IDENTIFICATION OF THE SECONDARY STRUCTURE OF POLYPEPTIDE CHAINS IN SOLUTION BY X-RAY DIFFUSION SCATTERING

A.I. GRIGORYEV, L.A. VOLKOVA, O.B. PTITSYN

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

and

Institute of High Molecular Compounds, Academy of Sciences of the USSR, Leningrad, USSR

Received 20 January 1971

1. Introduction

The study of X-ray diffusion scattering by macromolecules in solution was generally restricted to a region of small scattering angles in which the scattering indicatrix depends only on the size and shape of the macromolecule as a whole and not on the details of its conformational structure. At the same time, the maxima of intensity in the region of comparatively large scattering angles, which should contain information on the type of the conformational short-range order in macromolecules, were observed by some authors both for synthetic polymers [1, 2] and for globular proteins [3, 4].

The present paper is devoted to the identification of the secondary structure of polypeptide chains in solution by studying X-ray diffusion scattering at comparatively large angles. Synthetic polypeptides with an α-helical structure, β-structure and a non-regular (coil-like) structure in solution were investigated for this purpose. It is shown that α- and β-structures are characterized by the maxima on the scattering indicatrix in the region of $\mu \equiv (4\pi/\lambda)\mathrm{Sin}\,\vartheta$ corresponding to 1.6 and 1.3, while the scattering indicatrix of the non-regular structure has no maximum in this region.

2. Materials and methods

Na-salt of poly-L-glutamic acid (PGA), purchased from Koch-Light Laboratories, and converted into the acid form, hydrochloride of poly-L-lysine (PL), Ferak Company and Na-salt of poly-*S*-carboxymethyl-L-cysteine (PCMC), synthesized at the Institute of Protein Research, were investigated. The experimental conditions are given in the legends to figures. The scattering curves were plotted at room temperature ($\sim 22^{\circ}$C) except in specially stipulated cases.

The curves of the angle dependence of X-ray scattering intensity were plotted as described in [2] in the scattering angle regions of $2\vartheta = 2\text{–}13^{\circ}$ at $\lambda = 0.71$ Å (K_{α}Mo). Slit collimation of the source and the receiver was used. Collimation correction was not introduced since it is negligible in this scattering angle region (cf. [2]). To eliminate the difference in X-ray absorption by a solution and a solvent, two identical cells were filled one with the solution and the other with solvent. One of the cells was used as a sample and the other was placed directly before the counter and was used as an absorber (cf. [5]). Because the difference of the scattering intensities by the solution and the solvent at large angles was only a few percent, the differential scattering curve was obtained by gathering tens of thousands of counts for every angle which took up to 2,500 sec. The stability of the X-ray source was controlled and if necessary corrected every 400 sec. Thus, although the spread

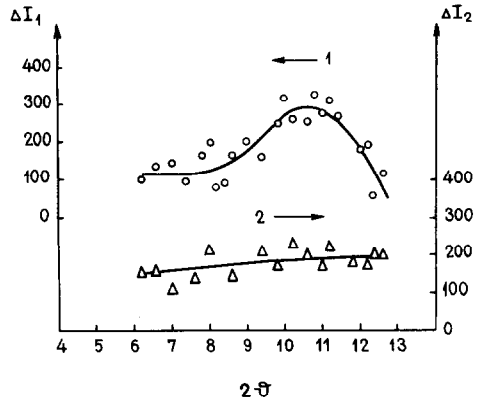

Fig. 1. 2% aqueous PGA solution scattering curves: (1) 0.2 M LiCl, pH 4.65; (2) 0.1 M LiCl, pH 7.0.

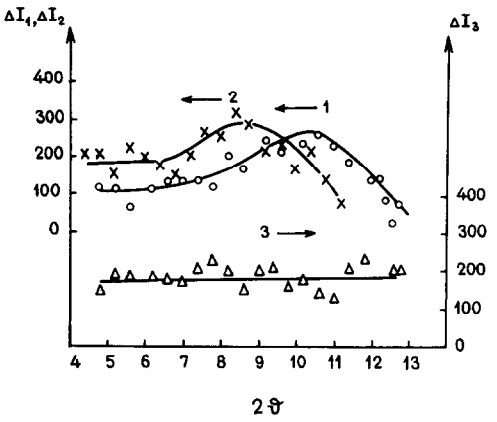

Fig. 3. 4% aqueous PL solution scattering curves: (1) 0.22 M LiCl, pH 10.8, 12–14°C; (2) 0.17 M LiCl, pH 11.5, after heating for 30 min at 50°C; (3) 0.22 M LiCl, pH 7.0.

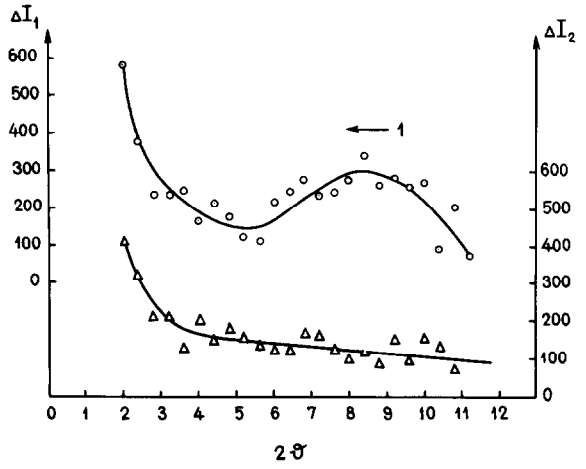

Fig. 2. 3% aqueous PCMC solution scattering curves: (1) 0.06 M NaCl, pH 4.5; (2) 0.06 M NaCl, pH 9.0.

of points on the differential curves sometimes reaches 30%, we succeeded in obtaining quite distinct differences between the scattering curves of helical, β-structural and coil-like macromolecules.

3. Results and discussion

Figs. 1–3 show the differential scattering curves of PGA, PCMC and PL in the coordinates of $\Delta I_k(2\vartheta)$ where ΔI is the difference of the scattering intensities of the solution and the solvent at an angle of 2ϑ (in counts per 400 sec) and k is the number of the appropriate curve. It is seen from figs. 1 and 2 that the scattering curves of PGA and PCMC in the uncharged state (curves 1) have definite maxima correspondingly at $2\vartheta = 10.5 \pm 0.5°$ and $8.5 \pm 0.5°$, i.e. at $\mu = 1.62 \pm 0.08$ and 1.32 ± 0.08. This difference of the scattering curves reflects the difference of the secondary structures of these polypeptides in the uncharged state: the α-helical structure for PGA [6] and the β-structure for PCMC [7]. With the ionization of PGA and PCMC accompanied by their transition to the coil-like state [6, 7] the maxima on the scattering curves disappear (see figs. 1 and 2, curve 2).

In order to ascertain that the observed differences in the scattering curves of uncharged PGA and PCMC are due to the difference of their secondary structure and not to the difference of the side chains, we also investigated the indicatrices of PL scattering which may have an α-, β- and coil-like structure depending on temperature and pH of the medium. Curves 1 and 2 in fig. 3 show that the non-ionized PL with an α-helical structure [8] at low temperatures is characterized by a maximum at $2\vartheta = 10.5 \pm 0.5°$, while after heating PL to 50°C accompanied by a transition to the β-structure [8], the maximum shifts to $2\vartheta = 8.5 \pm 0.5°$. The maximum on the scattering curve disappears with the ionization of PL effecting its transition to the coil-like structure [8].

Thus, the secondary structure of polypeptide

chains in solution directly manifests itself on the indicatrices of X-ray diffusion scattering. It will be shown in a following communication that the correlation established by us between the indicatrix of scattering and the secondary structure is also valid for globular proteins.

Acknowledgements

The authors express their thanks to B.A. Fedorov for valuable discussions of the work, to K.Kh. Zikherman and T.M. Mosina for synthesizing PCMC, to T.N. Nekrasova and N.G. Illarionova for help in preparing the samples for the investigation, and to T.B. Kuvshinkina and A.G. Raiher for translating the manuscript into English.

References

[1] W. Wunderlich and R.G. Kirste, Ber. Bunsengesell. Phys. Chem. 68.(1964) 645.
[2] A.I. Grigoryev, L.A. Volkova and O.B. Ptitsyn, Vysokomolekul. Soyedin. B11 (1969) 232; A12 (1970) 1363.
[3] G.H. Echols and J.W. Anderegg, J. Am. Chem. Soc. 82 (1960) 5085.
[4] W.W. Beeman, in: Small-Angle X-ray Scattering, ed. H. Brumberger (Gordon and Breach, New York, London, Paris, 1967) p. 197.
[5] O. Kratky, J. Pilz and P.Y. Schmitz, J. Colloid Interface Sci. 21 (1966) 24.
[6] P. Doty, A. Wada, J. Yang and E. Blout, J. Polymer Sci. 23 (1957) 851.
[7] N.G. Illarionova, I.A. Bolotina, B.Z. Volchek, V.I. Kalikhevich, Yu.V. Mitin, O.B. Ptitsyn and A.V. Purkina, Biofizika 11 (1966) 762.
[8] B. Davidson and G.D. Fasman, Biochemistry 6 (1967) 1616.