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Small-angle X-ray study on the structure of ribulose-1,5-bisphosphate carboxylase-oxygenase from *Rhodospirillum rubrum*

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Abstract. The quaternary structure of ribulose-1,5-bisphosphate carboxylase-oxygenase (rubisco) from *Rhodospirillum rubrum,* an enzyme consisting of two large subunits, L_2, was investigated by small-angle X-ray scattering. In the presence of HCO_3^- and Mg^{2+}, rubisco is in the active state and displays a radius of gyration of 2.96 nm, a maximum diameter of 9.5 nm and a volume of 170 nm^3. A model is presented where the subunits are arranged back-to-back, rotated relative to each other by 90°, and shifted by 1.3 nm. Upon inactivation by removal of HCO_3^- and Mg^{2+}, the model swells slightly without any distinct changes in configuration. This contrasts with our previous observations with rubisco from *Alcaligenes eutrophus,* an enzyme composed of small (S) and large (L) subunits, L_8S_8, where inactivation gives rise to substantial changes in configuration.

Key words: Small-angle X-ray scattering, solution structure, ribulose-1,5-bisphosphate, carboxylase/oxygenase

Introduction

Ribulose-1,5-bisphosphate carboxylase/oxygenase catalyzes the reactions

$$RuBP + CO_2 + H_2O \rightarrow 2\,3\text{-PGA} + 2H^+$$

and

$$RuBP + O_2 \rightarrow 2\,\text{Phosphoglycolate} + 3\text{-PGA} + 2H^+.$$

The enzyme from *Rhodospirillum rubrum* is a dimer with a molecular weight of about 112,000.

Small-angle X-ray scattering is a useful method for determining the size and shape of biological macromolecules in solution. Although not sensitive to small changes in local conformation, it proves valuable in the detection of larger conformational variations or of configurational changes brought about by relative shifts or rearrangements of subunits in quaternary assemblies (Glatter and Kratky 1982). In previous studies, we have investigated by small-angle X-ray scattering the quaternary structure of ribulose-1,5-bisphosphate carboxylase/oxygenase (rubisco; EC 4.1.1.39) from *Alcaligenes eutrophus,* an enzyme consisting of eight large (L) and eight small (S) subunits, L_8S_8 (Meisenberger et al.)[1]. It was found that the arrangement of the subunits obeys point group symmetry 422 in the active enzyme, with added HCO_3^- and Mg^{2+}, as had already been indicated by electron microscopic and X-ray diffraction studies (Bowien et al. 1980). In a series of ultracentrifuge sedimentation studies, it could be demonstrated that the enzyme changes shape if it is inactivated by depletion of HCO_3^- and Mg^{2+} (Bowien and Gottschalk 1982). Subsequent studies by small-angle X-ray scattering showed that in the inactive form the overall fourfold symmetry of the L_8S_8 enzyme is destroyed by squeezing the molecule into a diamond-shaped quaternary structure with 222 symmetry (Meisenberger et al. 1984).

Based on these experiments we were interested in the quaternary structure of the active and inactive states of the simpler enzyme from *Rhodospirillum rubrum* which consists only of two large subunits, L_2 (Tabita and McFadden 1974).

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Abbreviations: RuBP Ribulose-1,5-bisphosphate, 3-PGA 3-phosphoglyceric acid

1 In the paper on *A. eutrophus* carboxylase (Meisenberger et al. 1984) the molecular parameters (radius of gyration and maximum dimension) were given in Å rather than in nm, as erroneously indicated

Experimental

a) Preparation of active and inactive enzyme

Ribulose-1,5-bisphosphate carboxylase/oxygenase (rubisco) was isolated from the photosynthetic bacterium *Rhodospirillum rubrum* according to procedures described by Schloss et al. (1979). The active enzyme preparation contained 25 mg protein/ml, 20 m*M Tris*, 50 m*M* $NaHCO_3$, 10 m*M* $MgCl_2$, 1 m*M* dithioerythritol and 1 m*M* EDTA at pH 8.0. For inactivation, the enzyme solution was exhaustively dialyzed at 4 °C against the same buffer without $NaHCO_3$ and $MgCl_2$.

b) Small-angle X-ray scattering

Scattering experiments were performed using a Kratky camera with a slit collimation system on a Philips PW 1130 generator with copper tube, operated at 50 kV and 30 mA, as described previously (Meisenberger et al. 1984). The enzyme solutions were kept at 4 °C during the measurements and the scattering curves for each concentration were recorded up to 15 times in the angle range $0.1 \leq h \leq 4.0\ nm^{-1}$, where $h = (4\pi \sin\theta)/\lambda$ (2θ: scattering angle; $\lambda = 0.154$ nm, wavelength of the CuK_α line). Data evaluation, desmearing and indirect Fourier transformation were done as described by Glatter and Kratky (1982).

Results and discussion

Molecular parameters

A series of six concentrations (between 6.7 and 21.5 mg/ml) was measured, both of the active and the inactive enzyme. Below 17 mg/ml no concentration dependence was detected in the scattering curves.

The scattering curves (Fig. 1 a) were corrected for smearing effects and transformed to distance distribution functions $p(r)$ (Fig. 1 b) according to Glatter (1977). Because the scattering curves for active and inactive enzymes do not differ appreciably, the derived parameters are comparable. Maximum particle dimensions (D_{max}) and radii of gyration (Rg) are, for the active enzyme, $Rg = 2.96 \pm 0.02$ nm, $D_{max} = 9.5 \pm 0.5$ nm and for the inactive enzyme, $Rg = 3.02 \pm 0.02$ nm and $D_{max} = 10.0 \pm 0.5$ nm. From the invariants of the scattering curves (Porod 1951), the (hydrated) correlation volumes were calculated as $170 \pm 5\ nm^3$ for the active and $172 \pm 5\ nm^3$ for the inactive enzyme.

Model calculations were performed with the MULTIBODY program (Glatter 1980), which permits the construction of models from arbitrary small

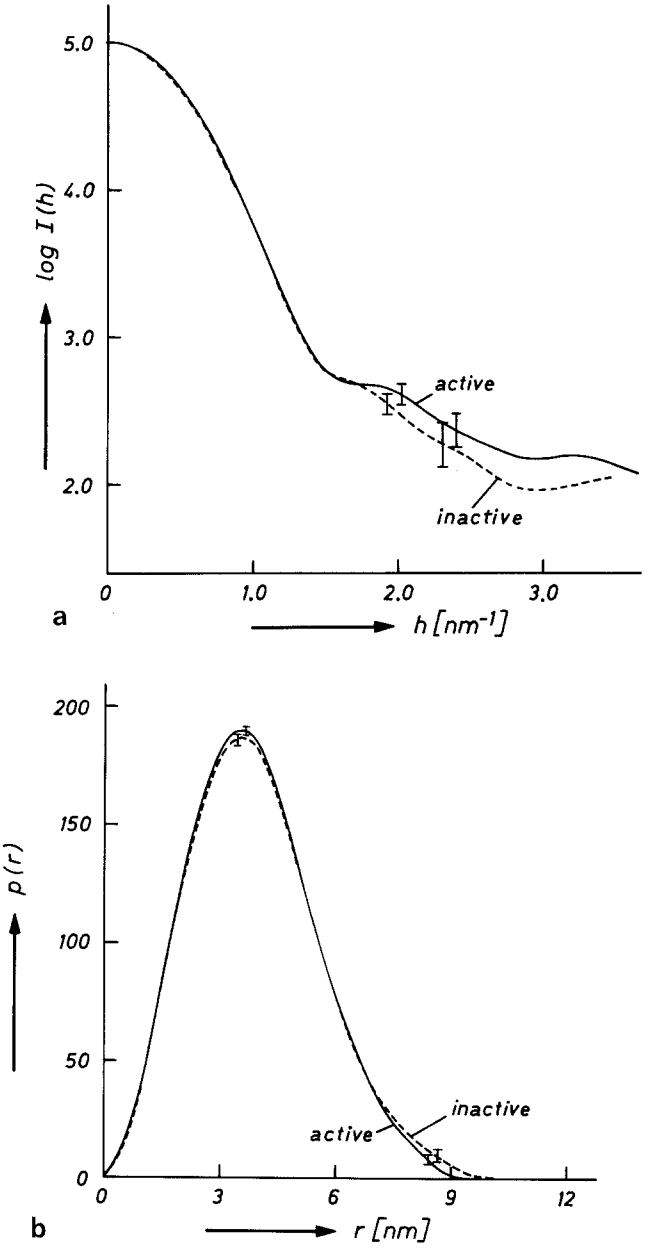

Fig. 1 a and b. Experimental X-ray scattering functions of rubisco from *Rhodospirillum rubrum*. **a** $I(h)$ functions: $h = 4\pi \sin\theta/\lambda$, 2θ = scattering angle, $\lambda = 0.154$ nm (wavelength of the CuK_α-line), I = scattering intensity (normalized to 10^5 at $h = 0$). **b** $p(r)$ function (electron pair distance distribution function in arbitrary units, normalized to an area of 10^5), r = distance in real space

spherical elements. Based on these models, the program generates the $I(h)$ and $p(r)$ functions which are compared with the experimentally obtained X-ray scattering curves (Fig. 2 a, b).

The fit of the $p(r)$ functions was considered of most importance and the positions of the minima and maxima of the $I(h)$ functions were used as an additional criterion for the quality of the derived models. Differences in the intensities of the observed

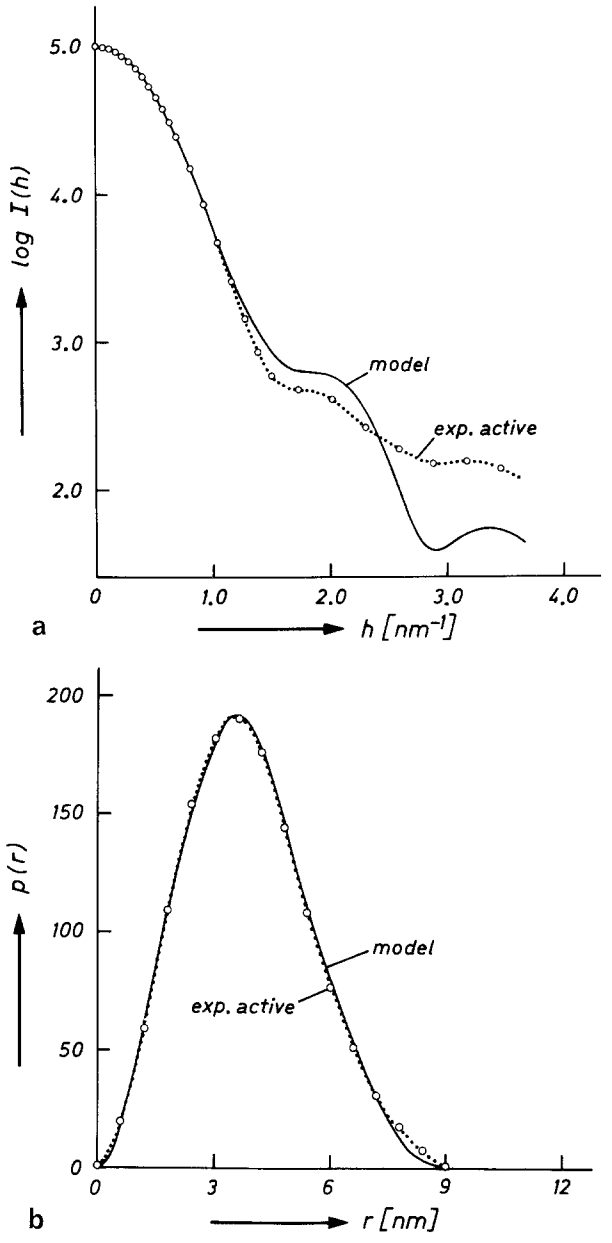

Fig. 2 a and b. Comparison of experimental X-ray scattering functions for the active rubisco from *Rhodospirillum rubrum* and the scattering function calculated on the basis of our model. **a** $I(h)$ functions, **b** $p(r)$ functions; (○○○○) experimental, (——) model

and calculated $\boldsymbol{I(h)}$ functions at larger angles $\boldsymbol{h}$ (Fig. 2a) are probably due to minor variations in electron density within the protein. These had to be neglected in the present study because both the comparatively low resolution of the X-ray scattering experiments and lack of additional information about the internal local structure of the enzyme permitted only the construction of electronically homogeneous models with uniformly weighted spheres.

Table 1. Structural parameters of rubisco from *Rhodospirillum rubrum*

		Active	Inactive
Radius of gyration	Rg =	2.96 ± 0.02 nm	3.02 ± 0.02 nm
Maximum diameter	D_{max} =	9.5 ± 0.5 nm	10.0 ± 0.5 nm
Hydrated volume	V_{hyd} =	170 ± 5 nm³	172 ± 5 nm³
Parameters of best model (Fig. 3a):			
Subunit (L):	radius of gyration		2.17 nm
	max. dimensions	x	3.94 nm
		y	5.25 nm
		z	5.25 nm
	max. diameter		7.20 nm
	depth and width of groove		1.31 nm
	contact area between subunits		6.90 nm²
Model (L_2):	radius of gyration		2.96 nm
	max. diameter approx.		10.0 nm
	relative shift of subunits		1.31 nm
	relative rotation of subunits		90°
	radius of the spherical elements		0.41 nm

Since the large subunits of all rubiscos studied so far have similar molecular weights and show sequence homologies, the shape of the *Rhodospirillum rubrum* rubisco subunit was assumed to be comparable to that of the large subunit of *Alcaligenes eutrophus* rubisco. As electron micrographs had indicated, the overall appearance of the latter may be that of a *U* (Bowien et al. 1976). In our calculations models with spherical and ellipsoidal rubisco subunits were constructed, too, but did not give acceptable results. A large number of dimeric arrangements were constructed with rotational or translational symmetry, and with combinations thereof including screw rotation symmetries. The best model, displayed in Fig. 3a was obtained when a sequence of operations was performed as illustrated in Fig. 3b. First, the *U*-shaped subunits were arranged back-to-back so that a complex resulted with point group 222. In a second step, one of the subunits was rotated by 90° as indicated in Fig. 3b, so that another complex with 222 symmetry was obtained. In a final adjustment, one of the subunits was shifted by 1.3 nm so that the symmetry of the complex broke down.

Concerning the inactive form of the L_2 enzyme, the best model is not distinctly different from that of the active enzyme. We have to conclude therefore that the overall shapes of inactive and active enzyme are similar. Upon inactivation, some swelling occurs as indicated by a marginal increase in radius of gyration, maximum dimension and hydrated volume (Table 1). In addition, the internal electron density changes somewhat as illustrated by the different

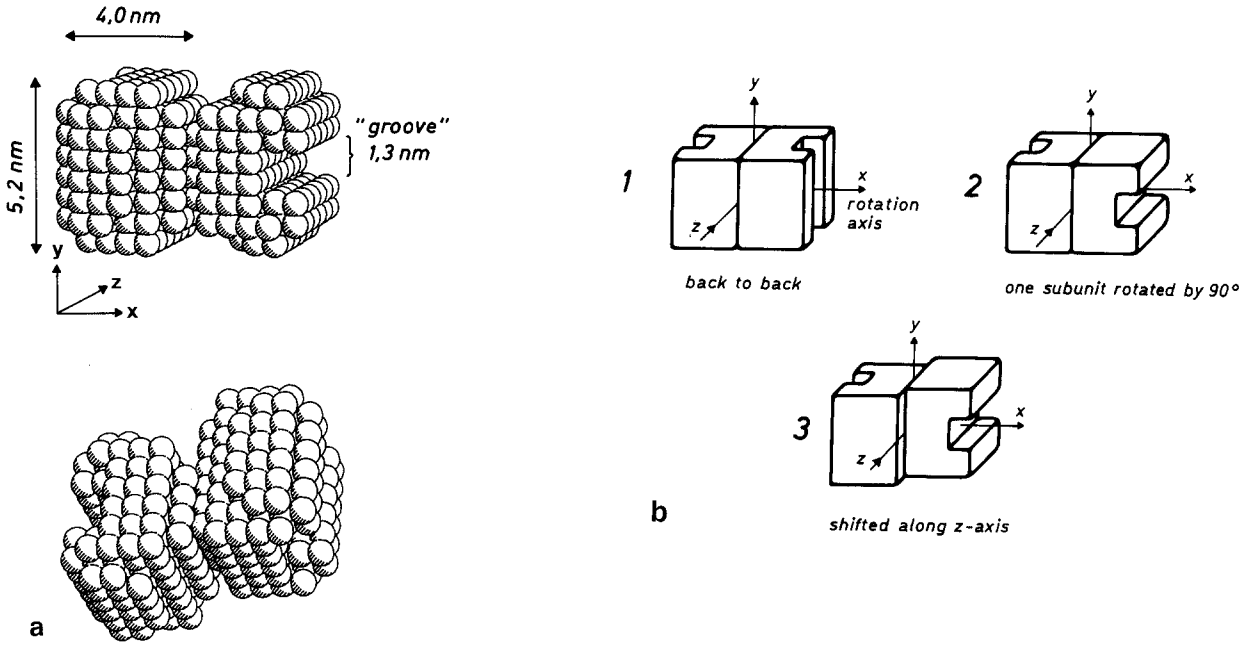

Fig. 3 a. The final model for the active rubisco from *Rhodospirillum rubrum* whose scattering and distance distribution functions are shown in Fig. 2, consisting of arbitrary spheres with a radius of 0.41 nm. **b** The model displayed in **a** consists of two monomers arranged back-to-back, rotated by 90° and shifted 1.3 nm relative to each other

intensity distribution at higher resolution, see Fig. 1 a. A definitive interpretation is impossible at present because detailed X-ray analyses are not yet available.

The structural stability observed for active/inactive L_2 rubisco from *Rhodospirillum rubrum* was also found for the larger L_8S_8 enzyme from spinach (Schwarz and Pilz, unpublished results). This contrasts with the sedimentation and X-ray scattering studies on the L_8S_8 from the H_2-bacterium *Alcaligenes eutrophus* where significant configurational reorganization of the subunits occurs (Meisenberger et al. 1984). Since the L_8S_8 rubiscos from phylogenetically more distant H_2-bacteria also display a dependence of the sedimentation values $S_{20,w}$ upon activation/inactivation (Bowien, unpublished results), it appears that there are (at least) two subgroups of the L_8S_8 enzyme: those with a stable, and those with a variable L_8S_8 quaternary structure. However, more experiments are needed to put these preliminary data on a firm basis.

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