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ON THE MECHANISM OF MUTAROTATION AND GEL FORMATION IN GELATIN SOLUTIONS

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The study of X-ray diffraction patterns of collagen and of the physicochemical properties of its solutions has made it possible to establish the molecular structure of this fibrillar protein. The collagen molecule is formed by three polypeptide chains helically twisted relative to one another, each of which, in turn, is also coiled into a helix of the poly-L-proline II type ([1]). Denaturation of collagen in solution, for example by heating to 40°, leads to the disintegration of its molecule into individual chains (gelatin), which at this temperature have the conformation of a statistical coil ([2]).

Owing to the helical conformation of the chains, the molecules of native collagen make a significant contribution to the optical activity of its solutions. In the denatured state the optical activity is due only to the presence of asymmetric carbon atoms, and the values of the specific levorotation in this case are considerably lower than for native collagen ([2,3]). It is well known, however, that when gelatin solutions are kept in the cold, the levorotation increases (mutarotation) to values close to those for native collagen. This is one of the reasons that allows many authors to speak of the possibility of restoration of collagen-like structures under these conditions ([2–5]). The mechanism of this process, considered in our work, is much less clear.

In the work, gelatin of the "Photo" grade was used. The method of protein purification was described earlier ([3]). Aqueous protein solutions (pH 4.9) and solutions in 0.2 M citrate buffer (pH 4.0) were studied. Measurements of the optical rotation of gelatin solutions with concentrations from 0.05 to 3.0 g/100 ml of solution were carried out on a "Zeiss" polarimeter with a reading accuracy up to 0.01° in monochromatic light ($\lambda = 546$ mμ), and on a spectropolarimeter with a reading accuracy up to 0.002° for solutions with concentrations from 0.016 to 0.128 g/100 ml of solution. Thermostated polarimetric cuvettes of length 2.0 and 0.5 dm were used. Thermostating was carried out with an accuracy of

Fig. 1

Figure 1: Fig. 1

$±$0.05°.

First, the dependence of the specific optical rotation of gelatin on time was studied for solutions of various concentrations (from 0.016 to 3.0 g/100 ml of solution) after rapid cooling from 40 to 6–8°. In order to eliminate the possible influence of different ionization states of gelatin molecules over a wide range of solution concentrations ([6]) on the kinetics of mutarotation, analogous experiments were carried out in 0.2 M citrate buffer at pH 4.0. On the basis of these data, approximate values of the initial rates of change of rotation in the process of renaturation were obtained by graphical extrapolation. These results are presented in Figs. 1 and 2. It is evident from the figures that the rate of mutarotation in gelatin solutions depends substantially on the protein concentration. It is known, however ([2]), that the final values of specific rotation for different concentrations differ very little. This may indicate the absence of any fundamental difference in the renaturation processes occurring at different concentrations.

Increasing the concentration of protein in solution naturally increases the viscosity of the system, which, in turn, hinders the course of relaxation processes and should lead to a decrease in the rate of helix formation and, consequently, of mutarotation. The results obtained (Figs. 1 and 2), however, contradict this assertion. The increase in the rate of mutarotation with increasing concentration of protein in solution indicates that the process of renaturation does not proceed, as was assumed ([7]), through the stage of formation of a helix by an individual chain, which, in the helical conformation, apparently cannot exist at all in gelatin solutions, but rather attests to the decisive role of intermolecular interactions in these processes. The rate of the process is then determined by the probability, increasing with concentration, of effective collisions of different molecules in the gelatin solution. Evidently, effective collisions will be those that lead to the formation, by segments of three different chains, of a region with the collagen-like conformation of a triple helix, which is what brings about the increase in levorotation.

Fig. 1. Dependence of the initial rate of mutarotation on the concentration of gelatin in solution at 6°. *a* —in aqueous solution, pH 4.9; *b* —in 0.2 *M* citrate buffer, pH 4.0

If the process of helix formation is indeed intermolecular, it is natural to suppose that renaturation will proceed faster in the case of chains previously linked by cross bonds. Recent experiments with α- and γ-gelatins ([8]) confirm this supposition well. α-Gelatin is an individual polypeptide chain having the conformation of a statistical coil at the temperature of collagen denaturation. γ-Gelatin consists of three chains linked to one another by transverse covalent bonds, and at

Fig. 2

Figure 2: Fig. 2

this temperature likewise does not form ordered conformations. Under conditions favoring the formation of collagen-like structures, the rate of mutarotation in the case of γ-gelatin, as was to be expected, proved higher.

The dependence we obtained of the initial rate of mutarotation on concentration, as well as the linear increase of the rate with increasing molecular weight of gelatin in solution ([9]), permits the conclusion that the number of regions in which a triple helix can arise is sufficiently large and that these regions are not unique; i.e., a triple helix can exist when three individual chains are displaced relative to one another in comparison with their arrangement in the native collagen molecule.

Fig. 2. Dependence of the initial rate of mutarotation on the concentration of gelatin in 0.2 M citrate buffer, pH 4.0, at a temperature of 8° for low protein concentrations

The results obtained by us and the assumptions put forward in connection with them are in considerable agreement with recently published data ([10]) and in many respects explain the behavior of gelatin solutions and gels.

Only segments of three separate chains take part in the formation of the helical collagen-like region. The remaining free parts of these polypeptide chains, in turn, become helical with the participation of other chains, which ultimately leads to the formation in solution of aggregates spatially linked by regions of collagen-like triple helices. This explains why, in cold solutions of various gelatins, highly asymmetric formations (in contrast to solutions of native collagen) or individual polymer chains are not detected; instead, large symmetric aggregates are always present ([11,12]), rapidly formed upon cooling of the solution and practically not dissociating upon dilution. At the same time, the values of specific levorotation in gelatin solutions are always lower than the values characteristic of native collagen, since some part of each chain remains disordered. Such aggregates have previously also been regarded as microgels, and it has been assumed that they are stabilized in the same way as gelatin gels ([11]).

At sufficiently high concentrations of gelatin in solution and at the corresponding temperature, spatial cross-linking due to helical regions formed by segments of three chains, in our opinion, determines to a considerable extent the formation and stabilization of the gel throughout the entire volume. This is consistent with the linear dependence between the square root of the shear modulus and the specific rotation of gelatin gels corresponding to a given temperature ([13]).

The proposed mechanism of the gelatin renaturation process also explains the formation of so-called globular gelatin upon rapid cooling of dilute solutions from 40 to 5° and subsequent evaporation of the solutions at low temperature

([14]). An increase in the concentration of the solution during evaporation in the cold should, at some point, lead to the formation of a spatially cross-linked gel, from which, upon further evaporation, a film would be obtained. It turned out, however, that during such evaporation up to concentrations corresponding to gel formation, no gel is formed; with further evaporation of the solution a powder is obtained, partially soluble again at reduced temperature. This powder was called globular gelatin. It was believed that, owing to the rapid cooling of such solutions, it had been possible to fix a statistical coil. In reality, during evaporation, stable aggregates are formed in the solution, fixed by regions of triple helices. Therefore, upon evaporation of such solutions, not a film but a powder is obtained, consisting of aggregates that were present in the solution. Since the aggregates are stable in cold solution, under the same conditions they pass back into solution comparatively easily, whereas in dissolving gelatin films one must denature by heating the collagen-like regions that mainly stabilize the swollen film. If the concentration of a solution obtained in the cold from globular gelatin is sufficiently high, then upon heating to 12–15° (when the triple helix of the collagen type still exists) a gel may form. This phenomenon is associated with "annealing" of the helical structure and corresponds to the known fact that more perfect collagen-like structures are formed when gelatin solutions are held not constantly in the cold, but alternately at 5 and 20° ([5]).

Thus, the rate of mutarotation in gelatin solutions depends substantially on the protein concentration over a wide concentration range. The increase in levorotation in gelatin solutions with time is due to the formation, by segments of three separate polypeptide chains, of collagen-like helical regions.

On the basis of the results obtained, it may be assumed that the helix formation of segments of three individual gelatin chains in solution and their formation of a triple helix proceed simultaneously.

After the formation, by segments of three gelatin molecules, of a section of a triple collagen-like helix, the remaining disordered parts of these molecules can then spiralize with segments of other chains. This process determines the stabilization of aggregates in gelatin solutions and, essentially, the spatial fixation of its gels.

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