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ELECTRON-MICROSCOPIC INVESTIGATION OF THE SUPRAMOLECULAR STRUCTURE OF POLY-γ-BENZYL-*L*-GLUTAMATE

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The high resolving power of the electron-optical method makes it possible to use it for purposes of direct structural analysis. Therefore electron microscopy finds especially broad application in the study of the structure of synthetic and natural polymers, which is connected with the unique possibility of direct observation of the mutual arrangement and shape of large molecules whose dimensions lie within the resolving power of modern electron microscopes. In turn, this advantage of electron microscopy makes it possible to use it for studying the processes of formation of secondary structures from individual large molecules. This approach ([1]) was used in the present work to investigate the structure of poly-γ-benzyl-*L*-glutamate (PBG), whose molecular structure has been studied in detail by other methods ([2]). As the object we chose a synthetic polypeptide whose molecular chains, depending, for example, on the solvent used, can assume the form either of ordered α-helices or of a disordered statistical coil. This feature of the molecular chains of PBG, associated with the possibility of their existence in two different forms, which is characteristic in particular of natural proteins, was used in the present work to study the connection between the initial conformation of the macromolecules and the character of the macrostructures arising in the two cases.

The structure of PBG was investigated in a YEM-5U type electron microscope. To prepare the specimens, solutions of the polypeptide in various solvents with concentrations from 0.01 to 0.1% were used. A drop of solution was placed on collodion and quartz supports, and after evaporation of the solvent the resulting PBG structures were examined in the electron microscope. Benzene, chloroform, and trichloroacetic acid were chosen as solvents. In choosing the solvents we proceeded from the known principles that in nonpolar solvents (benzene, chloroform) PBG chains exist in the form of α-helices (18/5). In polar solvents, for example dichloroacetic acid, as a result of interaction of the dissolved polypeptide with solvent molecules, the chains acquire the form of a statistical coil. Our measurements of the values of the specific rotation $[\alpha]_D$ of polypeptide solutions in trichloroacetic acid with small additions of water, as well as in benzene and chloroform, and the change in $[\alpha]_D$ with change in

Schematic drawing of the transversely striated structure of polypeptides

Figure 1: Schematic drawing of the transversely striated structure of polypeptides

temperature indicate that, indeed, in the case of solutions in benzene and chloroform the PBG macromolecules are in the form of ordered α-helices, whereas in trichloroacetic acid they are in the form of a statistical coil. In studying the structures arising from PBG solutions in chloroform and benzene, it was found that at low concentrations of the order of 0.01% the polypeptide chains aggregate, forming an ordered supramolecular structure of the fibrillar type. In an electron-microscopic photograph (Fig. 1a, see insert, p. 871), individual elementary fibrils or their aggregates are clearly visible, the smallest transverse dimensions of which are of the order of 100 Å.

To the article by P. V. Kozlov, N. V. Bakeev, R. K. Shmyreva, and A. B. Zezin, p. 905

Fig. 1. Electron-microscopic photographs of structural formations of poly-γ-benzyl-L-glutamate: *a*—from a 0.01% solution in chloroform; *b*—from a 0.01% solution in trichloroacetic acid; *c*—from a 0.1% solution in chloroform; *d*—from a 0.1% solution in chloroform, treated with a mixture of chloroform and hexane 1 : 1; *e*—from a 0.1% solution in chloroform, shadowed with chromium in a direction parallel to the direction of the folds; *f*—the same in the perpendicular direction.

The electron diffraction pattern obtained for such structures indicates the amorphous state of the specimen. The formation of such aggregates is apparently analogous to the process of ordering of amorphous polymers (1). For a large number of amorphous polymers, the existence of similar ordered bundles of chains has been found, the causes of whose appearance are associated with the fluctuation character of swarm formation in liquids. The results of the study of structures arising in the case of polypeptide solutions in trichloroacetic acid lead to the same conclusion. In this case one can also observe the appearance of ordered fibrils very similar in their form to the aggregates observed for solutions in benzene and chloroform (Fig. 1b). This indicates that the appearance of the observed ordered structures is not connected with any conformational transitions of the polypeptide molecules in solution, i.e., with the formation of α-helices or disordered coils, but is due to the long-chain nature of the PBG polymer molecules.

Fig. 2. Schematic representation of the transversely striated structure of polypeptides

As the concentration of the solutions under study increases and, consequently, the pattern of structure formation becomes more complex, interesting features and differences are observed in the behavior of the polypeptide depending on the solvent used. In the case of polypeptide solutions in chloroform and benzene, i.e., when the PBG chains form α-helices, at concentrations of about 0.1% an ordered aggregation of fibrils is observed, leading to the appearance of structures with a well-pronounced transverse striation (Fig. 1c). Morphologically, such a structure very much resembles the transversely striated structures previously found for a large number of fibrillar proteins (3). However, in the case of PBG the magnitudes of the repeat periods of the stripes are not constant and usually, for different specimens, and sometimes even for the same specimen, fluctuate within the range from 1000 to 4000 Å. To clarify the nature of the structure of the transverse striation, the method of etching such structures directly on the support and the method of shadowing were used. Etching was carried out with a mixture of chloroform and hexane in a ratio of 1:1 for one minute. In Fig. 1d one of numerous electron micrographs of etched specimens is presented. It is evident from the photograph that the transverse striation is formed from individual elementary fibrils, analogous to the fibrils arising from a dilute solution. In the same photograph one can observe how individual fibrils bend regularly, forming a smooth zigzag. The same picture is observed when examining preparations shadowed with chromium. Figures 1d, e show micrographs of striated structures shadowed with chromium in two mutually perpendicular directions. When shadowing along the direction of the stripes, it is evident that the striated structure is formed from individual densely packed fibrils (Fig. 1d). When examining regions shadowed in the direction perpendicular to the direction of the transverse stripes (Fig. 1e), it may be concluded that the plane of the zigzag does not coincide with the plane of the support. This can be judged from the appearance of shadows at the bends, and the different length of the shadows indicates nonuni-

height of neighboring folds. On the basis of these results, the transversely striated structure can be represented by the scheme shown in Fig. 2.

It is quite obvious that the appearance of alternating light and dark bands on unstained specimens will occur even in the case when the thickness of the fold is the same in all regions. The striation arises because, for inclined or perpendicular positions of the zigzag plane, absorption and scattering of the electron beam at the convexities and depressions, on the one hand, and on the slopes, on the other, will naturally be different. This, in turn, will lead to the appearance of the transverse striation that we observe in the electron microscope.

A different character of fibril aggregation is observed in the case when PBG chains exist in the form of a statistical coil. In studies of concentrated solutions of the polypeptide in trichloroacetic acid or in benzene and chloroform at high temperatures, when thermal melting of the α-helices occurs, structures with the characteristic transverse striation cannot be detected. In these cases, fibril aggregation is random in character and, as a result, "felt-like" structures arise, analogous to those shown in Fig. 1b.

Thus, from the results set forth above, it may be concluded that the formation of transversely striated structures is associated with the α-helical form of the polypeptide molecules.

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REFERENCES CITED

[1] V. A. Kargin, N. F. Bakeev, *Koll. zhurn.*, **19**, No. 2, 133 (1957).
[2] C. H. Bamford, A. Elliott, W. E. Hanby, *Synthetic polypeptides*, 1956.
[3] *Proteins*, edited by G. Neurath and K. Bailey, **3**, IL, 1956.

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