X-RAY DIFFUSE SCATTERING OF GLOBULAR PROTEIN SOLUTIONS: CONSIDERATION OF THE SOLVENT INFLUENCE

B.A. FEDOROV, O.B. PTITSYN and L.A. VORONIN

Institute of Protein Research, Academy of Sciences of the USSR, Poustchino, Moscow Region, USSR

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1. Introduction

It was recently shown [1–3] that X-ray diffuse scattering of globular proteins in aqueous solutions at comparatively large angles is characterized by a number of pecularities reflecting the specific features of the structure of globular proteins. Thus, diffuse scattering at large angles is potentially a powerful method of studying the structures of globular proteins not in a crystallic state but in aqueous solutions. The interpretation of the results obtained, however, is difficult because of the necessity of taking into account the effect of the solvent on the indicatrix of diffuse scattering [1, 3–5].

The present paper briefly describes a method of calculation of the indicatrix of diffuse scattering of globular proteins in a medium with a non-zero electronic density (for a detailed description of the method see [6]). The method is applied for calculating the indicatrices of scattering of native sperm-whale myoglobin in different solvents; the indicatrices obtained are near to the experimental ones [7].

2. Method of calculation

For a solution of macromolecules at a low concentration the difference of solution and solvent scattering intensities is represented as

$$I(\vec{\mu}) = |F(\vec{\mu}) - \Phi(\vec{\mu})|^2 \qquad (1)$$

where $\vec{\mu}$ is the vector of reciprocal space ($|\vec{\mu}| = \mu = (4\pi/\lambda)\sin\theta$, where λ is the X-ray wavelength, 2θ – the scattering angle),

$$F(\vec{\mu}) = \sum_k f_k(\vec{\mu}) e^{i(\vec{\mu}\vec{r}_k)} \qquad (2)$$

is the amplitude of scattering of the macromolecule in vacuum ($\vec{r}_k$ – coordinates of the k^{th} atom of the molecule, $f_k(\vec{\mu})$ – its atomic factor) and $\Phi(\vec{\mu})$ – the amplitude of scattering of the substance with an average electronic density of the solvent ρ_0, uniformly filling all the volume of the macromolecule. The expression (1) is also valid in the presence of a short range order in the solvent if the structure of the latter does not undergo essential changes in the presence of the macromolecule (approximation of "solid bodies") [8].

For calculating $\Phi(\vec{\mu})$ we have modelled the protein molecule with a system of cubical blocks adjoining each other, the compact packing of which ensured the uniform density within the particle. To decrease the number of arrays in calculations the adjacent cubes along one of the coordinates (e.g., Z) were joined into parallelepipeds. Then

$$\Phi(\vec{\mu}) = \rho_0 \sum_j \phi(\vec{\mu}, a, b_j) e^{i(\vec{\mu}\vec{r}_j)}, \qquad (3)$$

where $2a$ is the rib of the cube (in our case $2a$ = 1.5 Å) b_j and $\vec{r}_j$ – the length of the j^{th} parallelepiped and coordinates of its center and

$$\phi(\vec{\mu}, a, b_j) = [8 \sin(\mu_x a) \sin(\mu_y a) \times \sin(\mu_z b_j)/\mu_x\mu_y\mu_z]$$

is the amplitude of scattering of the appropriately oriented j^{th} parallelepiped with a single electronic density [9]. Calculations by formulae (1)–(3), including averaging of $I(\vec{\mu})$ over random orientations of

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the vector $\vec{\mu}$, as well as calculations b_j and $\vec{r}_j$ for parallelepipeds filling the particle were done on the M-220 M (USSR) computer with a number of ALGOL-programs described in [6].

For comparison, the consideration of the solvent influence was also performed by a modification of the Langridge method [10]. In this case the volume of the protein molecule was modelled by a system of spheres connected with the centers of all non-hydrogen atoms, the volume of the k^{th} sphere $V_k = (4/3)\pi R_k^3$ being regarded as the sum of the "van der Waals volumes" [11] of the k^{th} non-hydrogen atom and of the hydrogen atoms joined to it. Due to the spherical symmetry of the amplitude of scattering from the sphere, the expression (1), averaged by random orientations of the vector $\vec{\mu}$, may be presented in a form suitable for Debye calculation

$$I(\mu) = \sum_{k,j} A_k(\mu) A_j(\mu) \frac{\operatorname{Sin} \mu r_{kj}}{\mu r_{kj}} \qquad (4)$$

where

$$A_k(\mu) = f_k(\mu) - 4\pi\rho_0 \frac{\operatorname{Sin}(\mu R_k) - \mu R_k \operatorname{Cos}(\mu R_k)}{\mu^3} .$$

3. Results and discussion

Calculations were done of X-ray diffuse scattering of native sperm-whale myoglobin (Mb) solutions in different solvents with electronic densities ρ_0 from 0.334 (water) to 0.42 (el/Å^3), approximately corresponding to the experimental interval of electronic densities reported in paper [7]. The Cartesian coordinates of 1260 non-hydrogen atoms of myoglobin were taken from paper [12], the hydrogen atoms were put into the atoms joined to them by chemical bonds.

Figs. 1 and 2 represent theoretical curves obtained respectively by the first and second methods, as compared with the experimental curves of paper [7]. Besides this, figs. 1 and 2 show "vacuum" Mb scattering curves, correspondingly calculated by formulae (1) and (4) at $\rho_0 = 0$.

It is evident from the figures that the indicatrices of scattering calculated by both methods sufficiently well reflect the experimental picture in a wide interval of angle scattering (up to $\mu \sim 1$): the plateau at $\mu = 0.2$–0.7 and the minimum at $\mu \approx 1$ for water; the

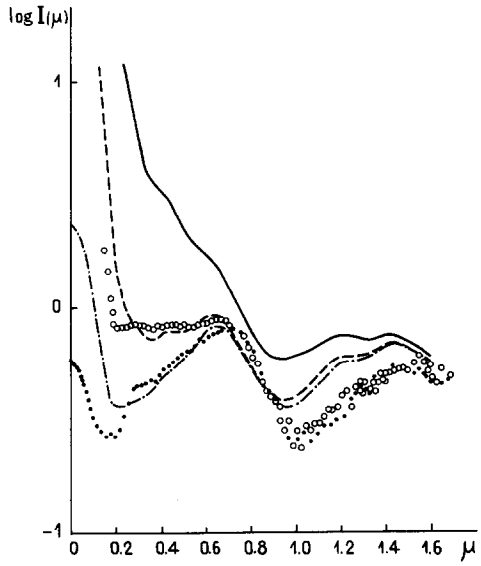

Fig. 1. Theoretical scattering curves, obtained by the first method; circles show experimental data [7]: —— "vacuum"; – – – and ○, $\rho_0 = 0.334$ (el/Å^3) (water); –·–·– and •, $\rho_0 = 0.41$ (el/Å^3).

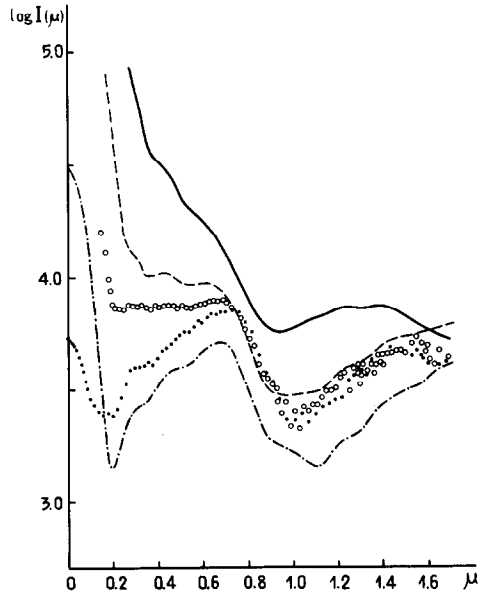

Fig. 2. Theoretical scattering curves, obtained by the second method, for details see legend to fig. 1.

minimum at $\mu = 0.2–0.3$, the maximum at $\mu = 0.7$ and the minimum at $\mu \approx 1$ for more dense solvents. At the same time the "vacuum" curves in the region of $\mu = 0.2–0.7$ essentially differ from the experimental ones (see also [1, 13]). The differences in the shapes of the theoretical and experimental curves at small angles of scattering are not major and can be eliminated by a more precise choice of the protein volume. In our case the volume of Mb was taken as ~22,500 Å^3.

Comparison of the theoretical curves obtained by the two methods shows that the "Langridge method" leads to considerably greater differences of scattering indicatrices in different solvents than found experimentally. In particular it does not describe the coincidence of curves for all solvents at $\mu > 0.7$, observed experimentally and described by our method. The divergence of the theoretical curves obtained by our method from the experimental ones at $\mu > 1$ probably can be explained by an incorrect account of the contribution of hydrogen atoms (see above).

Thus, this paper describes for the first time theoretical indicatrices of diffuse scattering of globular protein in agreement with experiment. Proceeding from this agreement the following conclusions can be made:

i) The dependence of the shapes of the curves of scattering on the solvent is mainly explained by the non-specific effect of the latter, reducing simply to a change of the average electronic density of the surrounding medium.

ii) The calculations taking into account the non-specific solvent influence explain the experimentally observed independence of scattering curves from the solvent at sufficiently great angles.

iii) The reason for the appearance of the plateau on the indicatrices of globular proteins in an aqueous medium [1, 2, 7] or the maximum in media with a greater electronic density [7] (including concentrated solutions or gels [14]) is the inhomogeneity of electronic density in the region of 10–20 Å. This follows from the fact that an increase of the electronic density of the medium leads to a decrease of scattering intensity at very small angles, while scattering at comparatively large angles ($\mu > 0.7$), as has been noted above, remains practically unchanged. The source of this inhomogeneity may be not only that postulated by Echols and Anderegg [14], a "tendency of the coiled polypeptide chains to pack at a more or less fixed distance apart", but also other general effects of large-scale peculiarities of globular protein structure, e.g. the occurrence of a hydrophobic core and hydrophilic shell.

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