ON THE QUATERNARY STRUCTURE OF NATIVE RABBIT MUSCLE PHOSPHOFRUCTOKINASE

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ABSTRACT Small angle X-ray scattering measurements on solutions of native rabbit muscle phosphofructokinase (EC 2.7.1.11; ATP: D-fructose-6-phosphate 1 phosphotransferase) show that the dimer has a radius of gyration of 32.5 Å and a molecular weight of 160,000, and that the biologically active tetramer has a radius of gyration of 51.5 Å and a molecular weight of 320,000. A possible model was calculated from scattering curves of the dimer and tetramer suggesting two hollow cylinders with cell dimensions for the dimer of a height of 78.0 Å and a long half axis of 38.0 Å, and for the tetramer of a height of 155.0 Å and an outer radius of 35.0 Å. The tetramer is formed along the 78.0 Å axis of the dimer by means of an end-to-end aggregation. The overall particle dimensions of the protomer of molecular weight 80,000 is calculated to be 35.0 x 30.0 x 55.0 Å, assuming an elliptical molecule. The distance between the centers of the two dimeric units within the tetramer is 104.5 ± 1.5 Å.

Rabbit muscle phosphofructokinase (EC 2.7.1.11; ATP: D-fructose-6-phosphate 1 phosphotransferase) is a key enzyme in the regulation of glycolysis. The complex kinetic properties exhibited by phosphofructokinases from various sources (1) lie in the fact that in addition to its multimolecular forms it is regulated by a certain number of ligands. Both substrates and products are allosteric modifiers (2, 3). The active enzyme consists of four identical subunits of molecular weight 80,000 (4, 5). Recently, Telford et al. (6) determined the shape of the dimer and tetramer by means of electron microscopic techniques, suggesting an end-to-end aggregation of two dimers believed to be the fundamental unit for polymerization. In the present work we report the first results of small angle X-ray scattering measurements on the dimer and tetramer of muscle phosphofructokinase in solution under physiological conditions.

EXPERIMENTAL SECTION

Materials. The enzyme was obtained from Boehringer as an ammoniumsulfate suspension. Further purification was performed on DEAE-cellulose (Whatman) and Bio-Gel A-1.5 m (BioRad Laboratories) column (1.5 x 120 cm) chromatography (7). The concentration of the enzyme stock solution was 15 mg/ml in 0.1 M potassium phosphate, pH 8.7, containing 5 mM EDTA. The specific activity at 25°C, pH 8.7, was 160 units/mg, applying the spectrophotometric assay, using coupling enzymes (2, 3). A unit of enzyme activity is defined as the amount that catalyzes the production of 1 µmol fructose-1.6-biphosphate per min. At pH 8.7 in 0.1 M potassium phosphate buffer, containing 5 mM EDTA, the enzyme has a sedimentation coefficient of 12.8S, obtained from analytical ultracentrifugation in a Spinco Beckman Model E ultracentrifuge. The dimer of phosphofructokinase was prepared by incubating the enzyme at pH 6.5 with 10 mM citrate, 5 mM 2-mercapto-ethanol, followed by chromatography on a Bio-Gel A-1.5 m column (1.5 x 120 cm) at pH 6.5 (4°C) in order to separate the dimer from higher aggregates. The dimer has a sedimentation constant of 7.5S.

Small angle X-ray scattering. The native phosphofructokinase was examined under conditions where it has the sedimentation constant of 7.5S or 12.8S. The X-ray scattering measurements of dilute solutions were performed using a Kratky camera with divergent-beam geometry, adapted to a highly stabilized Müller-Micro X-ray generator. The entrance slit-width was normally 100 µ with a corresponding counter slit-width of 210 µ, resulting in a resolution of approximately 1000 Å at a sample-to-detector distance of 220 mm. Monochromaticity was achieved by using a bent quartz monochromator. The radiation was nickel-filtered CuK_α-radiation (λ = 1.54 Å). The scattered radiation was detected with a proportional counter equipped with an electronically programmed step scanning device. The enzyme samples were mounted in 1 mm diameter thin-walled glass capillaries and the particle scattering curves were derived by subtraction of the solvent from the solution scattering curves. The scattering curves were corrected for collimation errors, slit desmearing was performed using the methods of Taylor and Smith (8). X-ray scattering curves from concentrated enzyme solutions (20.0 mg/ml) were obtained by using either toroidal or Frank optics. The X-ray source was a GX 13 rotating anode (Elliot) with a high intensity focal spot of 0.1 mm^2. The apparent radius of gyration (R_g) was determined according to Guinier (9).

RESULTS

The overall molecular parameters for the two different forms of phosphofructokinase are listed in Table 1. Since the molecular weight is directly proportional to $(I/c)_{c=o}$ it is determined by extrapolation of the Guinier plot to infinite solution. A partial specific volume of $\bar{v}$ = 0.732 ml/mg was applied for both forms of enzyme. The volume of the particles was determined according to Porod (10) from the invariant

TABLE 1. Molecular parameters of phosphofructokinase from small angle X-ray scattering in solution.

	Dimer	Tetramer
Radius of gyration, R_g (Å)	32.5 ± 0.5	51.5
Radius of gyration of the cross section, R_s (Å)	23.9 ± 0.5	41.9 ± 0.5
pH	6.5	8.7
Molecular weight	160,000	320,000
Stokes' radius, R_o (Å) †	44.2 ± 1.5	79.4
Volume (Å^3)	337,720	810,000
Degree of hydration		
g H_2O/g protein	0.40	0.25
a/b		
from $(S \cdot \frac{R_g}{V})$ †	1.1	2.01
from $(\frac{3V}{4\pi R_g^3})$ †	1.2	1.98
Actual dimensions of the molecular assemblies:		
height, (Å) H	78.0	155.0‡
long half axis	38.0	
outer radius, (Å) R		35.0‡
short half axis	17.6	
inner radius, (Å) R_i		9.0‡

† = assuming a prolate ellipsoid of revolution with long half axis, a, and short half axis, b; S = surface area of the particle.

‡ = assuming a rectangular tetrameric assembly with threoretical geometric dimensions of A = 165 Å and B = 80.0 Å (see Fig. 2).

term of the scattering curve and the scattered intensity at zero angle.

The experimental scattering curves were compared with theoretical curves obtained for solid cylinders, hollow cylinders, and tetrameric assemblies of ellipsoid of revolution of various axial ratios. Figure 1 shows the experimental and theoretical scattering curves of the dimer (a) and of the tetramer (b and c) of the enzyme, assuming different shapes. In the case of the tetrameric form of the enzyme the data are consistent with a cylinder with a length to diameter of V = 2R : H = 0.55 : 1. The distinct first maximum of the experimental scattering curve lies higher than the theoretical curve for a cylinder, which implies that this model can be regarded only as a crude approximation. By comparing scattering curves of hollow cylinders of fixed axial ratio, but with variable ratios of the radii (p = 0.1-0.5), the experimental scattering curve can be approximated by a hollow cylinder with a ratio of inner to outer radii V_i/V_o = 0.25 (Fig. 1b). Furthermore, comparisons of the experimental scattering curves of the tetrameric enzyme with theoretical scattering curves of hollow cylinders with given ratio of the radii at variable axial ratios V = 2R : H reveal, that the description of a hollow cylinder with an axial ratio of 0.55 and a ratio of the radii of 0.25 is the best approximation in order to fulfill the first side maximum and minimum (Fig. 1c).

Determinations of the detailed shape of the subunits and their arrangement within the tetrameric assembly were carried out by comparing theoretical scattering functions of the dimer with its experimental scattering curves (Fig. 1a). The dimeric enzyme can be described as an elliptic cylinder with heigth of H = 78.0 Å, long half axis of a = 38.0 Å and short half axis of b = 17.6 Å (Table 1). Moreover, the distance between the centers of the two dimeric units within the tetramer was calculated from the radii of gyration of the dimer and tetramer to be 89.5 Å, if the particles were of spherical shape. Inspection of the radial Patterson function from the tetrameric enzyme show pronounced extrema at d = 107.0 Å (center-to-center distance of the two dimers), at d = 78.0 Å (nearest neighbor distance of adjacent particles in the tetramer), and at d = 35.0 Å (interparticle correlation of the

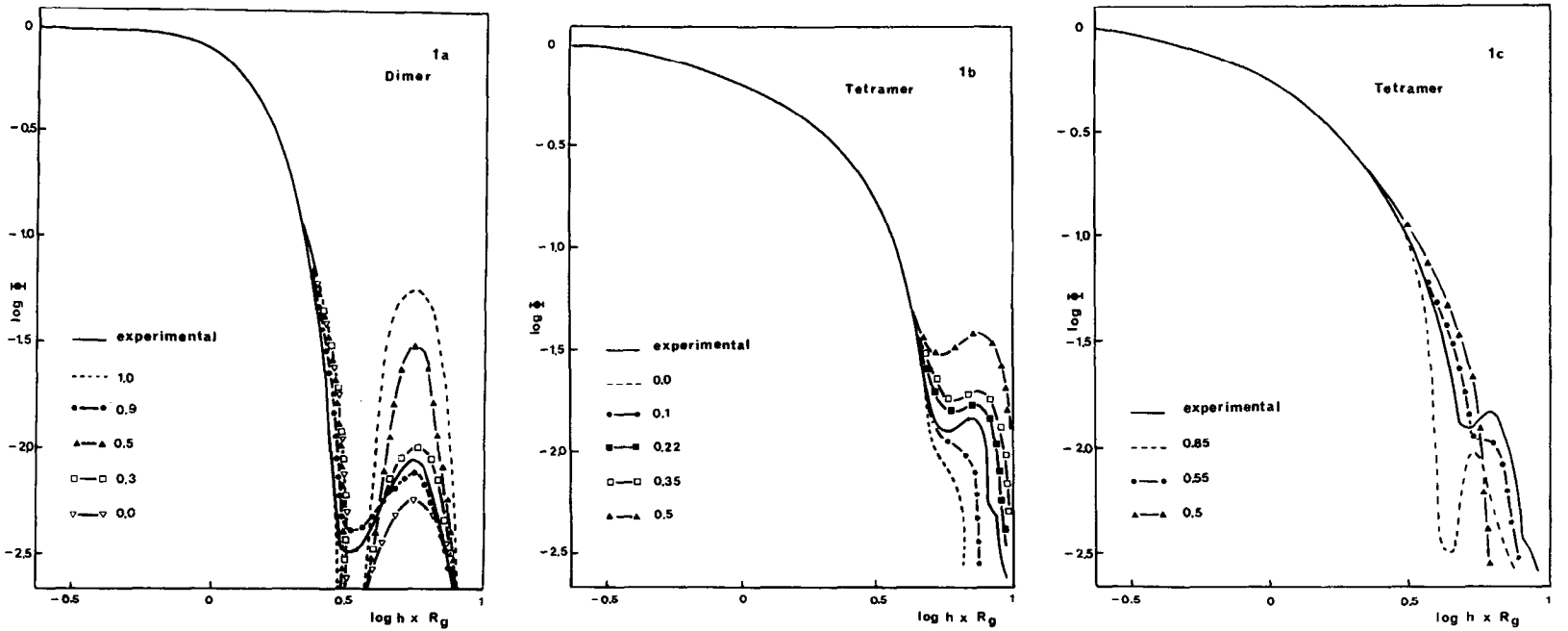

FIG. 1: Log-log plots of experimental and theoretical scattering curves for various models of phosphofructokinase. R_g = radius of gyration; Φ = scattered intensity normalized to units for h = 0; and h = $4\pi/\lambda \sin\theta$ with λ = 1.54 Å and θ = half of the scattering angle.

a) Dimer of phosphofructokinase with fixed outer radius and variable axial ratio V = 2R : H = 1.0, 0.9, 0.5, 0.3 and 0.0 for hollow cylinders.

b) Tetramer of phosphofructokinase with fixed axial ratio V = 2R : H = 0.55 and variable ratio of inner to outer radius (0.0, 0.1, 0.26, 0.35, 0.5) for circular cylinder.

c) Tetramer of the enzyme with variable axial ratio V = 2R : H = 0.85, 0.55, 0.5, and fixed ratio of inner to outer radii.

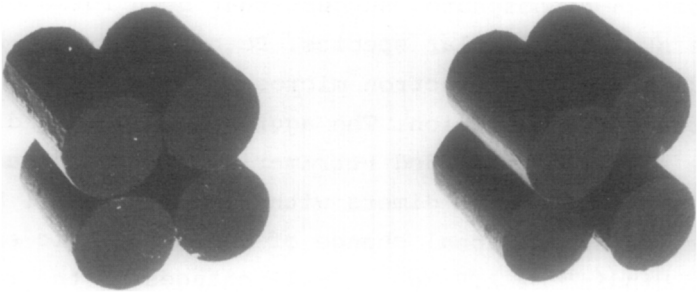

FIG. 2: Three dimensional assembly of the four subunits of the biologically active tetramer of phosphofructokinase (left) and the refined model for the tetramer of phosphofructokinase (right). The refined model shows the degree of overlap of the dimers in forming the tetramer as a rectangle with the dimensions A = 165 Å and B = 80.0 Å.

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monomer domain of phosphofructokinase). These data imply that the monomer of phosphofructokinase is asymmetric with overall cell dimensions of approximately 35.0 x 30.0 x 55.0 Å.

Comparison of experimental and theoretical scattering curves of tetrameric, tetrahedral, and rectangular arrangements of the subunits are consistent with the model shown in Figure 2. The dimer is composed of two equivalent cylinders of molecular weight 80,000. The tetrameric enzyme is composed of four unimpinging ellipsoids arranged in a rectangular assembly as deduced from the radii of gyration, volume, and scattering curves. The aggregation of the dimer is formed by isologous bonding of two monomers where each monomer is shifted about 7.0 Å along the short axis. The long axis of 70.0 Å can only be interpreted by this shift of the two monomers along the twofold axis of symmetry. Therefore, the more compact and spherical tetramer is built up of two different isologous associations with two binding regions per subunit (Fig. 2). Furthermore, the geometry of the cross sections of the dimer and tetramer that was obtained from Guinier plots of log (I/c x 2 θ) vs. $(2\theta)^2$, giving the radius of gyration of the cross section (Table 1), made the proposed model for the tetrameric enzyme possible (R_{cs} = 23.9 Å for dimer, R_{cT} = 41.9 Å for tetramer).

The small angle X-ray experiments, even in the presence of fructose-1.6-biphosphate, suggest that both forms of the enzyme are stable molecular species. Both images have been identified by means of electron microscopy (6) and small angle X-ray scattering in solution. The aggregation of the dimer to the stable and closely bonded tetrameric state requires isologous portions of the dimers within the tetramer. Moreover, we suppose a conformational change of the tetrameric enzyme induced by binding of ATP Mg^{2+}. Small changes of the radii of gyration are observed by different allosteric ligands, such as fructose-6-phosphate (0.15 m, pH 8.5) and fructose-1.6-biphosphate (10 mM). The subunits are obviously asymmetric and related by three two-fold axes (Fig. 2). By arranging the subunits of the tetramer at the corners of a rectangle with two different isologous bonds we obtain three two-fold axes of symmetry that are virtually perpendicular, resulting in D_2-symmetry.

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