PHYSICAL CHEMISTRY

V. P. MERZLOV, V. A. PCHELIN

1964

SovietRxiv

Abstract

Full Text

PHYSICAL CHEMISTRY

V. P. MERZLOV, V. A. PCHELIN

ON THE QUESTION OF THE ROLE OF IONOGENIC GROUPS IN CONFORMATIONAL TRANSFORMATIONS OF GELATIN

(Presented by Academician P. A. Rebinder, 31 I 1964)

It is well known that many physicochemical and biological properties of protein molecules—native ones, as well as synthetically obtained models—depend to a large extent on the acid-base equilibrium, i.e., on pH.

At present we have more detailed ideas about the nature of this dependence, and nevertheless it requires further investigation. In the case of synthetic polypeptides (polyglutamic acid and polylysine) it has been shown that a change in pH causes conformational changes of their molecules of the helix-coil type ([1,2]). This is readily detected by the method of measuring optical rotation. Also known is a very interesting dependence of the optical rotation of aqueous gelatin solutions on pH. However, the available literature data do not permit an unambiguous explanation of this dependence ([3,4]). In considering the phenomenon of optical activity of gelatin, one should bear in mind not only the presence of asymmetric carbon atoms in the polypeptide chain, but also the possibility of a change in optical activity as a result of a change in the form (conformation) of the polypeptide chain of macromolecules, which may be caused by the influence of a changed number and ratio of electrically charged functional groups.

The change in the specific rotation of gelatin solutions and gels with time, the process of so-called mutarotation, has often been explained by the formation in gelatin solutions and gels of crystal-like formations. As we have shown, "mutarotation" is not associated with processes of structuring of the system ([5]); nevertheless, it must be taken into account in measurements. In the present work an attempt is made to relate the conformational transformations of gelatin to the pH and ionic strength of the solution, taking into account temperature and time (mutarotation).

In the work we used "Photo"grade gelatin, purified and brought to the isoelectric state by Loeb' s method ([6]). The procedure for preparing the solutions has been described previously ([5]). Dilute solutions were studied, with concentration $C = 0.26$ g/100 ml of solution.

Optical rotation was measured on a Zeiss polarimeter with a reading accuracy of 0.01° in monochromatic light ($\lambda = 546$ mμ). For the work, thermostated

Fig. 1 and Fig. 2: graphs of the dependence of specific optical rotation on pH.

Figure 1: Fig. 1 and Fig. 2: graphs of the dependence of specific optical rotation on pH.

polarimetric cuvettes 2.0 and 0.5 dm long were used. Thermostating was carried out with an accuracy of ±0.05°.

The dependence of the specific optical rotation of gelatin solutions on pH was measured in aqueous solutions and in 1 M sodium chloride solutions at 6 and 37°. The pH of solutions kept at 6° was brought to the required values beforehand at room temperature.

The influence of the pH of the medium on the optical activity of gelatin solutions, both in aqueous solutions and in 1 M sodium chloride solutions, for different conformational states of its molecules was studied at different temperatures: 1) At a temperature of 37°, when the gelatin molecules are in the conformation of a statistical coil. 2) At a temperature of 6°. Che

after 10 min had elapsed following attainment of this temperature by solutions preliminarily heated to 40°. In this case, apparently, only certain primary processes of helix formation have time to occur, and only an imperfect structure is obtained (3). At a temperature of 6°, after the solutions had been kept for 150 h at this temperature, when the main renaturation processes* associated with the formation of highly ordered helical structures had already taken place.

The results of these measurements are presented in Figs. 1 and 2.

As is seen from Fig. 1 *I* and Fig. 2 *I*, at a temperature of 37° the specific rotation is practically independent of the pH and ionic strength of the solution. The viscosity, however, studied under analogous conditions (7) (determined in this case only by the asymmetry of individual molecules), depends substantially on pH. Thus, changes in the asymmetry of gelatin molecules in solution that are not associated with helical conformations do not affect the specific rotation. The optical activity in this case ($[\alpha]_{546}^{37^\circ} = -150$ to -160°) is due only to the presence of asymmetric carbon atoms in the gelatin molecule.

Fig. 1. Dependence of the specific optical rotation on pH for aqueous gelatin solutions; $C = 0.26$ g/100 ml of solution: *I*—at 37°, *II*—at 6° after 10 min following preliminary heating to 40°; *III*—at 6° after 150 h of standing at this temperature.

Fig. 2. Dependence of the specific optical rotation of gelatin in 1 *M* NaCl; *I*, *II*, and *III*—as in Fig. 1.

The curve of the dependence of the specific rotation on pH at reduced temperature (6°) is presented in Fig. 1 *II*. The maximum negative value $[\alpha]_{546}^{6} = -250^\circ$ at pH $= 5$ is associated with the maximum rate of mu-

* The term "renaturation" (restoration of collagen-like structures), recently used

in connection with the aging of gelatin solutions in the cold, evidently correctly reflects the state of the molecules in such solutions, since many of their physicochemical characteristics and the X-ray patterns of films dried at reduced temperature are entirely identical with those for native collagen.

rotation in the isoelectric state (^{3}) and, consequently, the formation of a more perfect helical conformation. In the preceding paper (^{6}) we already stated that changes in the optical activity of gelatin solutions at temperatures below 35° depend mainly on the conformational state of its molecules. Therefore, a sharp change in specific rotation when the pH is changed to either side of the isoelectric point indicates disruption of the intramolecular structure. The cause of disruption of the helical structure of the molecules is evidently electrostatic repulsion of like charges on the molecule. This is confirmed by experiments carried out in 1 M sodium chloride solutions. From Fig. 2 II it is evident that, in a solution with increased ionic strength owing to shielding of the protein charges by electrolyte ions, in this case no maximum of levorotation in the isoelectric state is observed.

Next, the dependence of the specific rotation of aqueous gelatin solutions on pH was investigated at a temperature of 6° after 150 hours of holding at this temperature (Fig. 1 III). In this case the main processes of helix formation had already ended (^{8}), and therefore, by comparing the results represented by curves I—II and I—III, one can judge the influence of the total charge of gelatin molecules on their ability to restore highly ordered (collagen-like) conformations. As is seen from Fig. 1 III, renaturation processes proceed actively at almost all pH values, except for a narrow region at pH 3. This pH region evidently corresponds to the conditions most unfavorable (from the standpoint of electrostatic interactions) for the formation of helical structures. However, under the given temperature-time conditions helix formation occurs even at the above-mentioned pH values; the specific levorotation at pH 3.2 is equal to −257°, which is considerably above the level characteristic of the statistical coil ($[\alpha]_{546}^{37^\circ} \simeq -160^\circ$). When the charges on the gelatin molecule are shielded, i.e., in solutions with increased ionic strength (1 M NaCl), renaturation of helical structures proceeds to a considerable extent at all pH values, as indicated by the high values of levorotation (Fig. 2 III).

Thus, measurements of the specific rotation of aqueous gelatin solutions at elevated temperature show that the optical rotation at 37° is practically independent of pH and ionic strength of the solution and is determined by the optical activity of asymmetric atoms in the molecule.

The data obtained by measuring the optical activity of solutions at reduced temperature as a function of pH, ionic strength of the solution, and renaturation time show that the conformation of gelatin molecules in solution at 6° (a temperature corresponding to the ordered conformation) depends on the magnitude of the total charge on the molecule and on the renaturation time. The results of measuring the specific rotation of gelatin solutions at 6° after 150 hours of holding in the cold show that significant disruptions of the structure of the molecules under the indicated conditions occur only in a narrow range of pH values 3. In 1 M sodium chloride solutions, helix formation proceeds to a considerable extent at all pH values of the medium.

Moscow State University
named after M. V. Lomonosov

Received
27 I 1964

CITED LITERATURE

[1] P. Doty, A. T. Wada et al., J. Polymer Sci., **23**, 851 (1957).
[2] J. Applequist, P. Doty, Abstracts, Am. Chem. Soc., Meeting, San—Francisco, April, 7—11, 1958.
[3] E. O. Kraemer, J. R. F. Fanselow, J. Phys. Chem., **32**, 894 (1928).
[4] J. Bello, H. Bello, J. R. Vinograd, Biochim. et biophys. acta, **57**, 214 (1962).
[5] V. A. Pchelin, V. N. Izmailova, V. P. Merzlov, Vysokomolek. soed., **5**, No. 9, 1429 (1963).
[6] Zh. Loeb, *Proteins and the Theory of Colloidal Phenomena*, 1933, p. 52.
[7] A. G. Ward, J. Photogr. Sci., **9**, 56 (1961).
[8] W. F. Harrington, P. H. Hipel, Arch. Biochem. and Biophys., **92**, 100 (1961).

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