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Comparison of the structure of the 7 S globulin from *Phaseolus vulgaris* in solution with the crystal structure of 7 S globulin from *Canavalia ensiformis* by small angle X-ray scattering

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The structure of the 7 S globulin from *Phaseolus vulgaris*, phaseolin, was determined by small angle X-ray scattering. The molecule consists of 3 Y-shaped subunits which are arranged around a 3-fold symmetry axis. Though phaseolin is structurally very similar to canavalin, the 7 S globulin from *Canavalia ensiformis*, there are considerable differences between the structure of crystalline canavalin and that of phaseolin in solution. The differences may be caused by tryptic treatment of canavalin before crystallization.

Small angle X-ray scattering *Seed globulin* *Phaseolin* *Canavalin*

1. INTRODUCTION

Phaseolin, the major storage protein in *Phaseolus vulgaris* seeds, has a number of interesting biochemical and physical properties which indicate that it is very similar in structure to the protein canavalin, which is a major storage protein in jack beans. Both proteins are soluble in weak salt solutions and have sedimentation coefficients $s^0_{20,w} = 7.1$ [1] and $s^0_{20,w} = 7.0$ [2], respectively. The molar mass of phaseolin in solution, determined by small angle X-ray scattering and quasi-elastic light scattering, is 1.45×10^5 g/mol [3]. After crystallization with trypsin, the molar mass of the redissolved canavalin molecule was found to be $1.13-1.5 \times 10^5$ g/mol [4–10]. Both phaseolin and canavalin are composed of 3 subunits and have similar compositions of amino acids, as well as an identical, unusual reactivity of exposed sulfhydryl groups in the molecule [9]. In this paper, we compare the structure of 7 S globulin from *Phaseolus vulgaris* in solution with the crystal structure of 7 S globulin from *Canavalia ensiformis*.

2. MATERIALS AND METHODS

The 7 S globulin from *P. vulgaris* was investigated in citric acid/disodium hydrogen phosphate buffer at pH 7.0 and ionic strength of 0.40 at 5–70 mg/ml by small angle X-ray scattering. The preparation of the protein, the small angle X-ray equipment and the data processing, as well as first results determined by combined X-ray and quasi-elastic light scattering measurements, have already been described [3].

3. RESULTS

Fig.1 shows the desmeared scattering data extrapolated to zero concentration of the 7 S globulin of *P. vulgaris*. The scattering curve has two barely visible shoulders at $s = 1.15$ nm^{-1} and $s = 1.45$ nm^{-1} as well as two pronounced subsidiary maxima at $s = 2.75$ nm^{-1} and $s = 5.75$ nm^{-1} ($s = 4\pi \sin \theta/\lambda$; 2θ is the scattering angle and λ is the wavelength). The following molecular parameters were determined from the scattering curve (fig.1) and from the distance

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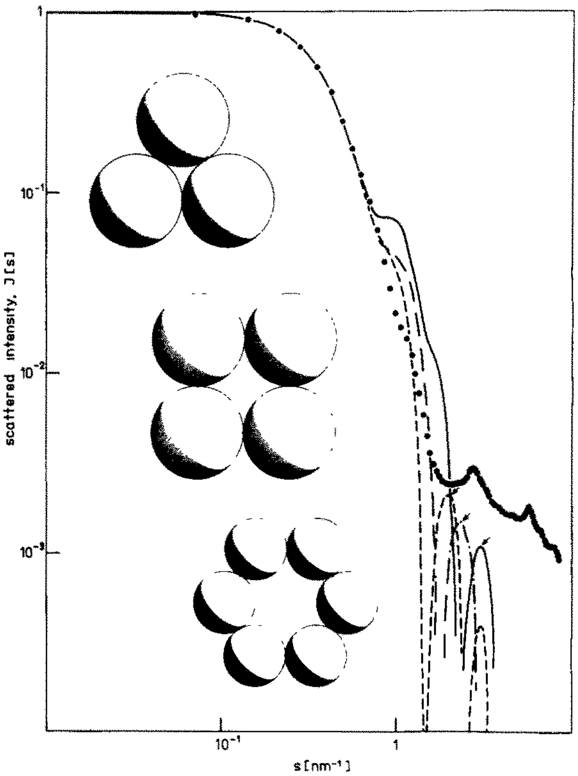

Fig.1. Comparison of the scattering curve for phaseolin from *P. vulgaris* (···) with the scattering curves for models consisting of 3 (– – –), 4 (-----) and 6 (——) spherical subunits.

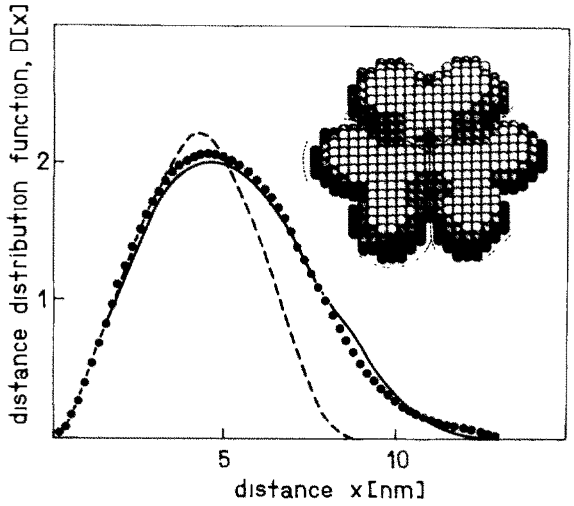

Fig.2. Comparison of the distance distribution function of the phaseolin from *P. vulgaris* (···) with that of the quaternary structure model of phaseolin in solution (——) and with the distance distribution function of canavalin from *C. ensiformis* (– – –), calculated from the theoretical scattering function for the canavalin molecule in crystal [7].

distribution function (fig.2), calculated by Fourier transformation of the scattering curve: radius of gyration R_G = 4.05 nm, maximum dimension of the molecule L = 13 nm, correlation volume V_c = 300 nm^3 [3]. When s is not larger than 1.65 nm^{-1}, the scattering curve can be approximated by the curve for an oblate ellipsoid of revolution with the axial ratio r = 0.3 and axes (12.5 × 12.5 × 3.75) nm [3].

To find a model with a higher resolution, we calculated the theoretical scattering curve of models consisting of different numbers of spheres. Fig.1 shows the scattering curve for trimeric, tetrameric and hexameric models with radii R = 2.91 nm, R = 2.51 nm and R = 1.89 nm, respectively. When we varied the form of the subunits to approximate the shape of the oblate molecule we obtained no better agreement up to s = 1.6 nm^{-1} between the models and the experimental data than between the scattering curve of the oblate ellipsoid of revolution and the experimental curve. However, this refinement of the trimeric model leads to the correct position of the first subsidiary maximum. Modification of models with other symmetries always gives a first subsidiary maximum at a position which disagrees with the experimental data. Therefore, we infer a trimeric structure of the phaseolin molecule with a 3-fold axis as a symmetry element.

We determined the model shown in fig.3 by stepwise approximation of the trimeric model to the shape of the phaseolin molecule. The calculated shattering curve approximates the inner part of the experimental data up to s = 1.6 nm^{-1} and has its first subsidiary peak at s = 2.75 nm^{-1}, as in the experimental curve (fig.3). However, the intensity of the maximum is 5-times smaller. Nevertheless, the distance distribution function of phaseolin is in good agreement with the distance distribution function of the model in fig.3 (fig.2). The scattered intensity of structures with homogeneous electron density is proportional to s^{-4} for large scattering angles [11]. The scattering intensity of the model in fig.3 also has this behaviour. The 7 S globulin of

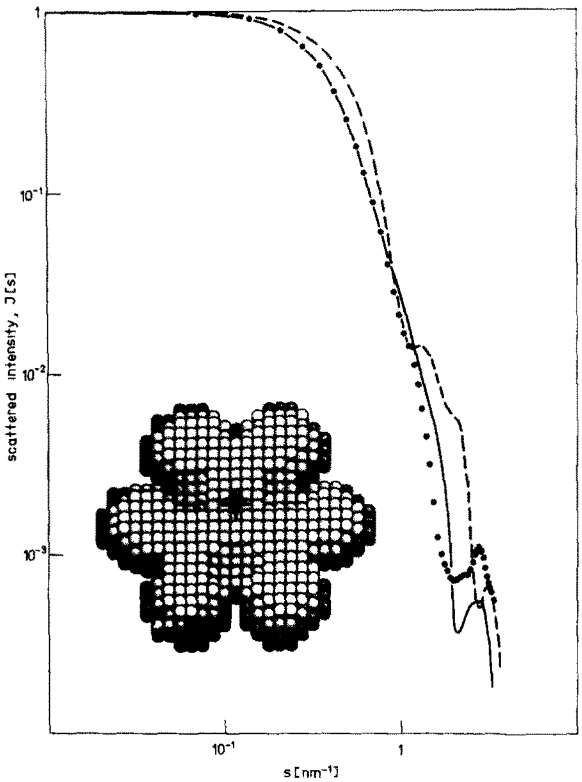

Fig.3. Comparison of the scattering curve of phaseolin for *P. vulgaris* (···) with the scattering curve for the quaternary structure model of phaseolin (——) and with the scattering curve for canavalin of *C. ensiformis*, calculated from the structure of canavalin in the crystal [7]. The constant $K = 1.3 \times 10^{-3}$ was subtracted from the scattering curve of phaseolin (section 3).

P. vulgaris has an inhomogeneous electron density distribution in the interior of the molecule, as a result of its atomic structure. This inhomogeneity leads to an additional scattering, which is superimposed on the scattering of the homogeneous substitutional body of the phaseolin molecule [12]. If we subtract this scattered portion from the scattering intensity of the 7 S globulin by the method described in [13], the scattering intensity of the first subsidiary maximum of the experimental data reduces to 1.1×10^{-3} and thus is in approximate agreement with the experimental scattering intensity for phaseolin in the region of the first subsidiary maximum (fig.3). Therefore, it is likely that the model in fig.3 is similar to the structure of the 7 S globulin.

Fig.3 shows the calculated scattering curve for the electron density function of canavalin, the 7 S seed globulin from *C. ensiformis*, in the crystal. The shape of the canavalin molecule in the crystal was approximated by 2198 spherical scattering centers with radius $R = 0.202$ nm, lying on cubic lattice points within the shape of the molecule. The shape of the canavalin molecule in the crystal was taken from the figures and dimensions shown in [7]. The scattering curve for this approximation was calculated by means of the Debye-equation [14]. The radius of gyration and the maximum dimension of the canavalin molecule in the crystal, calculated from the theoretical scattering function and from the distance distribution function are $R_G = 3.2$ nm and $L = 8.5$ nm (fig.2). The volume of the shape of the canavalin in the crystal is $V = 2198 \times (2R)^3 = 144.9$ nm^3. McPherson [7] states that the outer diameter of the canavalin molecule is 7.5 nm. Our approximation of the molecule in the crystal gives $L = 8.5$ nm. In spite of this somewhat larger maximum dimension, the molecular parameters of the canavalin in the crystal are much smaller than those of phaseolin in solution. In fig.3 and fig.2, the scattering curve and the distance distribution function of phaseolin in solution and of canavalin in the crystal are compared. The figures show that the structure of the phaseolin molecule in solution differs from that of the canavalin molecule in the crystal.

4. DISCUSSION

The phaseolin molecule in solution consists of 3 structural subunits, separated by deep solvent clefts. Each subunit has a distinctive *Y*-shape and is seemingly built up by 2 domains of similar size. It is tempting to speculate that the 3 structural subunits are the 3 different polypeptide chains with molar masses of $(4.8–5.3) \times 10^4$ g/mol into which it dissociates in the presence of sodium dodecylsulfate [9,15]. However, in this angular region our technique cannot resolve differences between the structural subunits.

The phaseolin molecule has a 3-fold symmetry axis. The 3 subunits are arranged in the molecule either with the cyclic point group symmetry 3 (C_3) or with the dihedral point group symmetry 32 (D_3). Therefore, phaseolin in solution has a symmetry similar to that of the canavalin molecule in the crystal [7].

A comparison of the scattering curves (fig.3) and distance distribution functions (fig.2) shows that the canavalin molecule in the crystal has much smaller dimensions than the phaseolin molecule in solution. The dry volume V_T of the canavalin molecule, calculated from the molar mass $M = 1.45 \times 10^5$ g/mol and the partial specific volume $\bar{v} = 0.729$ ml/g, is 175 nm^3. It thus is larger than the volume $V = 144.9$ nm^3 of the approximate model of canavalin in the crystal. However, in general we would expect, that the latter volume would be larger than the dry volume. A molar mass of canavalin in the crystal smaller than $M = 1.45 \times 10^5$ g/mol and thus smaller than the molar mass of phaseolin is one possible explanation of this contradiction. The molar mass obtained for redissolved canavalin by indirect methods does not exclude this possibility. This mass varies from $1.17-1.5 \times 10^5$ g/mol [4–9]. The smaller molar mass of canavalin in the crystal could be caused by the treatment of canavalin with trypsin for crystallization [16]. Additional support for this assumption comes from the results of Sumner and coworkers [10], who determined that for the canavalin molecule, redissolved from the crystal, the molar mass is 1.13×10^5 g/mol and also $s^0_{20,w} = 6.4$, in contrast $s^0_{20,w} = 7.0$ for the molecule before tryptic treatment [2].

Our results show clear-cut differences between the structure of phaseolin in solution and the structure of canavalin in crystal. Because both molecules are seemingly very similar [1–3,9], two interpretations of the differences are possible:

(i) The mass of the canavalin molecule may be lowered by the tryptic treatment before crystallization. This interpretation seems likely.

(ii) The alternative possibility of a change of the molecular structure by the crystal field seems improbable.

If neither interpretation is correct, we must assume that there are greater structural differences between phaseolin and canavalin than is obvious from the indirect results for the structures [1–3,9].

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REFERENCES

[1] Pusztai, A. and Stewart, J.C. (1980) Biochim. Biophys. Acta 623, 418–428.

[2] Derbyshire, E., Wright, D.J. and Boulter, D. (1976) Phytochemistry 19, 3–24.

[3] Plietz, P., Damaschun, G., Zirwer, D., Gast, K. and Schlesier, B. (1983) submitted.

[4] McPherson, A. and Rich, A. (1973) J. Biochem. (Tokyo) 74, 155–160.

[5] McPherson, A. and Spencer, R. (1975) Arch. Biochem. Biophys. 169, 650–661.

[6] McPherson, A. and Smith, S.C. (1980) Phytochemistry 19, 957–959.

[7] McPherson, A. (1980) J. Biol. Chem. 255, 10472–10480.

[8] Smith, S.C., Johnson, S., Andrews, J. and McPherson, A. (1982) Plant Physiol. 70, 1199–1209.

[9] Johnson, S., Grayson, G., Robinson, L., Chahade, R. and McPherson, A. (1982) Biochemistry 21, 4839–4843.

[10] Sumner, J.B., Gralén, N. and Eriksson-Quensel, I. (1938) J. Biol. Chem. 125, 45–48.

[11] Porod, G. (1951) Kolloid Z. 124, 83–114.

[12] Stuhrmann, H.B. and Kirste, R.G. (1965) Z. Physikal. Chem. (Neue Folge) 46, 247–250.

[13] Luzzati, V., Tardieu, A. and Aggerbeck, L.P. (1979) J. Mol. Biol. 131, 435–473.

[14] Debye, P. (1915) Ann. Phys. 46, 809–818.

[15] Bollini, R. and Vitale, A. (1981) Physiol. Plant. 52, 96–100.

[16] Nakamura, S. and Suzuno, R. (1965) Arch. Biochem. Biophys. 111, 439–505.