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Helix-Coil Transition of Albumin-Bovine and Gelatin

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Viscosity, hydrogen ion equilibria and specific optical rotation of albumin-bovine and gelatin in 8 M urea solution have been measured. The shape of a protein molecule easily changes by changing pH of the medium. Reduced viscosity increases with increasing pH of the medium upto a certain limit. After this limit, the viscosity decreases with the increase of pH. This is only because, at a certain pH value the concentration of ions in the solution increases sharply and they are attracted by the protein ions. This causes the screening of the chain charges, decreasing electrostatic repulsion and resulting in partial coiling of the protein molecules and hence in a decrease in viscosity of the solution. The different proteins have different pH region for helix-coil transition. Albumin-bovine and gelatin undergo helix-coil transition in the pH range of 6 and 3-4, respectively. Helix-coil transition takes place only in the states of denaturation.

Key Words: Helix-Coil transition, Albumin-bovine, Gelatin.

INTRODUCTION

The confirmations of polypeptide chains stabilized by hydrogen bonds are stable only under certain conditions. A change of temperature, solvent, pH of a medium leads to order-disorder transitions, to the transformation of regular conformation of the chain to a random coil. Dody[1,2] has found that the helix-coil transition are very sharp and similar to phase transition.

High concentration of urea solution causes an unfolding of proteins by weakening the hydrophobic bonds that maintains the ternary structure. The change in protein conformation leads to a less compact molecule with a large viscosity than the native protein[3].

Proteins have the helical structure in native state. Each peptide bond of a protein participates in the hydrogen bonding. This confirms maximum stability. The hydrogen bondings produce a regular coiled arrangement, called helix. Denaturation of proteins ruptures hydrogens, hydrophobic and electrostatic bonds, but not peptide or sulphide bonds. Due to denaturation, helical structure of the protein changes into the random coil. This phenomenon is understood as helix-coil transition[4]. So, in helix-coil transition, viscosity will also be affected as latter depends upon the shape and size of the molecule[5]. Interaction of ribonucleic acid with albumin-bovine and casein have already been communicated[6,7]. In the present study, the helix-coil transition of albumin-bovine and gelatin have been reported.

EXPERIMENTAL

Albumin-bovine and gelatin were taken as protein samples. These chemicals were obtained from the biochemical unit of Patel Chest Institute, Delhi University, Delhi. All the other chemicals used were B.D.H. AnalaR.

The viscosity measurement of the sample solutions were performed in Ostwald viscometer having a flow time of 80 s, of 10 mL of the distilled water at 25 °C. All the viscosity measurements were carried out in a thermostat having sensitivity of ± 0.01 ºC. The time of flow was measured with the help of a stopwatch, Jaquet, made in Switzerland having the accuracy to measure the time of 0.1 s.

pH measurement was done at room temperature. M/20 NaOH solution was used as alkali to increase the pH of the sample solution in 8 M urea solution. M/20 NaOH solution was prepared in air free doubly distilled water. 20 mg sample was dissolved in 20 mL of 8 M urea solution. Alkali solution was added dropwise to increase the pH of the solution and then its viscosity was measured by Ostwald viscometer. The volume of one drop of liquid was found to the 0.06 mL. pH was measured by systronic pH meter type 322.

Specific optical rotation at different pH was measured by a polarimeter having 1 dcm polarimeter tube with sodium lamp. All the three samples were dried over P_2O_5 for 48 h. 1 g/L stock solution of protein sample was prepared in 8 M urea solution. The pH of the sample solution was varied by the addition of M/20 NaOH solution and then specific optical rotation was measured.

RESULTS AND DISCUSSION

Protein solutions are a good model for studying the effect of macromolecular shape on the viscosity of dilute solutions. The shapes of protein molecules easily change[8] by changing the pH. The results so obtained in the case of albumin-bovine and gelatin have been shown in the Tables 1-3. It has been found that the reduced viscosity increases with increasing pH. This is only because due to the denaturation of the albumin-bovine in 8 M urea solution, the hydrogen bonds get dissociated. So the large number of negative charges form along the chains of the albumin-bovine at fairly close spacing and electrostatic repulsion arises between these charges. Electrostatic repulsion tends to straighten the chain, whereas the thermal motion tends to coil it. If alkalis are added to the denatured protein solution to change the pH of the solution, for each pH value, an equilibrium exists between electrostatic repulsion and thermal motion, corresponding to a definite shape of the protein molecule. During the gradual addition of alkali, the degree of dissociation of the protein molecule also increases. As the degree of dissociation of the protein increases, the protein molecule assume all the intermediate conformations from tight coil to a straight rod, as a result of which the specific viscosity of the denatured protein increases with increasing pH.

TABLE-1
REDUCED VISCOSITY (η_{sp}/C) OF ALBUMIN-BOVINE (C = g/100 mL) IN 8 M UREA SOLUTION AT DIFFERENT pH

pH ↓ / Conc. →	0.1	0.2	0.3	0.4	0.5
3	4.94	4.94	4.94	4.94	4.94
4	4.94	4.94	4.94	4.94	4.94
5	7.41	7.41	7.41	7.41	7.41
6	24.70	24.70	24.70	24.70	24.70
7	64.22	98.80	143.26	214.71	266.76
8	74.10	113.62	163.02	229.71	288.99
9	71.63	108.68	158.08	217.36	276.64

TABLE-2
DEPENDENCY OF INTRINSIC VISCOSITY OF ALBUMIN-BOVINE (C = g/100 mL) ON pH IN 8 M UREA SOLUTION

pH	Intrinsic viscosity (η)
4.0	0.578
4.6	0.612
4.9	0.624
5.4	0.612
5.7	0.496
6.0	0.390
6.2	0.318
6.8	0.390
7.3	0.418
8.7	0.412
9.8	0.418
10.7	0.418

TABLE-3
DEPENDENCY OF INTRINSIC VISCOSITY OF GELATIN ON pH IN 8 M UREA SOLUTION

pH	Intrinsic viscosity (η)
1.5	0.64
2.0	0.69
2.6	0.73
3.3	0.69
3.4	0.62
3.4	0.48
3.6	0.30
4.0	0.13
4.7	0.24
5.2	0.35
6.5	0.39
7.2	0.40
9.0	0.40
10.2	0.39

In the case of albumin-bovine (Table-2), the values of (η) fall sharply near pH 6. At pH < 6, the albumin-bovine has a helical conformation, but at the pH > 6, it has a coil conformation. Thus the albumin-bovine undergoes helix-coil transition nearly at pH = 6. Similar phenomenon has been found in case of gelatin also. In the case of gelatin, the sharp fall of (η) value take place at pH = 6. At the pH value less than 4, gelatin has the helix conformation, whereas at the pH higher than 4, it has coil conformation. Thus the gelatin undergoes helix-coil transition at the pH region of 4 (Table-3). Dependence of specific optical rotation on pH in the case of albumin-bovine has been presented in Table-4. From the Table-4, it is found that the specific optical rotation of albumin-bovine falls sharply near pH 5.6. At pH < 5.6, albumin-bovine has a helical conformation and at pH > 5.6, it has a coil conformation.

TABLE-4
DEPENDENCY OF SPECIFIC OPTICAL ROTATION (η_{sp}) OF ALBUMIN-BOVINE ON pH IN 8 M UREA SOLUTION

pH	Specific optical rotation (η_{sp})
4.4	4
4.8	8
5.3	6
5.6	-8
5.8	-28
6.3	-66
6.8	-76
7.3	-76
8.7	-76
9.4	-76

TABLE-5
DEPENDENCY OF SPECIFIC OPTICAL ROTATION (η_{sp}) OF GELATIN ON pH IN 8 M UREA SOLUTION

pH	Specific optical rotation (η_{sp})
1.3	40
2.0	46
2.5	44
2.8	20
2.9	0
3.0	-14
3.3	-40
4.0	-58
5.0	-58
6.0	-58
7.0	-56
8.0	-56
9.0	-56
10.0	-56

The experiment concerning with the dependence of specific rotation and pH has been made also in the case of gelatin.

From the Table-5, it is found that the sharp fall of specific optical rotation of the gelatin takes place near the 3 pH range. At pH < 3, the gelatin has a helical conformation, whereas at pH > 3, it has a coil conformation. Hence it can be inferred that in the case of gelatin, helix-coil transition takes place at the pH range of 3. The sharpness of the transition and the sigmoidal character of the corresponding curves (Tables 4 and 5) are an indication of the co-operative nature of the transition.

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