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Thermodynamic Stability (Proteins)

DENATURATION OF α-AMYLASE AND CASEIN

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The denaturation of α-amylase and casein, have been studied at temperatures 20–90 °C measuring viscosity in suitable solvents. A single state of transition has been observed at 55 °C, for α-amylase in urea plus HCl and casein in guanidine carbonate. The intrinsic viscosity for nature and denatured part, calculated at 30 and 55 °C for α-amylase and casein are respectively 3.53, 3.73, 3.85 and 0.16, 0.18, 0.20cc/gm. Two transition states have been observed for α-amylase in urea with unfolding of helices, at 55 and 70 °C. Casein in HCl undergoes a small expansion in the molecular domain with the weakening of intermolecular hydrogen bonds.

Keywords : Denaturation; α-Amylase; Casein; Intrinsic Viscocity; Huggin's Constant

INTRODUCTION

NATIVE protein molecules are known to be folded into essentially a rigid three dimensional structure, which can be disrupted by sufficiently drastic changes in both chemical and physical environment. The process of transformation may be reversible or irreversible. Mostly, spectroscopic and calorimetric studies have been reported using denaturants like urea, HCl and guanidine carbonate etc. The range of temperatures was very often from 0 to 90 °C. The specific viscosity of chymotrypsinogen has been reported by Brandts & Lumry (1963). Besides assigning possible conformational states, the studies also reveal the values for molecular constants. Presently, it is therefore intended to report the work on above proteins measuring their viscosity in good solvents.

ANALYSIS

Solutions were prepared from pure chemicals at strength (1) 0.67M urea in 1 : 3 HCl, (2) 0.67M urea, (3) 3 : 1 HCl and (4) 0.44M guanidine carbonate in distilled water. Viscosity values have been obtained by falling spheres method with Höpller's viscometer using spheres of glass, and the studies were limited over a range of temperatures from 20 to 90 °C. The observations were repeated with different spheres having different ball constants. The temperature control was maintained by an universal thermostat and the rate of heating was 10 °C per minute. Densities of protein solutions were determined before and after each experiment, with a 10cc specific gravity bottle and an accurate chemical balance. The calculations were made by the experimentally tested Faxén equation (Bacon, 1936) providing viscosity data

accurate to 0.005cP. There was no gel formation and *p*H of the solutions were obtained fairly constant to an accuracy of 0.05. Three protein strengths were taken in each case, and the variation in viscosity with temperature can be approximated for an inverse second order polynomial. Solvent viscosity was obtained by an extrapolation to infinite dilution. The intrinsic viscosity (Tanford, 1961) has been taken from fitting reduced viscosity values at different concentrations '*C*' for the equation :

$$\eta_r = [\eta] + K[\eta]^2 C,$$

where $[\eta]$ = intrinsic viscosity and K = Huggin's constant. No reversibility of the transformations could be observed, and so the solutions are non-Newtonian.

α-Amylase

Pure α-amylase, obtained from Sigma Chemical Co., St Louis, U. S. A. was weighed accurately for concentrations 0.05, 0.1, 0.2 per cent and 0.2, 0.5, 0.6 per cent respectively for the first two solvents. Their *p*H were recorded respectively as 0.65, 0.85, 1.20 and 7.7, 7.0, 6.8. The viscosity data (Table I) has been represented graphically in Fig. 1. It is apparent from the nature of the plot at the lowest concentration that a transition state exists at 55 °C with the unfolding of molecular helices. This curve at higher concentrations has broken into two parts resembling the nature and denatured part. These have further intersected each other indicating that the denaturation is also effective of changes in *p*H values. At higher *p*H probably swelling takes place, introducing new free volume within the protein at the expense of free volume in water with change in water protein interaction at the protein surface. The loss of the organised secondary structure occurs at segments of α-helices only after the side chains have become released from binding to parts of the protein. Beyond 80 °C, the viscosity value attains near constant indicating complete transformation. Having obtained the solvent viscosity at 30 and 55 °C, the intrinsic viscosity values calculated from above equation taking $K = 0.5$ have been given in Table V.

TABLE I

Viscosity with ten perature for α-amylase in urea plus HCl

Temperature °C	Viscosity in cP at strength		
	0.05%	0.1%	0.2%
30	1.211	1.434	1.911
40	0.865	1.285	1.358
50	0.713	1.212	1.173
60	0.693	0.781	0.768
70	0.715	0.612	0.634
80	0.808	0.606	0.585
90	0.808	0.606	0.585

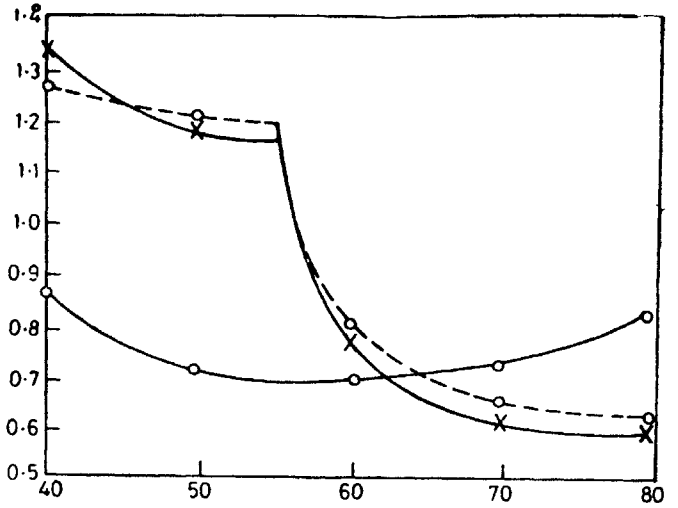

FIG. 1. Viscosity with temperature for α-amylase in urea plus HCl. Concentration : (*a*) 0.05 per cent –○–; (*b*) 0.1 per cent – –○– –; (*c*) 0.2 per cent –×–.

In the second case, the viscosity data (Table II) have been represented graphically for the basic and neutral solution in Fig. 2. When the solution is basic, a transition state is observed at 70 °C with descending viscosity values showing an upward trend. Here urea being a good hydrogen bonding reagent, the temperature dependence of the hydrophobic interaction for protein molecule is slowed down. The denaturation is accompanied by changes in polypeptide chain, side chains and void volume. In case of neutral solution, the viscosity values show an upward trend followed by a downward trend. This is rather unusual representing an additional state over and above the two distinctly resolved macroscopic thermodynamic states. Besides co-operative changes giving a cross-linked random coil, probably there is a primary bond rearrangement with contribution from amino acid side chains and this squeezing mechanism of "subtle change" takes place at 70 °C. The other turning point is at

TABLE II

Viscosity with temperature for α-amylase in urea

Temperature °C	Viscosity in cP at strengh		
	0.2%	0.5%	0.6%
40	0.835	0.842	0.970
50	0.714	0.720	0.844
60	0.632	0.725	0.769
70	0.612	0.750	0.759
80	0.685	0.703	0.715
90	0.685	0.703	0.715

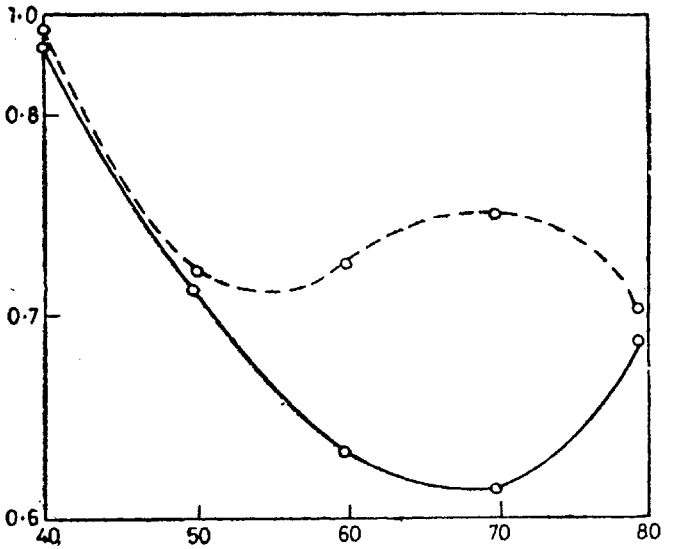

FIG. 2. Viscosity with temperature for α -amylase in urea. Concentration : (*a*) 0.2 per cent –○–; (*b*) 0.5 per cent – –○– –.

55 °C. In the acid solution there is no transition state, except that the molecule expands with loss of secondary structure.

Casein

Vitamin free casein, obtained from ICN Pharmaceuticals Inc. Cleveland, Ohio was weighed accurately for concentrations 0.05, 0.2, 0.4 and 0.4, 0.7, 1.0 per cent respectively for the rest two solvents. Their *p*H were recorded respectively as 2.3, 2.0, 1.9 and 11.8, 11.6, 11.4. In the first case, the viscosity values (Table III) follow a decreasing trend with the rise in temperature till a constant value is attained at 80 °C. The protein is denatured with some loss of secondary structure and a small expansion of the molecular domain. The transformation is complete and irreversible.

TABLE III

Viscosity with temperature for casein in HCl

Temperature °C	Viscosity in cP at strength		
	0.5%	0.2%	0.4%
40	1.227	1.251	1.285
50	1.069	1.087	1.122
60	0.942	0.961	1.014
70	0.840	0.866	0.913
80	0.766	0.792	0.834
90	0.766	0.792	0.834

The protein may be assumed to exist as a flexible coil with ionized groups distributed along the molecular chain. So the decrease in viscosity with dilution can be due to diminution in repulsive forces resulting into a more symmetrical molcule, weakening of hydrogen bonding and the binding of water to protein. With passing away of time, the solutions change in colour from pink to blue and then to greenish grey. However, no denaturation could be ascribed due to change in *p*H values. In the alkaline range, the viscosity values (Table IV) have been represented graphically in Fig. 3. Each of the three curves has broken into two parts representing the nature

TABLE IV

Viscosity with temperature for casein in guanidine carbonate

Temperature °C	Viscosity in cP at strength		
	0.4%	0.7%	1.0%
30	1.214	1.269	1.324
40	1.037	1.135	1.186
50	0.938	1.028	1.070
60	0.776	0.820	0.855
70	0.581	0.633	0.662
80	0.510	0.582	0.614
90	0.510	0.582	0.614

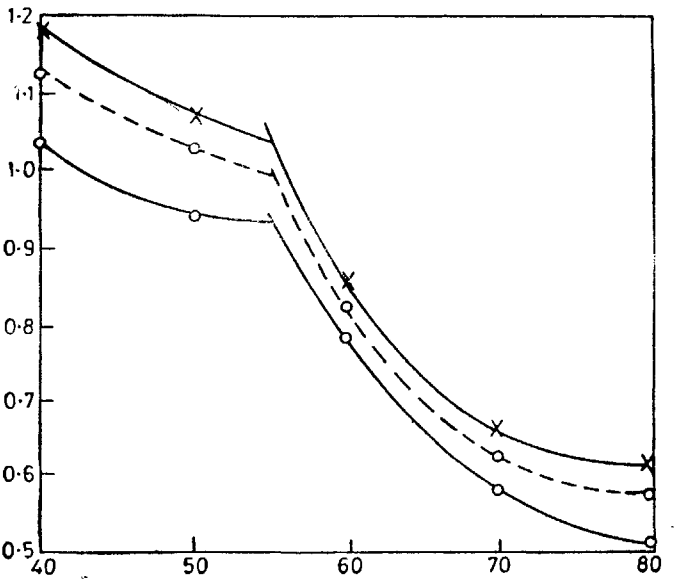

FIG. 3. Viscosity with temperature for Casein in guanidine carbonate. Concentration : (*a*) 0.4 per cent –○–; (*b*) 0.7 per cent – –○– –; (*c*) 1.0 per cent –×–.

and denatured part. There is a transition state at 55 °C, marked by an increase in the viscosity value. Hence the unfolding of molecular helices which probably refold into a new rigid structure of the denatured part. The transformation is said to be complete. Having obtained the solvent viscosity at 30 and 55 °C, the intrinsic viscosity calculated from above equation taking $K = 0.5$ have been given in Table V.

TABLE V

Intrinsic viscosity in cc/gm for α-amylase and casein

Temperature °C	α-amylase		Casein	
	Nature	Denatured	Nature	Denatured
30	3.53	–	0.16	–
55	3.73	3.85	0.81	0.20

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