EXPERIMENTAL AND THEORETICAL LARGE-ANGLE X-RAY DIFFUSE SCATTERING BY GLOBINS IN SOLUTION. SENSITIVITY OF THE METHOD

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1. Introduction

In recent years detailed experimental and theoretical investigations were made of the application of large-angle X-ray diffuse scattering to the study of protein internal structure in solution [1–4]. It was established that an unspecific consideration of the solvent influence is sufficient for a satisfactory agreement between theoretical and experimental curves in the region of scattering angles 2θ up to $\sim 13^\circ$ for CuK_α radiation [5]. After that arose the problem of the sensitivity of the X-ray scattering indicatrix in the range of angles to structural peculiarities of the proteins investigated. This question is very important as large-angle X-ray diffuse scattering can become a useful method of studying protein structure in solution only when there is sufficient sensitivity of the scattering curve to peculiarities of or, at least, to the type of proteins investigated.

This paper gives evidence of a high sensitivity of large-angle X-ray scattering by globular proteins with tertiary and quaternary structures. This conclusion is based both on the consideration of theoretical scattering curves of several globins with a known three-dimensional structure (horse haemoglobin (Hb-H), lamprey globin (GB-Lp), sperm whale myoglobin (Mb-SW)) and also on direct experimental data on large-angle X-ray scattering of Gb-Lp, Mb-SW and adult human oxyhaemoglobin (Hb-A).

2. Materials and methods

The samples were prepared according to the technique described earlier [6]. Measurements were made with CuK_α radiation from a highly stable TuRM 62 X-ray generator (VEB Freiberger Präzisionsmechanik) with a Rigaku Denki M2 slit camera. The slit width was 0.3 mm and 0.5 mm, the distance between the slits was 205 mm and 443 mm between the sample and counter. Evaluation of the slit width influence on the scattering indicatrix [7] has shown that this influence can be neglected in the range of the scattering angles studied. The solution m and the solvent s were in identical 1 mm diameter capillaries. Hb-Lp scattering curves were plotted up to concentration $c = 95$ mg/ml and the Hb-A scattering curves to $c = 260$ mg/ml. The scattering intensity j/c (μ)

$$(\mu = \frac{4\pi}{\lambda} \sin\theta,$$

λ, X-ray wavelength, 2θ, scattering angle)

for Hb-A was extrapolated to zero concentration. The scattering intensity of the sample was calculated according to the formula

$$j(\mu) = \frac{n_m(\mu)}{n_{Em}A_m} - \frac{n_s(\mu)}{n_{Es}A_s},$$

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where $n_m(\mu)$ and $n_s(\mu)$ are intensities (counts per second) for the solution and solvent, respectively, A_m and A_s are the absorption coefficients of the solution and solvent, n_{Em} and n_{Es} are the intensities of standard preparations used for measurement of absolute intensities. The count for each measured point was from 2×10^4 to 7×10^4. The collimation correction for the slit height was taken into account only for the internal part of the scattering curves.

Calculation of scattering indicatrices was based on a combination of methods described earlier [8], the 'cube method', and the 'sphere method'. Similarly to the 'sphere method', the solvent effect was taken into account by introducing effective atomic factors $\tilde{f}_i(\mu)$

$$\tilde{f}_i(\mu) = f_i(\mu) - \frac{4\pi}{3} r_i^3 \rho_o k\Phi(\mu r_i).$$

Here $f_i(\mu)$ is the atomic factor of the i-th atom, $\frac{4\pi}{3} r_i^3$ is its van der Waals volume, ρ_o is the solvent electronic density, k is the coefficient compensating the difference between the volume of the whole protein molecule and the sum of the van der Waals volumes of all its atoms, and

$$\Phi(\mu r_i) = 3 \frac{\sin(\mu r_i) - \mu r_i \cos(\mu r_i)}{(\mu r_i)^3}$$

is the scattering amplitude of the van der Waals volume of the i-th atom. To speed-up computer calculations, the scattering intensity was not calculated by the Debye formula, but, as in the 'cube method', by calculation of the scattering amplitude and intensity at the given point of reciprocal space with a following intensity averaging over the sphere of μ radius in reciprocal space. Published coordinates of atoms for Mb-SW [9] were used for calculation. Coordinates of atoms for Gb-Lp and Hb-H were kindly communicated by Professor Perutz and Professor Hendrickson.

3. Results and discussion

Fig.1 shows theoretical scattering curves Mb-SW and Gb-Lp in an aqueous environment calculated on the basis of the monomer form of both proteins. These curves significantly differ in the region of scattering

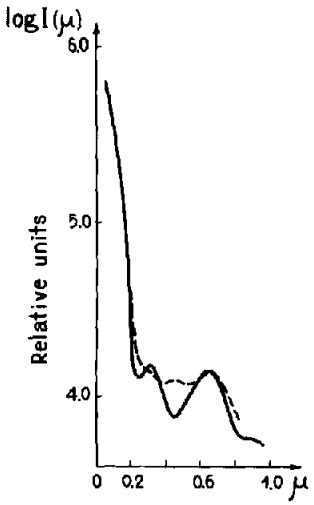

Fig.1. Theoretical scattering indicatrices of diffuse scattering by Mb-SW (– – –) and Gb-Lp (———) molecules.

angles corresponding to $\mu = 0.2$–0.7 Å^{-1}: here the Mb-SW scattering curve is almost a plateau, while the Gb-Lp scattering curve displays two prominent maxima at $\mu \approx 0.3$ Å^{-1} and $\mu \approx 0.7$ Å^{-1}. Such an essential difference in diffuse scattering curves for Mb-Lp and Gb-Lp, i.e. for proteins which have a very similar three-dimensional structure testifies to a high sensitivity of the method to the tertiary structure of globular proteins.

To determine the sensitivity of the large-angle scattering curves to the quaternary structure of proteins, we obtained theoretical indicatrices of scattering from an α-chain, a β-chain, a dimer $\alpha + \beta$, and a tetramer $2\alpha + 2\beta$ of Hb-H on the basis of its known three-dimensional structure. These indicatrices are given in fig.2. The pictures of scattering from α- and β-chains of Hb-H completely coincide. The dimer ($\alpha + \beta$-chains) displays a noticeable maximum in the region of the weak shoulder at $\mu \approx 0.3$ Å^{-1} and a sharp rise of the maximum at $\mu \approx 0.7$ Å^{-1}. The tetramer ($2\alpha + 2\beta$-chains) scattering curve coinciding with the scattering of the whole Hb-H molecule, exhibits a further increase of the maximum at $\mu \approx 0.3$ Å^{-1}, the appearance of a noticeable maximum at $\mu \approx 0.5$ Å^{-1} and a sharp maximum at $\mu \approx 0.2$ Å^{-1}. These significant changes in the scattering curve profile speak of a noticeable sensitivity of large-angle X-ray scattering to the quaternary structure of proteins.

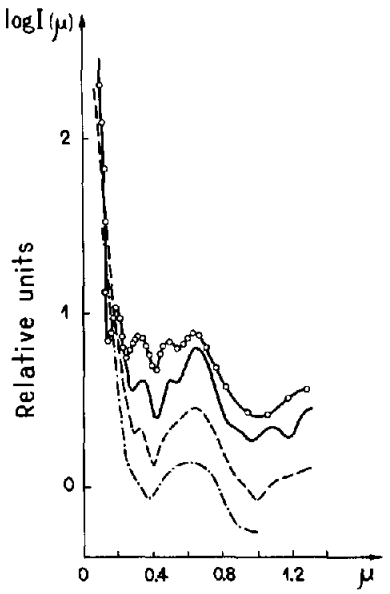

Fig.2. Experimental curve of Hb-A X-ray diffuse scattering (o———o) and theoretical curves of scattering of Hb-H (———) and its subunits: α(β)-chain (— . —) and α + β-chain (– – –).

Fig.2 also shows the experimental scattering curve of Hb-A in an aqueous solution. Analysis of the small-angle part of this curve at $c \to 0$ leads to a molecular weight of about 64 000 which corresponds to four subunits in the molecule. A comparison of the

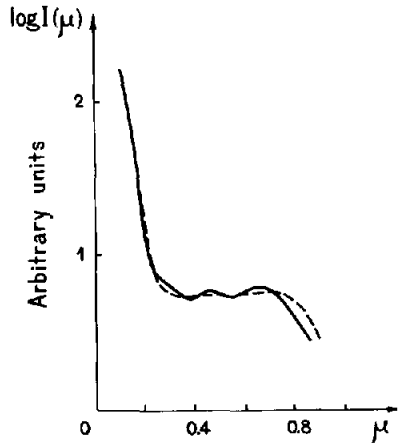

Fig.3. Experimental (– – –) and theoretical (———) curves of Mb-SW X-ray diffuse scattering.

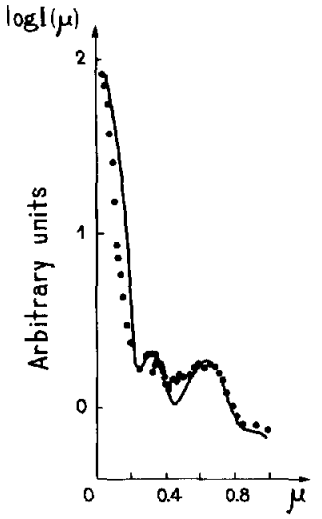

Fig.4. Experimental (• • •) and theoretical (———) curves of Gb-Lp X-ray diffuse scattering.

experimental scattering curve of Hb-A with the theoretical scattering indicatrix of Hb-H (these two proteins vary only in insignificant amino acid replacements in the subunits) shows that the theoretical scattering curve of tetramers reflects all the numerous features of the experimental curve up to $\mu \approx 0.8$ Å^{-1}. This fact as well as the practical coincidence of the calculated and experimental curves for Mb-SW established earlier [5,8,10], evidences of the effectiveness of the suggested method of calculating theoretical curves and, consequently, of the reliability of our analysis of experimental and theoretical curves.

Figs.3 and 4 show experimental scattering indicatrices of Mb-SW* and Gb-Lp in comparison with the corresponding theoretical curves given in fig.1. It is seen that the experimental scattering curves of Mb-SW and Gb-Lp which differ considerably from each other, are in good agreement with their corresponding theoretical curves. The most outstanding feature is the agreement of the experimental and theoretical scattering indicatrices of Gb-Lp. Indeed, the calculated curve refers to the monomer form of protein while the experimental curve refers rather to its dimer form, as an analysis of the small-angle

*The experimental X-ray scattering curve of Mb-SW in an aqueous solution was taken from the paper [11].

part of the experimental curve at $c \rightarrow 0$ gives a molecular weight of about 36 000 which corresponds to the dimer Gb-Lp*. At the same time the above consideration of the theoretical scattering curves of Hb-H shows that the formation of specific dimer complexes in this case leads to a noticeable change in the shape of the scattering curve in the large-angle region. Hence, a conclusion can be drawn that the dimerization of Gb-Lp molecules is not as 'rigid' as in the case of haemoglobin, but allows a certain flexibility between the subunits which leads to a suppression on large-angle scattering curves of the effects connected with the quaternary structure.

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* Higher concentrations give rise to reversible weak aggregates of Gb-Lp with a seemingly higher molecular weight.

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