Small Angle X-Ray Study on the Structure of Active and Inactive Ribulose-1,5-bisphosphate Carboxylase-Oxygenase from Spinach. Evidence for a Configurational Change

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Z. Naturforsch. **42c,** 1089–1091 (1987); received May 4/July 9, 1987

Small Angle X-Ray Study, Ribulose-1,5-bisphosphate Carboxylase-Oxygenase, Configurational Change

Small angle X-ray scattering studies on ribulose-1,5-bisphosphate carboxylase-oxygenase (Rubisco) from spinach reveal a configurational change in its quaternary structure upon the transition of the molecule from the activated form occurring in the presence of CO_2 and Mg^{2+} to the deactivated form obtained when CO_2 and Mg^{2+} are removed by extensive dialysis under nitrogen. Present structural models are comparable to models which were postulated previously for the same enzyme but isolated from the hydrogen bacterium *Alcaligenes eutrophus* [O. Meisenberger, I. Pilz, B. Bowien, G. P. Pal, and W. Saenger, *J. Biol. Chem.* **259,** 4463–4465 (1984)]. The radius of gyration is $R = 47.5 \pm 0.2$ nm for the active spinach Rubisco. Upon deactivation, R changes to 49.2 ± 0.2 nm, suggesting a more elongated quaternary structure. The observed difference in deactivation behaviour in ambient and in nitrogen atmosphere indicates a higher affinity of this spinach enzyme to CO_2 with respect to the same enzyme from *Alcaligenes eutrophus*.

Ribulose-1,5-bisphosphate carboxylase-oxygenase (Rubisco) catalyzes the competing pathways of photosynthetic CO_2 fixation and photorespiration. In both reactions, the enzyme needs activation *via* the formation of a ternary complex with CO_2 and Mg^{2+} [1]. In plants and in most bacteria this enzyme exists as a hexadecamer containing eight large (L) catalytic subunits ($M_r = 55{,}000$) and eight small (S) subunits ($M_r = 14{,}000$) of unknown function. Rubiscos of different subunit compositions are also known [2].

A previous small angle X-ray scattering study of Rubisco from *Alcaligenes eutrophus* showed a configurational change in its quaternary structure upon the transition of the molecule from the activated to the deactivated state *i.e.*, in presence or in absence of CO_2 and Mg^{2+} [3] which was correlated with sedimentation properties [4]. Rubisco from *Rhodospirillum rubrum* which is composed of only two large subunits (L_2) and lacks the small subunits showed no difference between the quaternary structures of the activated and deactivated forms [5]. These results agree with a neutron small angle scattering study [6], which, however, showed that the L_8S_8 Rubisco from spinach does not change its quaternary structure when CO_2 and Mg^{2+} are removed by dialysis.

In order to investigate this subject further, to evaluate a structure-function relationship and to classify the Rubiscos isolated from different sources, the present study on the quaternary structure of spinach Rubisco using small angle X-ray scattering has been undertaken.

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Verlag der Zeitschrift für Naturforschung, D-7400 Tübingen
0341–0382/87/0900–1089 $ 01.30/0

Materials and Methods

Isolation of Rubisco

Rubisco was isolated from fresh spinach leaves according to published procedures [7, 8] with some alterations in the buffer compositions and column dimensions. The active material was electrophoretically homogeneous with an absorbance ratio (280/260 nm) of 1.8 to 1.9. Enzyme activity was measured at 20 °C by the spectrophotometric assay [9]. The material was stored either as ammonium sulfate suspension at 4 °C for 1–2 weeks or at −20 °C in presence of 20% glycerol for a longer period.

Enzyme activation/deactivation

For activation, samples of ammonium sulfate suspension or glycerol containing Rubisco were exhaustively dialysed against 20 mM Tris-HCl, pH 8.0,

containing 50 mM $NaHCO_3$, 10 mM $MgCl_2$, 1 mM dithioerythritol and 1 mM EDTA; for deactivation the same buffer was used but without $NaHCO_3$ and $MgCl_2$. Deactivation was performed under ambient conditions and under nitrogen atmosphere using preboiled water.

Small angle X-ray scattering

The measurements were carried out employing a Kratky camera with slit collimation on a Philips PW 1130 X-ray generator operated at 50 kV and 30 mA using CuK_α radiation. During the measurements, enzyme solutions were kept at 5 °C. Scattered X-ray intensities were recorded at 90 different angles from $h = 0.01$ to $0.35\ nm^{-1}$ using an entrance slit of 120 μm, where $h = (2\pi/\lambda) \cdot \sin 2\theta$ [2θ = scattering angle], and $\lambda = 0.154$ nm.

Each scattering curve was recorded several times and about 200000 pulses were measured per angle in order to minimize the statistical errors. Details of the experimental techniques and the data evaluation procedure are described elsewhere [10, 11].

Results and Discussions

Two series of measurements were performed with the activated and deactivated samples of freshly prepared Rubisco, and in each series four different concentrations were used, ranging from 5 mg/ml to 40 mg/ml.

The spinach enzyme in the activated state produced virtually the same X-ray scattering curve as that obtained with the active Rubisco from *A. eutrophus*. The radii of gyration derived from these curves are for the active enzymes from spinach and *A. eutrophus* 47.5 ± 0.2 nm and 47.8 ± 0.1 nm respectively. Therefore the molecular model which was postulated for the *A. eutrophus* enzyme [3] also fits with the spinach Rubisco. On the other hand, unlike the *A. eutrophus* Rubisco, the deactivated spinach Rubisco in ambient conditions (see Materials and Methods) showed scattering curve and p(r) function practically identical to those derived for the activated sample.

In order to avoid traces of CO_2 which might keep Rubisco in the activated state, deactivated Rubisco was prepared under nitrogen atmosphere and by using preboiled water throughout the experiment. This enzyme showed clearly a different X-ray scattering behaviour in comparison to enzyme deactivated under ambient atmosphere (Fig. 1). The radius of gyration for the deactivated spinach Rubisco is 49.2 ± 0.2 nm, and its scattering curve and p(r)-function fits the curves calculated on the basis of the molecular model of *A. eutrophus* Rubisco in the deactivated state. The good fit of the p(r)-functions observed for spinach Rubisco and *A. eutrophus* suggests that the quaternary structures of both enzymes in the deactivated state are identical (Fig. 2).

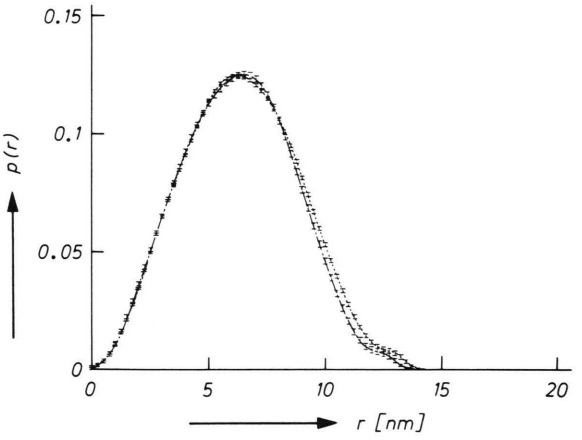

Fig. 1. Comparison of the p(r) functions of deactivated Rubisco from spinach; deactivated under ambient conditions (——) and in nitrogen atmosphere (----). The accuracy of the measurement is given by the calculated error bars.

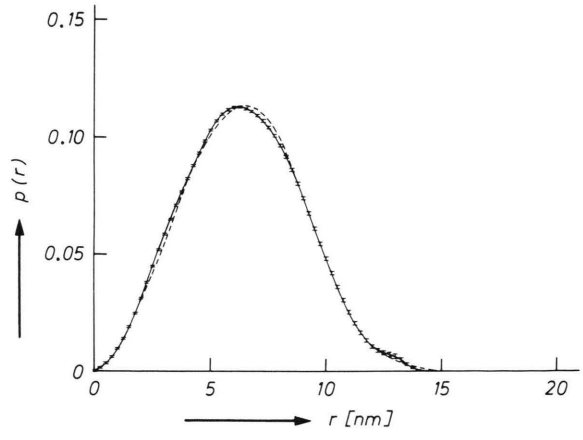

Fig. 2. The p(r) functions of spinach (——) and *A. eutrophus* (----) Rubisco in the deactivated state are nearly identical, suggesting comparable three-dimensional structures.

The X-ray scattering data suggest that the *A. eutrophus* and spinach Rubiscos have almost spherical quaternary structures in the activated state which become more ellipsoidal, elongated upon deactivation, *i.e.* removal of CO_2 and Mg^{2+}. Evidence for a structural difference in the activated and deactivated states of Rubisco from spinach was also obtained by cross-linking experiments [12] which indicated that in the activated state, the subunits are closer together. The point was emphasized that the fully deactivated state was never reached because CO_2 from the air was always present, even without addition of $NaHCO_3$ in the cross-linking experiments.

In sedimentation studies and small angle neutron scattering experiments on spinach Rubisco [6], no changes in quaternary structure could be monitored when going from the activated to the deactivated state. Because in these investigations, no precautions had been taken to totally remove CO_2 by working under nitrogen and with preboiled water, it appears that, in fact, the deactivated state of spinach Rubisco had never been reached. If we take the K_M values as measure for the binding affinities of the Rubiscos for CO_2, then bacterial Rubiscos such as *A. eutrophus*, $K_M \sim 66$ μM [13] have a much weaker affinity than the higher plant Rubiscos, such as the spinach enzyme, ~ 17.5 μM [14]. Because the concentration of CO_2 in air-equilibrated aqueous solution is about 10 μM [15], it is clear that even without added $NaHCO_3$ spinach Rubisco remains in the activated form whereas the *A. eutrophus* enzyme occurs in the deactivated state.

This view reconciles all the seemingly contradicting results concerning differences in quaternary structure of L_8S_8 Rubiscos in the activated and deactivated states. It suggests that they really undergo structural change with a transition point associated with the binding affinity for CO_2, which is not uniform but characteristic for each species.

Acknowledgements

The authors are indebted to Prof. B. Bowien, Göttingen, for fruitful discussions. I.P. and E.S. thanks the "Österreichischen Fonds zur Förderung der wissenschaftlichen Forschung" for generous support. W.S. and G.P.P. acknowledge financial support by the Deutsche Forschungsgemeinschaft in the frame of Sonderforschungsbereich 9 (Teilprojekt A7), and by Fonds der Chemischen Industrie.

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