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Flow behaviour of gliadin in absence and presence of ionic surfactants

R.P. SINGH[1], S. KUMAR[2], POONAM C. KUMAR[3] and R. CHAUDHARY[4]

[1-2]Chemistry Department, S.G.P.G. College, Sarurpur Khurd, Meerut (India)
[3-4]Chemistry Department, R.K. Goel Institute of Technology, Ghaziabad -201001 (India)

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ABSTRACT

In order to extent the rheological behaviour of protein-surfactant systems, the viscometric studies on gliadin with sodium dodecyl sulphate (SDS) and cetyl pyridinium bromide (CPB) were made and the effect of pH and temperature on viscosity behaviours were studied. Studies show a minimum point, which corresponds to the isoelectric pH (5.20) of these solutions, has been explained to be due to the contacted state of the molecule caused by the attractive forces between the balanced positive and negative charges on the protein molecule. On either side of isoelectric point, the viscosity depends on factors such as hydrodynamic volume, net charge on the molecule, existence of free ions in the solutions. It has been shown that the interaction of protein molecule with cationic and anionic surfactants depends on the pH-values of protein-surfactant system.

Key words: Sodium dodecyl sulphate, cetyl pyridinium bromide, Gliadin.

INTRODUCTION

The viscosity technique has been proved to be of great utility in investigating the colloid-chemical nature of polymers. The concept of intrinsic viscosity has made several advancements in elucidating the physico-chemical and structural of proteins and other related compounds. Many significant aspects of protein chemistry like, their shape and size, denaturation effect of pH and ionic strength etc. have been studied with the help of viscosity measurements. Mehl *et al.*,[1] and Edsall[2] have worked out the axial ratios of many proteins from this technique. Many other workers[3-5] have also applied this method to study the configurational changes accompanying the denaturation of proteins. On the basis of flow investigations Edsall and Mehl[6] have reported that myosin depolymerizes into smaller units in the presence of guanidine hydrochloride. Neurath and Saum[7] have reported that urea increases the viscosity number of serum albumin as a result of the change from rigid to flexible form.

The viscosity technique has also been employed in studying the surfactant-protein combination[8-10]. It has been observed that sodium caprylate and other similar salts of long chain fatty acids stabilize serum albumins against coagulation effects of heat[11]. Putnam and Neurath[9-10] found that surfactant which produce the same viscosity increase on both the acid and alkaline sides of the isoelectric point of a protein, precipitates it is the pH region where they carry charge opposite in sign to that on the protein. Harrap and Schulman[12] studied the unfolding of serum albumins by surfactants. Malik and Ashraf[13] have studied the binding of SDS with transfusion gelatin (TG) employing viscometric method. Arora *et al*. have also studied surfactant-protein interactions using viscometric and other related methods[14-28].

The extensive treatment of the dependence of viscosity on the shape and size of the polymers has been reviewed[29]. Several other workers have also studied the subject matter by this method[30-36]. The protein-ionic surfactant

complexes have been subject matter of many stuidies[37-46]. The stoichiometry of protein surfactant interaction has also been studied by measuring the flow properties of their mixed solution. This method has been used to test the validity of the mechanism of interaction as proposed by Lundgren[47]. With a view to enlarge our present knowledge of the rheological behavior of protein-surfactant systems, the viscometric studies forming the subject matter of this paper were undertaken using different combinations of ionic surfactants and gliadin (a fraction of glutein (wheat protein). The intrinsic viscosity and interaction index have been determined to gain an insight into the nature of particle-particle combination.

EXPERIMENTAL

Protein

Gluten power (BDH) was kept over night immersed in light petroleum ether to extract out fats and oils. The process was repeated several times to extract all the gliadin fraction of the powder. The fractions were isolated as following.

1. Extract in 50% alcohol contained the protein gliadin. Alcohol (solvent) was practically distilled off and the residue left behind was washed by double distilled water and dried. It was then dissolved in a very dilute KOH solution and dialysed to get an isoionic solution of gliadin protein. Its concentration was determined by colorimetric biuret method as well as gravimetrically. It was stored in a refrigerator under toluene to check and avoid the surface denaturation.
2. The residue left behind after alcohol separation was dried and dissolved in a minimum amount of very dilute KOH solution. The process was repeated many times to get a sample of pure glutenin. It was finally dissolved in dilute KOH solution and dialysed against double distilled water to get an isoionic protein solution. Its concentration was also determined by colorimetric biuret method. The solution of gliadin was prepared in double distilled water.

Reagents

Sodium dodecyl sulphate (SDS) and cetylpyridinium bromide (CPB) were Sigma products and their stock solutions were prepared in double distilled water. Potassium chloride (AR) solution was used for adjustment of ionic strength of reaction mixtures. Solution of hydrochloric acid and potassium hydroxide were used for the adjustments of pH. Acetic acid and sodium acetate were used for preparation of acetate buffers of lower pH values while phosphate and sodium carbonate buffers were used for preparation of buffers higher pH-values.

Techniques

pH-measurements

These were carried out on Systronic pH-meter using a wide range single glass electrode. The pH-meter was calibrated with the help of standard buffers of pH 4.00 and 9.20 for acid and alkaline ranges.

Viscosity measurements

These measurements were made by means of an Ostwald Viscometer of relatively long capillary tube (flow time for water was 80 seconds) at 25°±0.1° in a water thermostat. Mixtures were centrifuged at 16,000 rpm for 60 minutes to remove any suspended particles. The density of the solvent and solutions were determined with the help of relative density bottle. The viscosity values were calculated from the following expression.

$$\eta_{rel} = \frac{\eta}{\eta_o} = \frac{tp}{t_0 p_0}$$

where η_{rel} is relative viscosity, t and p are flow time and density of solution, while t_o and p_o are the flow time and density of the solvent.

Procedure

Following sets of mixtures were prepared for viscosity measurements:

1. 20 ml of 0.5 percent protein was taken in different boiling tubes and their pH was adjusted at 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12 by adding HCl and NaOH with the help of a pH-meter.
2. To a fixed concentration of gliadin (5g/l) different amounts of surfactants (1, 2, 4, 6, 8, 10, 12 and 14ml of 0.04 M concentration) were added to have an effective concentration of 2, 4, 6, 8, 10, 12, 15, 18, 20, 22, 25, 30, 35, 40, 45 and 50 millimoles

per litre. Total volume was made 20ml in each case and the viscosities of such sets were determined at pH values 2.0,3.0,6.0 and 8.0 in case of SDS-gliadin while at pH 2.0,3.0,8.0 and 9.0 in case of CPB-gliadin system.

3. Different concentration of gliadin (0.8 percent to 1.4 percent) were taken along with the same concentration of the surfactants solutions. Viscosity was measured at pH 8.0 with SDS and at pH 3.0 with CPB.
4. Varying amounts of 0.01 M SDS (0.06-1.3ml) were added to solution having 1.8 percent protein and total volume was made ml so as to obtain mixtures having different protein-surfactant ratio. Viscosity measurements were made at pH 8.0.

RESULTS AND DISCUSSION

The intrinsic viscosity, [η] at different surfactant concentrations has been obtained by plotting η_{sp}/ C cs C(η_{sp} is the specific viscosity and C the concentration of protein in g/ml) and then extrapolating the curves to zero concentration. The interaction index 'K' has been calculated using Kramer's equation which has been found to be true for such systems over a wide range of concentrations. This equation is

$$\ln \eta_{rel}/C = [h] + k[\eta]^2 C$$

The value of [h] and k are compiled respectively in the tables 1 and 2.

Preliminary studies of set (i) have shown a minimum point in the viscosity -pH curve of pure gliadin solutions (Fig. 1). The minimum point, which corresponds to the isoelectric pH (5.2) of these solutions, has been explained to be due to the contracted state of the molecule caused by the attractive forces between the balanced positive and negative charges on the protein. On either side of the isoelectric point, the viscosity depends on such

Table 1: Relative viscosity of gliadin (0.5%) at different pH value at 25°C, viscosity of water = 0.80 centipoise

pH	2	3	4	5	6	7	8	9	10	11	12
Viscosity (η) of solution in centipoise relative Viscostiy to [ηrel]	1.37	1.75	1.70	0.82	1.4	1.5	1.55	1.55	1.40	1.25	0.94
	1.72	2.18	2.12	1.00	1.75	1.87	1.93	1.93	1.80	1.56	1.05

factors as hydrodynamic volume, net charge on the molecule, existence of free ions in the solution etc.

The work done here was carried out on both the acidic and basic sides of the isoelectric point. It is clear from the results that the interaction with cationic and anionic surfactants depends on the pH value of the protein-surfactant system. Cationic surfactant precipitated only anionic protein, i.e., protein in the pH range above the isoelectric point while anionic surfactant precipitates only cationic protein i.e, protein on the acidic side of the isoelectric pH. A decrease in viscosity is observed at the specified pH values in both cases consequent upon the addition of small amounts of the respective

Table 2: Binding constants from viscosity measurements

Conc. in millimoles per litre	SDS-Gliadin		CPB-Gliadin	
	[η]	K	[η]	K
0.00	39.8	6.1	39.8	6.1
10.0	51.0	5.0	45.0	5.4
15.0	58.8	4.0	56.0	4.2
20.0	70.0	3.3	68.0	3.6
25.0	58.0	2.5	75.0	2.8
30.0	108.0	2.0	96.5	2.5
35.0	122.0	1.8	117.0	1.9
40.0	134.0	1.5	123.0	1.7

surfactants until precipitation takes place. The precipitation zone is shown by a blank in the curves. on adding a larger amount of the surfactant, the precipitate initially formed redissolves and an increase in viscosity is observed. Gliadin molecule which initially existed in the expanded state either at the acidic or the alkaline side for the isoelectric pH gets tightened up with the progressive interaction with surfactant ions and therefore, viscosity decreases. Boyer *et. al.*,[11] have reported similar findings in their studies with serum albumin in the presence of sodium caprylate and explained them on the basis of the tightening up of the protein molecule. With further addition of the surfactant a stage is finally reached when precipitation takes place due to the orientation of the hydrophobic part of surfactant ions in solution. On adding more quantity of surfactant, a second adsorption layer is formed by Vander Waals attractive forces between carbon chains[38] which makes the molecule hydrophilic and as such, the precipitate again dissolves causing the viscosity of solution to increase again. The resulting dispersion shows nearly the Newtonian flow. It is further solution see from the results that SDS is more effective in precipitating the protein from solution than CPB. It is also observed that the changes in viscosity are more marked in SDS-gliadin mixtures than in CPB-gliadin mixtures. Beyond the precipitation zone, an increase in viscosity in caused by the addition of more amounts of SDS and CPB. A few workers[12,48] have reported stoichiometric interactions between surfactant and protein under these conditions. Such interactions involve two types of binding forces; firstly, the electrostatic attractions involving the salt linkages of the protein, and secondly, non-electrostatic forces which usually bind surfactant ions into micelles.

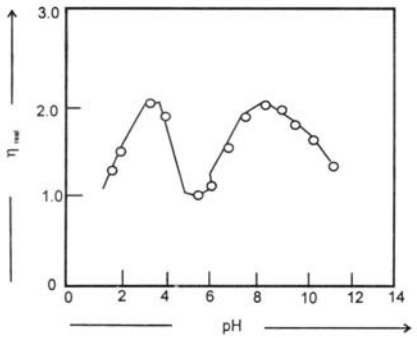

Fig. 1: Relative viscosity of 0.5% protein plotted against pH

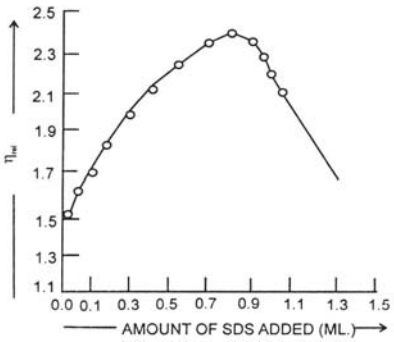

Fig. 2: Viscosigy of gliadin-SDS system of constant composition at pH 8.0

The viscosity values of varying amounts of the surfactants with a fixed amount of the protein (set ii) were used to determined the nature of the protein-surfactant interaction. The forgiven tables (from results of data of set (iii) show the variations of intrinsic viscosity, [η], of protein solutions as well as the interaction index, k, with using amount of SDS at pH 8.0 and for CPB at pH 3.0. It may be noticed from these results that the viscosity, as also the intrinsic viscosity, of gliadin-surfactant systems increases with increasing concentration of surfactant. The total effect of the anionic SDS or cationic CPB is to cause a regular unfolding of the protein molecule producing in its solubilization. The increase in intrinsic viscosity can be ascribed to the extension in the shape of the molecule bringing about an increase in the dissymmetry of the suspended units. In the initial stages, when smaller quantities of surfactant is added, the electrostatic force predominates and causes tis binding with gliadin. In the presence of larger amounts of SDS of CPB, a second type of interaction involving non-electrostatic forces is more probable to take place. Lundgren[47] has also proposed two types of interactions identifying the first one as stoichiometric linking and the second one as the secondary association of the extra surfactant. The size and structure of the paraffin (apolar) portion as well as the presence of an ionic group in the surfactant are two possible factors

controlling such combinations. The ionic group contributes to salt-like linkages between the oppositely charged groups of the protein and surfactant while the paraffin portion confers stability on the ionic bond and promotes then the linking of extra surfactant.

Support to the above discussed view also emerges from the data on the interaction index (K). A rapid decrease in the values of K in the initial stages indicates salt-like combinations while a smaller decrease in its values at higher surfactant concentrations shows a lesser amount of particle-particle interaction forces. It may be, therefore, concluded that the secondary association of extra surfactant is in the form of the loose combination due to apolar attraction with the already electrostatically bound surfactant-gliadin ratio of 1:3 corresponding to a complex of constant composition. Similar conclusions have been reported by Lundgren *et. al.,*[40] from electrophoretic studies. However, such plot could not be obtained with dilute solutions. The formation of protein-surfactant complex only at higher concentration shows the presence of rod-like particles in such system[39].

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