EFFECT OF THE SECONDARY STRUCTURE OF GLOBULAR PROTEINS IN SOLUTION ON THE INDICATRIX OF X-RAY DIFFUSION SCATTERING

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1. Introduction

We have previously shown [1] that the indicatrix of X-ray diffusion scattering reflects the secondary structure of synthetic polypeptides in solution: α- and β-structures are characterized by the maximal intensities of scattering at $\mu \equiv (4\pi/\lambda)\sin\theta$, which are equal to 1.6 and 1.3 respectively, while in the irregular coil-like structure the maxima are absent in the investigated region $\mu \leqslant 2$. Studies on the indicatrices of X-ray diffusion scattering of four globular proteins are reported in this paper and it is shown that the scattering indicatrices of native proteins with a prevalence of α-helical structure (myoglobin, lysozyme) and β-structure (α-chymotrypsin, immunoglobin) are characterized by maxima at $\mu = 1.5$ and 1.35 respectively. Consequently, the correlation which was earlier established by us on synthetic polypeptides is also found for these globular proteins. A plateau exists at smaller scattering angles on the indicatrices of all the proteins investigated. The denaturation of myoglobin and α-chymotrypsin is accompanied by a more or less complete disappearance of the above features.

2. Materials and methods

Sperm whale myoglobin was isolated by chromatography on carboxymethyl-Sephadex [2], hen egg-white lysozyme was obtained by double recrystallization of the commercial preparation and was verified for homogeneity by electrophoresis in polyacrylamide gel and by chromatography on Amberlite IRC-50, bovine α-chymotrypsin was a commercial sample with ∼95% content of active centers (according to titration by cinnamoyl-imidazole), human immunoglobulin G was isolated from the γ-globulin specimen obtained by the alcohol method of Cohn by chromatography on DEAE-cellulose (DE-32, Whatman) in a 0.0175 M phosphate buffer, pH 6.3. The nativity of the prepations was tested by their optical activity. The experimental conditions are given in the legends to the figures. The scattering curves (except curve 3 in fig. 2) were plotted at room temperature (∼22 °C).

The curves of X-ray diffusion scattering were plotted in the region of scattering angles $2\theta = 1°–12°$ ($\mu = 0.15–1.85$) at $\lambda = 0.71$ Å (K_αMo). The experimental procedure has been described [1]. The difference of intensities of scattering by the solution and the solvent was 5–10% at large angles, so several tens of thousands of counts were gathered at every scattering angle to increase the statistical accuracy. As a result the maximal spread of points on the differential scattering curve did not exceed 20%.

3. Results and discussion

Differential scattering curves of the proteins investigated in the native (curves 1 and 2) and denaturated (curves 3) states are given in figs. 1 and 2. The curves are shown in ΔI, where ΔI is the difference of intensities of scattering by the solution and the solvent at an angle of 2θ (in counts per 400 sec).

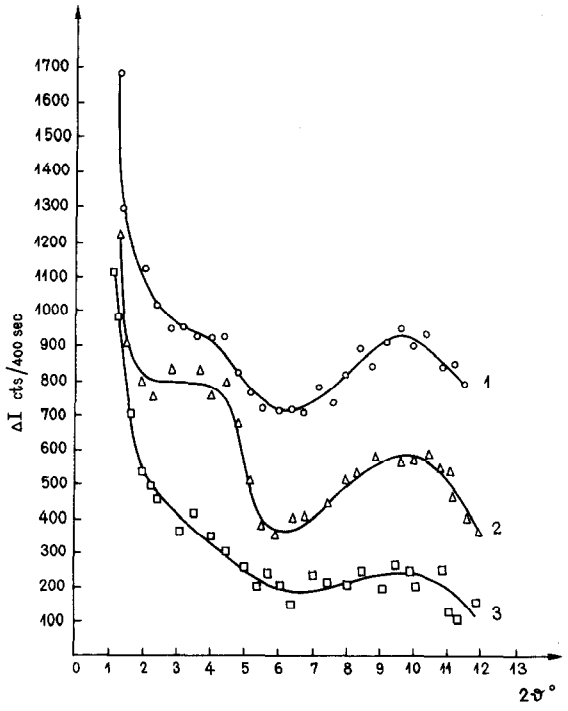

Fig. 1. Scattering curves:
(1) 7% hen egg-white lysozyme, aqueous solution 0.1 M NaCl, pH 3.2;
(2) 6% sperm whale myoglobin solution, phosphate buffer, pH 7.0;
(3) 3% sperm whale myoglobin, aqueous solution, pH 2.5.
The origin of the ordinate for curves 1 and 2 is shifted upwards by 100 and 500 counts per 400 sec correspondingly.

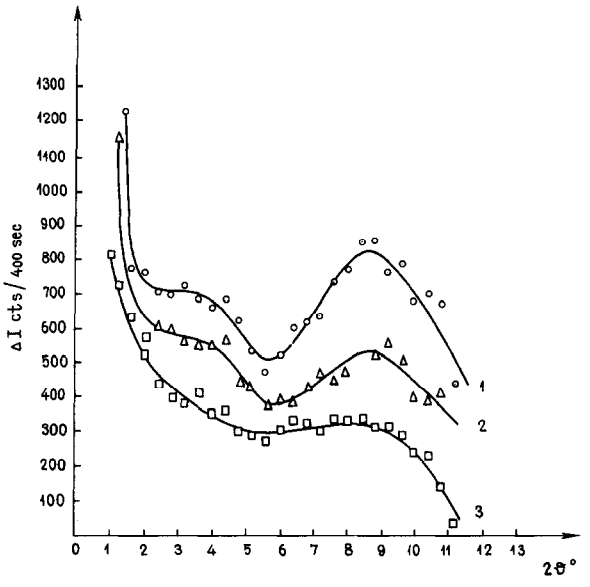

Fig. 2. Scattering curves:
(1) 8% human immunoglobulin G, aqueous solution, borate buffer, pH 7.5;
(2) 7% bovine α-chymotrypsin, aqueous solution, pH 6.0;
(3) 6% bovine α-chymotrypsin, aqueous solution, pH 1.5; 40 °C.
The origin of the ordinate for curves 1 and 2 is shifted upwards by 100 counts per 400 sec.

It is seen from fig. 1 that the scattering curves of native myoglobin (79% in α-helices; 0% in β-structure [3]) and native lysozyme (36% in α-helices; 8% in β-structure [4, 5]) are characterized by distinct maxima at an angle of $2\theta = 9.8° \pm 0.5°$ which corresponds to $\mu = 1.50 \pm 0.08$. It is also seen from fig. 2 that the scattering curves of native α-chymotrypsin (7% in α-helices; 26% in β-structure [6]) and native immunoglobulin G (mainly the β-structure [7]) have maxima at $2\theta = 8.7° \pm 0.5°$, i.e. at $\mu = 1.34 \pm 0.08$. Thus, the differences in the secondary structure of these proteins are directly reflected in the indicatrices of X-ray diffusion scattering though the difference of the scattering indicatrices is somewhat lesser than with synthetic polypeptides [1]. In the region of $2\theta = 2°–4°$ ($\mu = 0.3–0.7$) the scattering indicatrices of globular proteins have a rather clearly expressed plateau which is probably connected (cf. [8, 9]) with the packing effects in protein globules.

The scattering curve of sperm whale native myoglobin at $\mu \leqslant 1.8$ was earlier obtained with a high accuracy by Beeman [10]. The main features of our scattering curve (see curve 2 in fig. 1) are: the plateau at $\mu = 0.3–0.7$, the minimum at $\mu = 1.0$ and the maximum at $\mu = 1.5$ in keeping Beeman's curve [10]. The scattering curves of other globular proteins in the corresponding region were not previously investigated, with the exception of serum albumin for which a curve was obtained with a plateau at $\mu \simeq 0.3–0.7$, a minimum at $\mu \simeq 1.0$ and a maximum at $\mu \simeq 1.3$ [9].

Curves 3 in figs. 1 and 2 refer to denaturated myoglobin and α-chymotrypsin respectively. They show that the maxima on the scattering curves sharply weaken with a decrease of the secondary structure of these proteins as a result of their denaturation. Simultaneously the disappearance of the plateau is also observed, apparently connected with the packing effects in the native proteins.

Though the available experimental material is probably insufficient for final conclusions, it gives grounds for one to assume that an investigation of X-ray diffusion scattering at large angles may be a useful method of studying the secondary structure of globular proteins in solution. Inasmuch as a direct structural and not an indirect optical method of investigation is concerned, a further study of this problem seems to be called for.

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