SMALL-ANGLE X-RAY SCATTERING STUDIES ON THE QUATERNARY STRUCTURE OF PHOSPHOFRUCTOKINASE FROM BAKER'S YEAST

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1. Introduction

Phosphofructokinase (EC 2.7.1.11) from baker's yeast, a key enzyme in the regulation of glycolysis, has allosteric properties. Contrary to other enzymes of glycolysis the quaternary structure of the phosphofructokinase (PFK) is unknown [1]. From a comparison of the molecular masses of the undissociated enzyme with those of the two immunologically different kinds of subunit [2] a hexameric structure of the enzyme was originally suggested [3,4]. However, because of intramolecular crosslinking of the undegraded phosphofructokinase and after a more detailed analysis of the molecular masses of the subunits [5] an octameric structure of the enzyme was thought more likely [6].

From the small-angle X-ray scattering data we propose for the quaternary structure of the PFK a model consisting of 8 subunits arranged in the dihedral point group symmetry D_2 with a resolution of 2.5 nm. The suggested shape model [7] had only a resolution of 5.0 nm.

2. Material and methods

2.1. *Materials*

PFK from baker's yeast was prepared according to [8]. The buffer contained 0.1 M K-phosphate buffer, pH 7.0, 1 mM EDTA, 5 mM mercaptoethanol, 1 mM fructose-6-phosphate and 1–2 mM $(NH_4)_2SO_4$. The concentrations of PFK in the investigated solutions were 4–55 g/l.

2.2. *Methods*

All measurements were made at constant room temperature and humidity with a highly stabilized X-ray generator (VEB Freiberger Präzisionsmechanik, GDR) using a copper tube. A Kratky-diffractometer and an automated 4-slit diffractometer were used. Collimation effects were eliminated using a computer program described in [9,10]. Measurements of the absolute intensity were performed by using a calibrated Lupolen sample [11]. The scattering curves of models that contain subunits were calculated by means of a computer program developed in [12].

3. Results

It could be shown by means of scattering curve calculations of models that the arrangement of 6 spherical subunits in a 6-fold ring (cyclic symmetry C_6) or in an octahedron (D_3) is in contradiction to the experimental scattering data. Moreover the arrangements of eight subunits in a 8-fold ring (C_8), in a cube (D_4) and in a square antiprism (D_4) do not correspond to the quaternary structure of PFK. An octameric model (model 1) of spherical subunits is shown in fig.1. The model consists of 2 tetramers in a tetrahedral arrangement whose 4 interacting subunits are also arranged tetrahedrally. The calculated scattering curve of model 1 shows a maximum in that angular range, in which the experimental one has a shoulder.

A decision between extended and plain forms of the subunits can be made by comparing the subunit scattering curve with the experimental one in their

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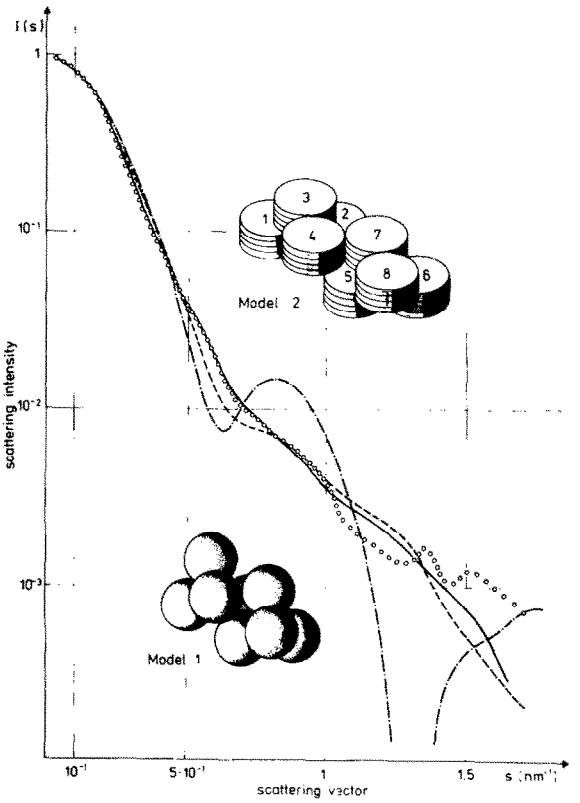

Fig.1. Comparison of the experimental scattering curve of PFK (ooooo) with the theoretical scattering curves of model 1 (.—.—.—), model 2 (– – – – –) and model 3 (——).

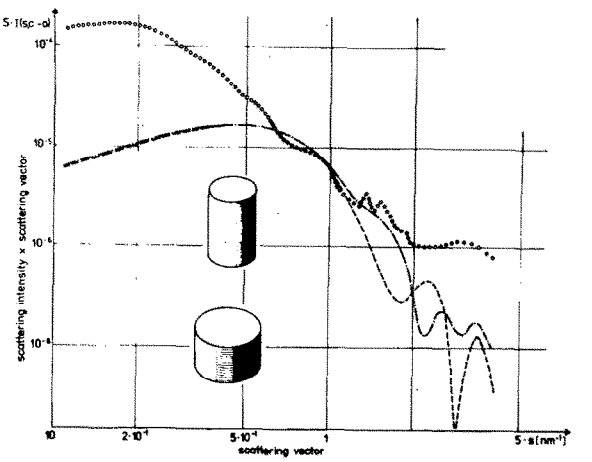

Fig.2. Comparison of the scattering of PFK with the scattering of oblate and prolate form of a subunit, oblate form (.—.—.—), prolate form (– – – – –).

outer parts (fig.2). The plain subunit's scattering curve approximated the course of the experimental curve between $s = 0.65$ nm^{-1} and $s = 2.5$ nm^{-1} better than the extended one. The axial ratios of the circular cylinders were calculated from the overall form, the radius of gyration R_s, the volume, and the largest diameter. Model 2 is composed of 8 circular cylinders (radius $R = 3.9$ nm and height $H = 4.0$ nm) in an arrangement similar to model 1. In contrast to model 1, in each case 4 of the subunits of model 2 lie at the corners of a distorted tetrahedron. The scattering curve of model 2 corresponds to the experimental one of PFK up to $s = 1.4$ nm^{-1}, except for the angular region between $s = 0.55$ nm^{-1} and $s = 0.75$ nm^{-1} (fig.1).

Model 3 (fig.3) is obtained by clockwise turning of the 4 subunits in model 2 by about 63° around their common mass centre. The scattering of this model is equivalent to the scattering of PFK. The theoretical scattering curve of model 3 is in good agreement with the experimental curve of yeast PFK up to $s = 1.4$ nm^{-1} (fig.1). This corresponds to a resolution of 2.5 nm. In table 1 the experimentally determined molecular parameters are compared with those of the proposed quaternary structure model. The parameters are calculated from the desmeared scattering curve after extrapolation to zero concentration.

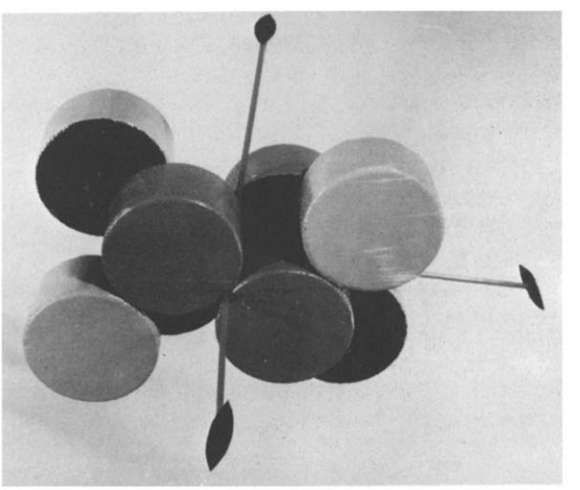

Fig.3. Representation of model 3 having three 2-fold axes.

Table 1
Experimentally determined molecular parameters of the PFK molecule and parameters of the proposed quaternary structure model

	Experimental parameters	Model parameters
Molecular mass M (g/mol)	$(7.42 \pm 0.4) \times 10^5$	
Radius of gyration R_S (nm)	7.45 ± 0.2	7.4
Volume V (nm^3)	1520 ± 150	1556
Surface S (nm^2)	1288 ± 140	1360
Largest diameter L (nm)	22.5 ± 0.7	23.2

4. Conclusion

The subunit arrangement of PFK corresponds to model 3 (fig.3) in which a dihedral point group symmetry D_2 was assumed. Either the 4 α-subunits lie in the centre of the molecule and the 4 β-subunits outside, or vice versa. Therefore the model possesses a $\beta_2\alpha_4\beta_2$ or an $\alpha_2\beta_4\alpha_2$ structure and does not agree with the proposed $\alpha_4\beta_4$ structure [6]. The difference between the 2 types of subunits must be of structural relevance because the point group symmetry C_8 and D_4 has been ruled out before.

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