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Abstract

Full Text

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THE EFFECT OF FIXING POLYPEPTIDE CHAINS IN TWO CONFORMATIONS

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A polypeptide chain ([1,2]), depending on the conditions, may have the conformation either of a rodlike helical spiral or of a randomly coiled globule (Random Coil). Both types of conformation are labile and capable of mutual transformation—spiral globule (Helix—Coil Transitions). This transformation occurs, for example, under the influence of changes in temperature, pH, the ionic strength of the solution, and the nature of the solvent. The spiral and the globule are two limiting cases; however, under appropriate conditions conformations of an intermediate type may exist, for example, a partially unfolded spiral or a partially spiralized globule.

Fig. 1. Effect of fixing gelatin in the spiral conformation *(A)*; the same in the globule conformation *(B)*

The conformation of a polypeptide chain in solution is readily and unambiguously determined by measuring the specific optical rotation, and by measuring the dispersion of the optical rotation in the ultraviolet region of the spectrum it is possible to calculate the degree of helicity in percent ([1]). The conformational transformation spiral globule has an extremely strong effect on the physicochemical properties of the solution. This is especially clearly manifested in gelatin solutions. The helical form of gelatin macromolecules, which exists at temperatures up to 20–25° (Fig. 1A, 1), is the cause of the structural viscosity and gelation of its solutions. These phenomena disappear when the temperature is raised and, beginning at 35–40°, a gelatin solution has the properties of a Newtonian liquid. In the interval 25–35° the spiral globule transformation occurs, accompanied by a sharp fall in the specific rotation (Fig. 1A, 1).

It was of interest to attempt to fix gelatin macromolecules in their spiral conformation and in the globule conformation. Interesting experiments on obtaining gelatin in the globular form were described as early as 1949 ([3]).

In the experiments described below, "Kodak" gelatin was purified and brought to the isoelectric state by Loeb' s method ([4]). The rotation of the plane of polarization was measured on a polarimeter with a reading accuracy of 0.05°. Chromium alum, chemically pure, was used as the fixing agent. The procedure and results of the experiments were as follows.

To avoid gelation, a dilute 0.25% solution was used.

gelatin. To 10 ml of a 1% gelatin solution, 20 ml of water at 30° was added; the solution was cooled to 15°, and 10 ml of a 1% solution of chromium alum, likewise cooled to 15°, was added to it. The solution was kept at 15-16° for two days and was then subjected to ordinary dialysis in a cellophane bag until the reaction for the ion SO_4^{2-} disappeared. After dialysis, the solution was brought back to its initial concentration by evaporating water through the walls of the bag until it reached its original weight. In parallel, a 0.25% solution of pure gelatin was prepared and kept at 15-16°. The dependence of the specific rotation on temperature for both solutions (Fig. 1) clearly shows the effect of fixation of the helical conformation of gelatin (curve 2). The slight decrease in specific rotation beginning at 30° is apparently explained by an incomplete reaction. It is interesting that after dialysis the fixed gelatin was completely colorless.

In the next experiment, a gel-forming 3% gelatin solution was taken. To 10 ml of a 6% gelatin solution, 10 ml of a 1% solution of chromium alum was added. The solution was prepared at 40° and kept for 24 hours at the same temperature in a thermostat. By maintaining the temperature at 40°, we kept the gelatin in the coil conformation throughout. If, after the addition of chromium alum, the solution is immediately cooled to room temperature, a gel is obtained that does not melt even at 100°.

In parallel, a 3% solution of pure gelatin was kept at 40°. As a result, it was found that the 3% gelatin solution containing chromium alum had lost its ability to gel upon cooling to room temperature (18°). This already indicated fixation of the molecular structure in the form of a coil. Objective proof of this effect was obtained after recording the curves for the dependence of specific rotation on temperature, shown in Fig. 1B. Here curve 1 refers to pure gelatin, and curve 2 to gelatin treated with chromium alum. It is clearly seen that, upon lowering the temperature, only a slight rise of curve 2 is observed. After curve 2 had been recorded, the solution was subjected to ordinary dialysis, as in the preceding experiment. In this case, removal of the free ions K^+, Cr^{3+}, and SO_4^{2-} led to an increase in pH from 4.4 to 4.9, which should have caused an increase in the basicity of the chromium alums bound to the gelatin and thereby enhanced the effect of fixation of the "coil." This is excellently confirmed by curve 3 in Fig. 1B, which may practically be regarded as a straight line. It is interesting that fixation of the coil is accompanied by a noticeable drop in specific rotation in the region 25-45°. This fact requires special study and is probably connected with the very mechanism of attachment of chromium complexes to the coil-

like gelatin molecule. Also important is the circumstance that after dialysis the solution retained the color of chromium alum, whereas in the preceding experiment it was colorless.

The effects of fixation of the structure of gelatin molecules described here may serve as new evidence for the correctness of the view of their conformation as a helix at temperatures up to 20° and as a coil at 40°. Furthermore, it is evident that the helix and the coil possess, in their structure, reactive groups situated so close to one another that reaction with chromium complexes gives rise to intramolecular cross-linking of the macromolecules. It may also be concluded that the structure of the randomly coiled coil is apparently sufficiently compact —at any rate, sufficiently so as is necessary for intramolecular cross-linking to occur.

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Note: Figure translations are in progress. See original paper for figures.