form II	Square plate	10% PEG 8000 100 mM $MgCl_2$ 100 mM Tris, pH 8.5	nd	nd	nd	nd

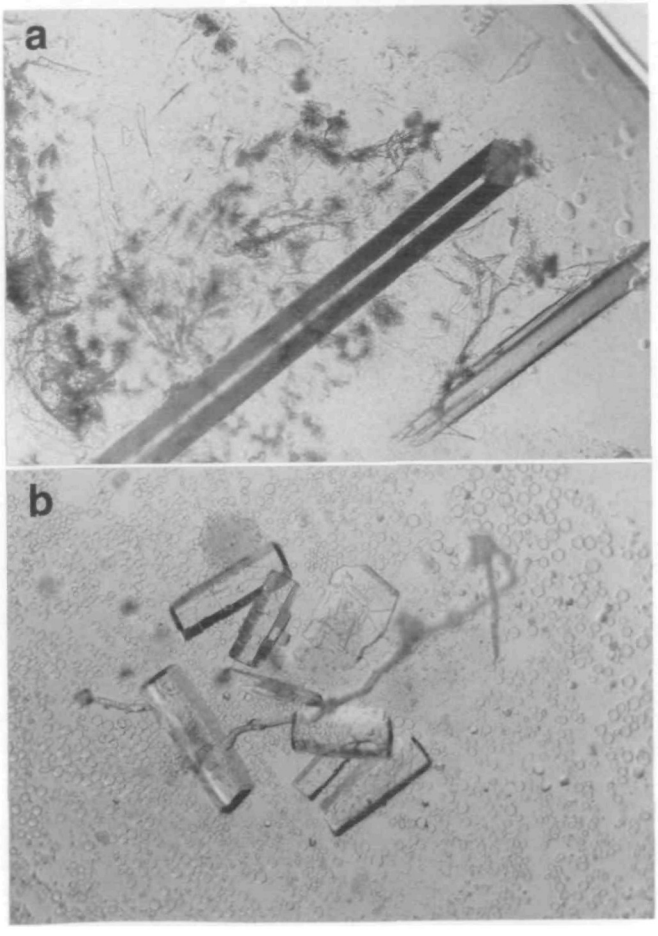

Fig. 1. **Crystals of RuBisCO from *Galdieria partita*.** (a) Form I and (b) Form II. The dimensions of these crystals are approximately 1.5×0.15×0.15 mm and 0.2×0.15×0.03 mm, respectively. In the droplet of the Form II crystal, small hexagonal plate crystals can also be observed.

Form II ones grew completely within three days at higher ion strength than that for Form I. Only the Form I crystal was suitable for X-ray work. X-ray diffraction from the Form II crystal was too weak to determine its crystal data.

Intensity data were collected for a Form I crystal. Ninety frames with a rotation angle of 2.0° were recorded and processed. The combined set was obtained from 307,530 reflections in total, which were reduced to 82,842 unique reflections with an R_{merge} of 11.1% and a completeness of 86% at 3.0 Å resolution. The R_{merge} value is rather high because of the weak diffractions at around 3.0 Å resolution (Fig. 2), and gradual X-ray damage to the crystal. The data obtained using synchrotron radiation may be necessary to carry out structural analysis at higher resolution.

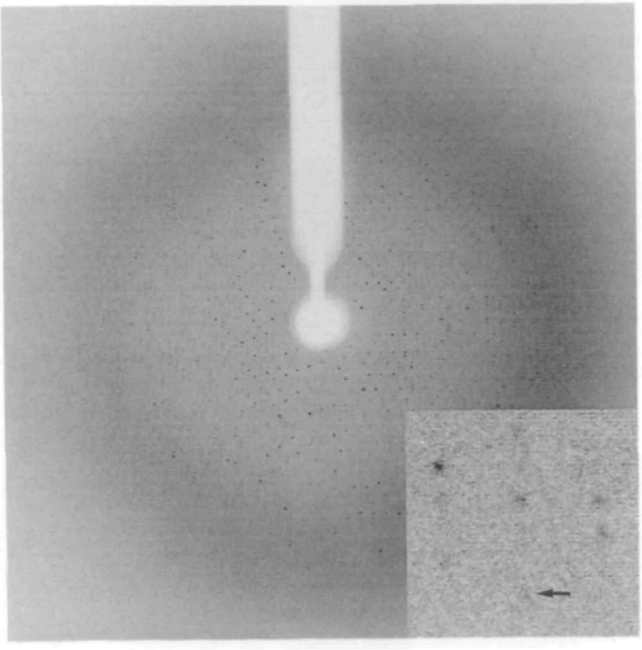

Fig. 2. **An oscillation diffraction pattern of a *Galdieria* RuBisCO crystal with a zoom window.** The image was obtained with a Rigaku R-AXIS IIC imaging plate detector system (crystal-to-detector distance, 160 mm). The black arrow in the zoom window indicates the diffraction spot at 3.0 Å resolution.

We are currently attempting to solve the structure of the Form I crystal by the molecular replacement method.

REFERENCES

1. Andrews, T.J. and Lorimer, G.H. (1987) Rubisco: Structure, mechanisms, and prospects for improvement in *The Biochemistry of Plants* (Hatch, M.D. and Boardman, N.K., eds.) Vol. 10, pp. 131-218 Academic Press, New York
2. Chapman, M.S., Suh, S.W., Cascio, D., Smith, W.W., and Eisenberg, D. (1987) Sliding-layer conformational change limited by the quaternary structure of plant RuBisCO. *Nature* **329**, 354-356
3. Chapman, M.S., Suh, S.W., Curmi, P.M.G., Cascio, D., Smith, W.W., and Eisenberg, D.S. (1988) Tertiary structure of plant RuBisCO: Domains and their contacts. *Science* **241**, 71-74
4. Curmi, P.M.G., Cascio, D., Sweet, R.M., Eisenberg, D., and Schreuder, H. (1992) Crystal structure of the unactivated form of ribulose-1,5-bisphosphate carboxylase/oxygenase from tobacco refined at 2.0-Å resolution. *J. Biol. Chem.* **267**, 16980-16989
5. Schreuder, H.A., Knight, S., Curmi, P.M.G., Andersson, I., Cascio, D., Sweet, R.M., Brändén C.-I., and Eisenberg, D. (1993) Crystal structure of activated tobacco rubisco complexed with the reaction-intermediate analogue 2-carboxy-arabinitol 1,5-bisphosphate. *Protein Sci.* **2**, 1136-1146
6. Knight, S., Andersson, I., and Brändén, C.-I. (1989) Reexamina-

tion of the three-dimensional structure of the small subunit of RuBisCo from higher plants. *Science* **244**, 702-705
7. Knight, S., Andersson, I., and Brändén C.-I. (1990) Crystallographic analysis of ribulose 1,5-bisphosphate carboxylase from spinach at 2.4 Å resolution. *J. Mol. Biol.* **215**, 113-160
8. Taylor, T.C. and Andersson, I. (1996) Structural transitions during activation and ligand binding in hexadecameric Rubisco inferred from the crystal structure of the activated unliganded spinach enzyme. *Nature Struct. Biol.* **3**, 95-101
9. Andersson, I. (1996) Large structure at high resolution: The 1.6 Å crystal structure of spinach ribulose-1,5-bisphosphate carboxylase/oxygenase complexed with 2-carboxyarabinitol bisphosphate. *J. Mol. Biol.* **259**, 160-174
10. Shibata, N., Inoue, T., Fukuhara, K., Nagara, Y., Kitagawa, R., Harada, S., Kasai, N., Uemura, K., Kato, K., Yokota, A., and Kai, Y. (1996) Orderly disposition of heterogeneous small subunits in D-ribulose-1,5-bisphosphate carboxylase/oxygenase from spinach. *J. Biol. Chem.* **271**, 26449-26452
11. Newman, J. and Brändén C.-I. (1993) Structure determination and refinement of ribulose 1,5-bisphosphate carboxylase/oxygenase from *Synechococcus* PCC6301. *Acta Crystallogr.* **D49**, 548-560
12. Newman, J. and Gutteridge, S. (1993) The X-ray structure of *Synechococcus* ribulose-bisphosphate carboxylase/oxygenase-activated quaternary complex at 2.2-Å resolution. *J. Biol. Chem.* **268**, 25876-25886
13. Read, B.A. and Tabita, F.R. (1994) High substrate specificity factor ribulose bisphosphate carboxylase/oxygenase from eukaryotic marine algae and properties of recombinant cyanobacterial Rubisco containing "algal" residue modifications. *Arch. Biochem. Biophys.* **312**, 210-218
14. Uemura, K., Suzuki, Y., Shikanai, T., Wadano, A., Jensen, R.G., Chmara, W., and Yokota, A. (1996) A rapid and sensitive method for determination of relative specificity of RuBisCO from various species by anion-exchange chromatography. *Plant Cell Physiol.* **37**, 325-331
15. Valentin, K. and Zetsche, K. (1989) The genes of both subunits of ribulose-1,5-bisphosphate carboxylase constitute an operon on the plastome of a red alga. *Curr. Genet.* **16**, 203-209
16. Kostrzewa, M., Valentin, K., Maid, U., Radetzky, R., and Zetsche, K. (1990) Structure of the rubisco operon from the multicellular red alga *Antithamnion* spec. *Curr. Genet.* **18**, 465-469
17. Valentin, K. and Zetsche, K. (1990) Structure of the Rubisco operon from the unicellular red alga *Cyanidium caldarium*: Evidence for a polyphyletic origin of the plastids. *Mol. Gen. Genet.* **222**, 425-430
18. Anwaruzzaman, Nakano, Y., and Yokota, A. (1996) Different location in dark-adapted leaves of *Phaseolus vulgaris* of ribulose-1,5-bisphosphate carboxylase/oxygenase and 2-carboxyarabinitol 1-phosphate. *FEBS Lett.* **388**, 223-227
19. Otwinowski, Z. (1993) *Data Collection and Processing. Proceedings of the CCP4 Study Weekend*, pp. 56-62, Daresbury Laboratory, Warrington, England
20. Matthews, B.W. (1968) Solvent content of protein crystals. *J. Mol. Biol.* **33**, 491-497