Investigation of Ribulose-1,5-bisphosphate Carboxylase-Oxygenase from Tobacco by Small Angle X-Ray Scattering: A Structural Model for the Enzyme in Solution

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The quaternary structure of ribulose-1,5-bisphosphate carboxylase/oxygenase from tobacco *(Nicotiana tabacum)* was investigated in solution by means of small angle X-ray scattering. The most important molecular parameters as the radius of gyration (Rg) and the maximum diameter (Dmax) were determined. Both the active and the inactive form of the enzyme were measured at 5 °C and at 20 °C. A more distinct difference in size could be detected between the inactive forms at these two temperatures (Rg = 4.80 nm (5 °C) and 4.68 nm (20 °C)) than between the active forms (Rg = 4.73 nm and 4.69 nm). The maximum diameters were determined to be 13.1 nm for the inactive form at 5 °C and 12.8 nm for the other forms.

A model is proposed consisting of eight large and eight small subunits arranged in the way that seems to be typical for this enzyme in higher plants.

Introduction

Ribulose-1,5-bisphosphate carboxylase-oxygenase (henceforth called Rubisco) catalyzes the reactions

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

and

$$\text{RuBP} + O_2 \rightarrow \text{2-phosphoglycolate} + \text{3-phosphoglycerate} + 2\ H^+.$$

The enzyme is abundant in the chloroplasts of all higher plants and also occurs in algae and in photosynthetic and chemosynthetic bacteria. The enzyme in higher plants always consists of eight large and eight small subunits [1].

The catalytic and most of the regulatory properties have been located on the large subunits whereas the role of the small subunit remains unclear, even if there have been successful attempts to demonstrate its regulatory functions recently [2].

A simple structural model based on electron micrographs has been presented several years ago [3, 4]. In this model the large subunits are arranged in two layers, each of them consisting of four large subunits arranged with symmetry 422. An equivalent model has been proposed for Rubisco from *Alcaligenes eutrophus* [5] and subsequent SAXS-studies [6, 7] lead to a model with somewhat U-shaped large subunits arranged similarly as in the previous models.

As SAXS permits investigation of the structure of macromolecules in solution its results are a good basis for models of an enzyme in its native state.

Abbreviations: RuBP, ribulose-1,5-bisphosphate; DTE, dithioerythritol; SAXS, small angle X-ray scattering.

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Materials and Methods

Rubisco from tobacco was prepared according to the method of Kung *et al.* [8] with some minor modifications. Young tobacco plants (*N. tabacum* var. Turkish samsun) were a gift from the Austria Tabakwerke AG and Saatzuchtanstalt Gleisdorf, Austria. The preparation proved to be homogenous in the analytical ultracentrifuge. The protein was stored at 4 °C in crystalline form. As storage buffer 25 mM Tris-HCl at pH = 7.5 was used, containing 1 mM EDTA, 1 mM DTE and 0.01% NaN_3. In order to dissolve the crystals the suspension was dialyzed against a Tris-HCl-buffer (25 mM, pH = 7.5) containing 100 mM NaCl, 2 mM DTE, 1 mM EDTA and .01% NaN_3. For activation this buffer additionally contained 10 mM $MgCl_2$ and 50 mM $NaHCO_3$.

Solutions of various concentrations in the range between 6 and 40 mg/ml were investigated at 5 °C

and 20 °C. Little damage of the protein by irradiation was noticed, but its effects could be minimized by separate measurement of the inner and outer part of the scattering curves. Scattering experiments were performed with a Kratky camera (improved compact type) with slit collimation system on a Philips PW1730 X-ray generator equipped with a copper tube which was operated at 50 kV and 30 mA. Each scattering curve was recorded up to 10 times in the range of $h = .1$ to $5.1\ nm^{-1}$ ($h = (4\pi \sin\theta)/\lambda$, 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 [9, 10].

Results

A series of four to six concentrations between 4 and 40 mg/ml was measured both of the active and the inactive form of the enzyme at 5 °C and 20 °C. The results were extrapolated to zero concentration. The structural parameters are listed in Table I. There is a distinct change in structure between the inactive enzymes at 5 °C and 20 °C as found by several other authors [11, 12] (Fig. 1). The inactive enzyme at 5 °C showed a radius of gyration which was 0.12 nm larger than that of the inactive conformation at 20 °C (note the shift of the p(r)-functions to larger *r*-values in Fig. 1). There is also a significant conformational change between the active and inactive forms at 5 °C whereas the differences of the radii of gyration of the active and inactive forms at 20 °C are within experimental error. At 5 °C the active enzyme is smaller than in the inactive state as already noticed in studies of Rubisco from *Alcaligenes eutrophus* and spinach [6, 7]. Dissociation effects could not be noticed. The molecule shrinks somewhat at higher temperatures but remains intact and no rearrangement or disruption of the symmetry of the enzyme takes place as may be seen from the scattering curves (I(h)-functions) and electron pair distance distribution functions (p(r)-functions).

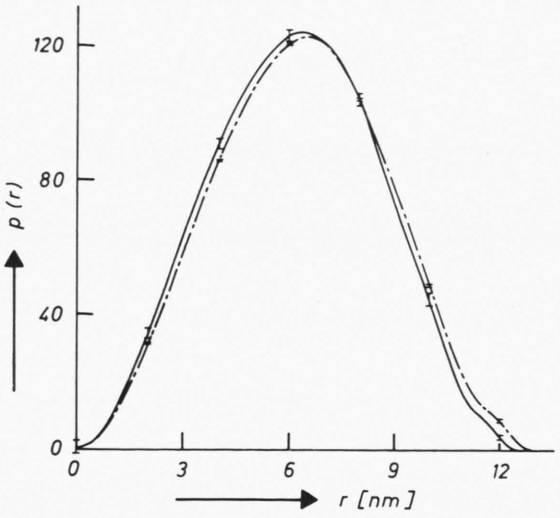

Fig. 1. Electron pair distance distribution functions (p(r)-functions) of inactive Rubisco from tobacco at 5 °C and 20 °C with error bars. (———): 5 °C; (– – – –): 20 °C.

Table I. Molecular parameters of Rubisco from tobacco. Experimental values of the radii of gyration (Rg) and maximum diameters (Dmax) as obtained from SAXS.

	Rg	Dmax
5 °C		
active	4.73 ± 0.04 nm	12.8 ± 0.10 nm
inactive	4.80 ± 0.04 nm	13.1 ± 0.10 nm
20 °C		
active	4.69 ± 0.04 nm	12.8 ± 0.10 nm
inactive	4.68 ± 0.04 nm	12.8 ± 0.10 nm

Model Calculations

Model calculations were performed using the MULTIBODY program [13] which calculates the scattering functions from models consisting of arbitrary spherical elements. These functions may be compared to the experimental curves. Usually the fit of the p(r)-function serves as main criterion for the goodness of fit as the I(h)-function is influenced at larger angles by minor variations in electron density which may not be represented by a model without further information about the interior structure of the protein. However the resolution of the experiment itself does not permit the construction of more detailed models.

As the scattering curves for active and inactive enzyme do not differ very much in shape we constructed only one model and recalculated it to fit the different radii of gyration. So the overall shape is the same for all conformations and only the actual dimensions are slightly different. The model scattering

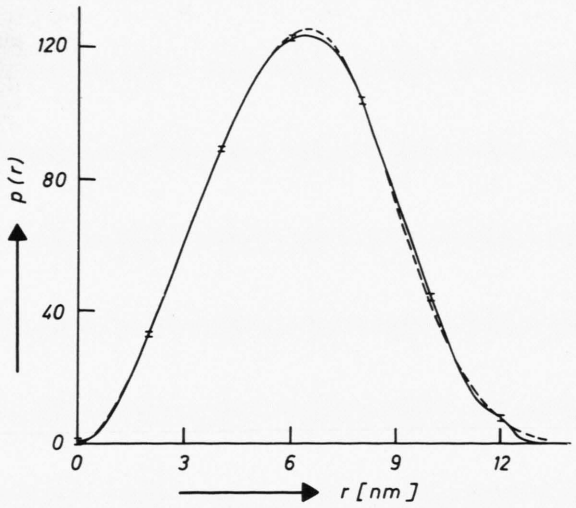

Fig. 2. Comparison of the experimental to the model p(r)-function (distance distribution function): (———): experimental, active, 5 °C; (– – – – –): model (Fig. 3).

functions may be compared to the experimental functions of the active enzyme at 5 °C in Fig. 2.

The model consists of eight large and eight small subunits arranged as shown in Fig. 3a. These subunits are constructed from spheres with a radius of 0.43 nm. There are two layers of four large subunits arranged cloverleaf-like around the *z*-axis (perpendicular to the drawing plane of Fig. 3b) in each layer. On top of each large subunit there is a small one (dotted spheres in Fig. 3a) to give the structure shown in Fig. 3a. The dimensions of the large subunit are approximately $4.84 \times 6.92 \times 4.15$ nm (Rg = 2.24 nm), those of the small subunit are $2.77 \times 2.77 \times 2.08$ nm (Rg = 1.31 nm). The channel through the center of the model (Fig. 3b) is 1.38 nm wide. The dimensions of the whole complex are $11.96 \times 11.96 \times 12.45$ nm.

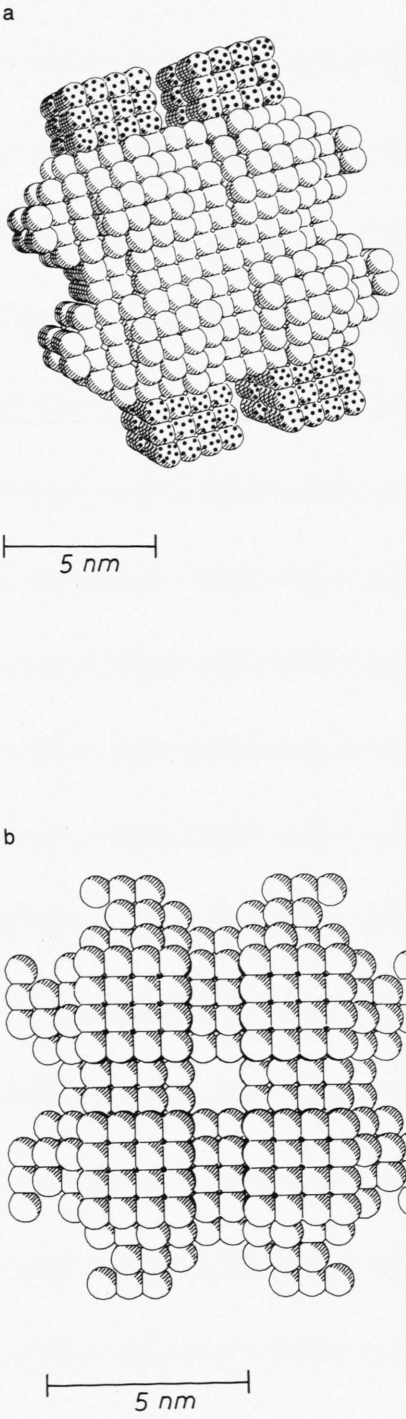

Fig. 3. The model for the enzyme in solution (parameters are given in the text).
a) Front view, the spheres of the small subunit are dotted.
b) Top view, to show the fourfold symmetry around the *z*-axis (perpendicular to the drawing plane).

Discussion

Regarding the overall shape of the L_8S_8-complex the model is in good agreement with those so far derived from electron microscopy, SAXS and neutron angle scattering experiments [6, 7, 14, 15].

It is always a problematical point to denominate structural features of a model as a certain functional entity. For Rubisco there are several ways of interpretation that lead to a consistent L_8S_8 arrangement of subunits. For spinach Rubisco there were

attempts to model the complex with the small subunits in the equatorial plane of the whole molecule [14, 15]. Taking into account the resolution of the methods used, the L_8S_8 complex might equally well be represented by an arrangement as we used it in the model presented in this paper. A similar arrangement was already found for the enzyme from *Alcaligenes eutrophus* [5, 6, 16]. It was originally deduced from X-ray crystallographic data for tobacco Rubisco [3] and has been most frequently used so far.

Acknowledgements

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