SMALL-ANGLE X-RAY SCATTERING OF D-RIBULOSE-1,5-DIPHOSPHATE CARBOXYLASE FROM *Dasycladus clavaeformis* ROTH (Ag.) IN SOLUTION

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Summary: D-Ribulose-1,5-diphosphate carboxylase from *Dasycladus* was purified, and the gross dimensions were obtained by means of small-angle X-ray scattering measurements in solution. Dissolved single crystals of this enzyme (called "fraction I protein") gave the same hydrodynamic parameters as the purified form. The molecular weight was found to be 535,000, and a radius of gyration of R_g = 45.5 Å was determined. The experimental scattering curves revealed a geometrical particle of D-Ribulose-1,5-diphosphate carboxylase with gross dimensions of that of a hollow sphere with outer radius of 56 Å and inner radius of 12 Å. Determinations of the diffusion coefficients lead to the conclusion that the enzyme has a spherical shape of almost uniform density.

D-Ribulose-1,5-diphosphate carboxylase (RuDP-carboxylase) plays an important role in photosynthesis (1, 2) in plants and photosynthetic bacteria (3), and accumulated interest in the enzyme properties with regard to kinetics, e.g. carboxylase and oxygenase activity (4), arose. Since the chemical papers were published on the carboxylation reaction by Quayle et al. (5) and Weissbach et al. (6) and on the purification of RuDP-carboxylase (7, 8), detailed structural investigations on the so-called "fraction I protein" were only reported by Trown (9) and Baker et al. (10). By means of determinations of the sedimentation constant, molecular weight and diffusion coefficient Trown (9) was able to show that the "fraction I protein" is consistent with the parameters derived from homogeneous preparations of RuDP-carboxylase. The crystallographic study of single crystals of RuDP-carboxylase, according to the method described by Wildman's group (11), revealed a possible space group of $I4_132$ with cell dimensions of a = 383 Å, showing a

subunit structure of L_8S_8, with L = large and S = small subunit.

We are able to show that homogeneous preparations of RuDP-carboxylase from Dasycladus, a marine green alga, and dissolved single crystals after spontaneous crystallization show the same hydrodynamic properties when determined by means of small-angle X-ray scattering measurements in solution. Moreover, from these measurements we determined the molecular weight of RuDP-carboxylase to be 535,000, the radius of gyration, as well as the shape of this enzyme in solution. From the small-angle X-ray experiments it was possible to show that the physico-chemical parameters of "fraction I protein" are identical with those of purified homogeneous RuDP-carboxylase, since no components of higher and/or lower molecular weight could be detected by means of small-angle X-ray scattering measurements in solution.

MATERIALS AND METHODS

The source of RuDP-carboxylase was the marine green alga Dasycladus clavaeformis ROTH (Ag.), cultivated according to Hämmerling (12) at 21°C and 2400 lux. After breaking the cells with ether at -20°C in potassium phosphate buffer, pH 8.5 (13), the enzyme was purified on a Bio-Gel-1.5 m column (100 x 1 cm) in 0.01 M TRIS-HCl, pH 7.5, containing 0.1 M NaCl and 6 mM ß-mercaptoethanol, after ammoniumsulfate fractionation and removal of nucleic acids by treatment with DNA'se and RNA'se. Fractions containing activity were pooled, precipitated with $(NH_4)_2SO_4$ (65 % saturation) and extensively dialyzed against 0.01 TRIS-HCl, pH 7.5, and eluted with a linear gradient ranging from 0-0.2 M NaCl (14). The obtained material had a specific activity of 1.5 under the assay conditions described in (15).

Crystallization of RuDP-carboxylase. Normally, single, 0.1 mm large, cube-like crystals can be obtained directly by pressing the algae with a pair of tweezers. In their concentrated mother liquid they sometimes grow in each direction to a size of 0.2-0.3 mm (14). These crystals were collected, washed several times with 0.01 M TRIS-HCl, pH 8.5, containing 6 mM ß-mercaptoethanol, 0.1 M NaCl and 0.01 M $MgCl_2$, and dissolved at 22-25°C to a homogeneous solution with a glass homogenizer. This prepared solution ("fraction I protein") was used throughout the small-angle X-ray scattering experiments after dialysis of the solution against 0.01 M TRIS-HCl, pH 7.5.

Small-angle X-ray scattering experiments. The small-angle X-ray scattering measurements were performed with CuK_α-radiation using a rotating anode from Elliot Marconi, G x 13, with a focal spot size of 0.1 x 1 mm, and a Kratky camera. The sample-to-receiving slit distance was 215 mm, the width of the entrance slit, scatter slit and receiving slit were close to 0.1, 0.3 and 0.1 mm, respectively. All measurements were

Table 1. Physical parameters for RuDP-carboxylase from Dasycladus derived from small-angle X-ray scattering in solution.

	purified	diss.cryst.
Radius of gyration, R_g (Å)	45.5 ± 0.5	46.5 ± 0.5
Volume, V (Å^3)	74.0 x 10^4	75.0 x 10^4
Surface, S (Å^2)	21.0 x 10^3	21.0 x 10^3
α (A^{-1})	0.0285	0.0280
$\bar{\rho}_i$ (eA^{-3})	0.4105	0.4100
$\bar{\rho}_i \cdot V$ ($10^3 \cdot e$)	304	307
Degree of hydration (g H_2O/g protein)	0.35	0.32
a/b from $3V/4\pi R_g^3$	1:1.4	1:1.6
a/b from $(\frac{S}{V} \cdot R_g)$	1:1.5	1:1.6
r_g (Å)	56.0 ± 0.1	56.5 ± 0.1
r_v (Å)	57.1 ± 0.5	57.0 ± 0.5
r_s (Å)	57.0 ± 0.5	57.1 ± 0.5
Cell dimensions‡ (Å), r_{out}	56.5	57.0
r_{in}	12.5	12.5
Molecular weight (x 10^{-5})	5.35 ± 0.05	5.2 ± 0.08
$\bar{v}$ (ml · g^{-1})	0.730 ± 0.003	0.730 ± 0.003

$\bar{\rho}_i$ = bouyant density; corresponding to an isopycnic density of 1.18-1.20 g/cm^3.

$\bar{\rho}_i \cdot V$ = the number of electrons of one solvated particle of RuDP-carboxylase.

α = the ratios of surface to volume of one particle.

r_g, r_v, r_s = the radii of spheres, determined from the radius of gyration, volume and surface of RuDP-carboxylase, respectively.

‡ = assuming a sphere with outer radius, r_{out}, and inner radius, r_{in}.

performed at 22°C. Normally, three scattering curves were obtained from the enzyme solution and the corresponding blank curves for the 0.01 M TRIS-HCl, pH 7.5, buffer. In order to detect possible small protein fraction in the preparations ("fraction I protein ") the entrance and detector slits were changed to 0.25 and 0.3 mm, respectively, thus giving a resolution of approximately 300 Å. Corrections for slit desmearing and calculations of scattering functions for various

geometrical bodies were performed as described in (16, 17).

The partial specific volume for RuDP-carboxylase was determined according to Lipkin (18) and was found to be 0.730 ± 0.003 $mg \cdot g^{-1}$.

Diffusion coefficients for RuDP-carboxylase were determined as described in (19) and by evaluation of the sedimentation velocity patterns according to Fujita (20). The values of the G^{-1}-function in Fujita's equations were calculated from expanded plots from the tabulated data given by Williams (21).

RESULTS AND DISCUSSION

The overall molecular parameters for the two different preparations of RuDP-carboxylase are listed in table 1. The radius of gyration for the purified form is almost the same as the one obtained from the dissolved crystals. The molecular weights, which are directly proportional to $(I/C)_{C=O}$ by extrapolation of the Guinier plots to infinite dilution, were found in the range of 520,000 to 535,000; this is compatible with the values reported by (7, 9). The hydrodynamic values of the particles, which were determined according to Porod (22) from the invariant term of the scattering curve and the scattered intensity at zero angle, are almost the same for the purified RuDP-carboxylase as for the dissolved crystals.

Comparison of the radii of gyration for the two forms give identical values, even when determining the radius of gyration by applying the correlation function H(x), or when making use of calculating the radius of gyration from the first isometric submaximum. Since the determination of the radius of gyration of densely packed particles from the correlation function requires the whole scattering curve (in practice, the integration up to a distance equal to the maximum diameter of the particles in question) and therefore is less unaffected by errors than the Guinier approximation, it yields a value of R_g = 45.0 Å. Furthermore, when taking the first submaximum of the scattering curve into account, R_g was found to be 46.0 Å, which is in good accordance with the experimental value of R_g = 46.5 ± 0.5 Å.

Comparisons of the experimental scattering curves with theoretical scattering curves of different geometrical particles that are equivalent in scattering the best description of the scattering particle of RuDP-carboxylase is that of a hollow sphere with a ratio of inner to outer radius

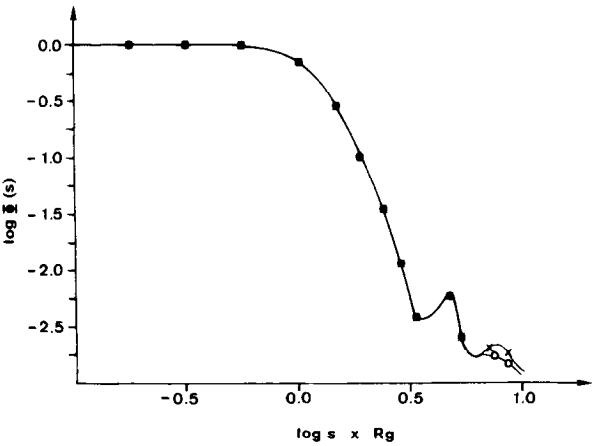

FIG. 1. Log $\Phi(s)$ vs. log s x R_g plot for comparison of the experimental scattering curve (x——x) of RuDP-carboxylase in the purified state, and of the dissolved crystals (o——o).

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of V_i/V_o = 0.22 (Fig. 1). This would yield dimensions of the RuDP-carboxylase particle of a radius of 56.6 Å (diameter = 113.2 Å) with an inner hole having a radius of 12.5 Å. Electron microscopic studies of RuDP-carboxylase particles give a diameter compatible with the dimensions obtained in solution (14).

The overall shape of the enzyme determined by the scattering curves shows that the molecule is not very anisometric when compared with the anisometric ellipsoids of revolution, E2 and E3. Moreover, theoretical scattering curves for spheres and hollow spheres with various radius of r_i/r_o follow the experimental scattering curves down to the second submaximum, but there are some uncertainties in the course of the outer parts of the scattering curves (Fig. 2). However, the most interesting feature of the two experimental scattering curves of the purified enzyme form and the dissolved crystals is the similarity in a log I(s) vs. log s x R_g plot, indicating that we are dealing with the same geometrical bodies when the radius of gyration, molecular weight and volume are taken into consideration. Further more, since the molecule has a certain molecular symmetry it should be possible to investigate the submaxima in more detail in order to calculate scattering curves of geometrical bodies

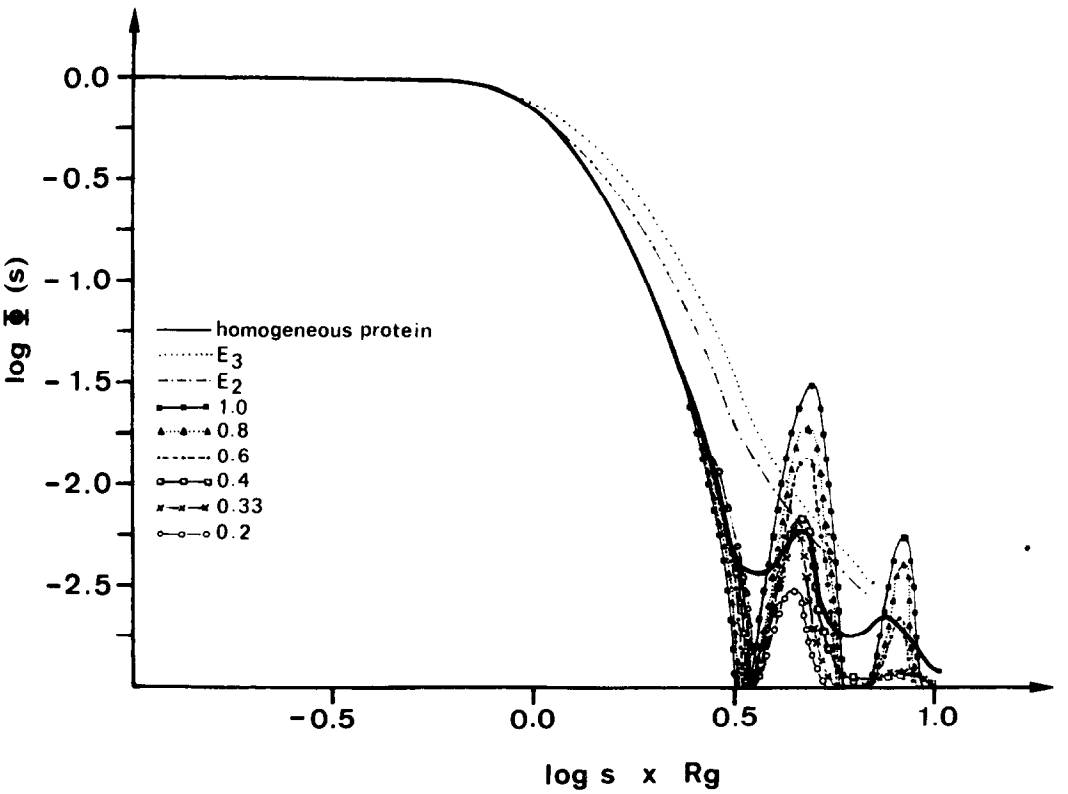

FIG. 2. Log $\Phi(s)$ vs. log s x R_g plot of theoretical scattering curves of hollow spheres with different ratios of inner to outer radii, r_i/r_o = 0.2, 0.6, 0.8 and 1.0, and of ellipsoids of revolution of axial ratios a:b:c = 0.6:1:1 (E 1), 0.2:1:1 (E 2) and 2.5:1:1 (E 3) with $s = 4\pi/\lambda \sin\theta$; λ = 1.54 Å and θ = half of the scattering angle. $\Phi(s)$ = scattered intensity normalized to units of h = 0.

composed of subunits of different size and arrangement. By considering an assembly of eight subunits, the possible geometries are: the cube, the square antiprism and the octagon, all possessing D_4 symmetry.

For spherical particles of uniform density the radius of gyration is related to the diffusion coefficient by $R_g = (3/5)^{1/2} \times \frac{k \cdot T}{6\pi\eta_o \cdot D}$. By comparison of the diffusion coefficients, measured with different independent methods, an average diffusion coefficient of $D = 3.02 \times 10^{-7}$ cm^2/sec (Table 2) is obtained. These physical properties agree with those obtained by Trown (9) when applying a partial specific volume of

Table 2. Diffusion coefficients for RuDP-carboxylase from Dasycladus.

Buffer	from R_g [+]	from R_o [‡] $cm^2 \cdot sec^{-1}$	from $S^o_{20,w}$ [†]
0.01 M TRIS-HCl, 0.1 M KCl, pH 7.5	2.95	3.1×10^{-7}	$(2.93 \pm 0.05) \times 10^{-7}$
0.1 M K_2HPO_4, pH 6.5	2.90	3.2×10^{-7}	$(2.98 \pm 0.05) \times 10^{-7}$
0.05 M CH_3COOK, pH 6.0, 0.1 M KCl	2.91	3.1×10^{-7}	$(3.01 \pm 0.05) \times 10^{-7}$

[+] = from $D = (3/5)^{1/2} \times \frac{k \cdot T}{6\pi\eta_o \cdot R_g}$

[‡] = determined from gel permeation chromatography on BioGel A-1.5 m in 0.01 M TRIS-HCl, pH 8.5; 0.1 M NaCl and 6mM ß-mercaptoethanol.

[†] = determined in an analytical ultracentrifuge (Model E Beckman) at 55,970 rpm in 2^o double sector cells.

0.730 mg/ml and reveal furthermore that RuDP-carboxylase from Dasycladus is almost spherical in shape and of uniform electron density. The degree of hydration of 0.35 g H_2O/g protein, determined by small-angle X-ray scattering experiments, show a relatively dense protein molecule with overall cell dimensions of a diameter of 120 Å. When using a description of a spherical body or the equivalent to that of an ellipsoid of revolution with cell dimensions of a half axis of a = 56.0 Å and b = 90.0 Å, having the same volume, one obtains an axial ratio of a:b = 1:1.6.

The measured radius of gyration of RuDP-carboxylase changes only slightly with increase of solvent density, e.g. when sugar, D_2O or phosphate is added to the enzyme solution. If there were a significant external hydration layer containing, for example, sucrose one would expect a decrease of the radius of gyration. While the hydration layer will have an electron

density less than that of a suger-water solvent, an immediately adjacent polypeptide chain layer will have a density greater than that of the solvent. As a consequence of these measurements, the total hydration of 0.35 g H_2O/g protein is an internal (structural) hydration.

The present evidence , in conjunction with the enzymatic activities of the dissolved crystals and the homogeneous preparation of RuDP-carboxylase, indicates that the crystals ("fraction I protein ") are identical with RuDP-carboxylase.

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