THE EFFECT OF SODIUM CHLORIDE ON TYPE-BASED DIFFERENCES IN GELATIN DESOLVATION BEHAVIOUR

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Introduction

Gelatin is the denaturation product of the protein collagen. The conversion of collagen to gelatin results in a heterogeneous product with a broad molecular weight profile, which is important in determining the behaviour of the protein in solution. Addition of successive increments of a non-solvent, such as ethanol, to gelatin solutions causes progressive desolvation of the polymer. When sufficient water molecules are removed, the gelatin molecules begin to aggregate, resulting in phase separation, and forming a coacervate or, if sufficient desolvation occurs, a precipitate.[1]

Modification of the net charge of the protein molecules, by adjusting the solution pH to values ranging about the iso-electric point (IEP), influences the degree of interaction between the different molecular weight fractions, and hence the response of the protein to non-solvent.[1] It can be hypothesised that the use of gelatin types with different IEP's, and alteration of the molecular charge intensity by changes in the ionic strength of the solution would affect the overall response of the protein. The objectives of this work were to determine whether different gelatin types (Type A vs. Type B) exhibit different pH dependencies in their desolvation behaviour, and whether such differences are effected by dilute NaCl concentrations.

Experimental Methods

Lime-cured gelatins from bovine skin (Type B) of bloom strength 75 and acid-cured gelatin from porcine skin (Type A) of bloom strength 175 were purchased from Sigma Chemical Co., USA. Unbuffered gelatin solutions were prepared by heating aqueous suspensions of undissolved gelatin to 40°C with stirring for 20 minutes. The pH was adjusted to 5, 7, or 9 using dilute HCl or NaOH.

The method used was that of Farrugia and Groves (1999).[1] The gelatin solutions prepared above were incubated at 39°C for 1.5 hours and mixed with ethanol / water mixtures that had been similarly incubated such that the final solutions contained 0.2% w/w gelatin and increasing ethanol concentrations (40 to 75% w/w). Similar mixtures containing 0.1, 0.5 or 0.9% w/v sodium chloride were also prepared. The three-component systems were incubated at the same temperature for a further 20 minutes and the turbidity of the solutions measured by percentage transmittance using a Shimadzu 160 UV/Vis spectrophotometer (Shimadzu Corporation, Japan) operated at 600nm.

The data obtained from the desolvation experiments was subjected to nonlinear regression analysis, using the equation:

$$T = Bottom + \frac{(Top - Bottom)}{1 + e^{\frac{V_{50} - C}{Slope}}}$$

where T represents % transmittance, C represents ethanol concentration (% w/w), *Top* is the plateau % transmittance value at the top of the curve, *Bottom* is the plateau % transmittance value at the bottom of the curve, and V_{50} is the ethanol concentration at the % transmittance midway between *Top* and *Bottom*. The changes in V_{50} with changes in experimental conditions were used to monitor the effects of the various experimental conditions on the phase behaviour of gelatin in solution, lower V_{50} values being indicative of a greater sensitivity to desolvation.

Results and discussion

The variations in the V_{50} values of gelatin solutions with no added salt with changing solution pH were observed to be dependent on gelatin type (r=0.6503, p>0.05). B75 gelatin solutions adjusted to pH 5, 7 and 9 exhibited increased turbidity with increasing ethanol concentration, with the solutions adjusted to pH 5 being the most sensitive (Table 1). A175 gelatin solutions exhibited a

similar behaviour except that the solutions adjusted to pH 7 were most sensitive (Table 1). The proximity of pH 5 to the IEP of B-type gelatins $(4.8 – 5.2)$[2] ensured that the B75 gelatin molecules in solution carried a reduced net charge. Thus, the electrical double layer surrounding each molecule was not efficient in inhibiting aggregation, and precipitation resulted. Solutions at pH's 7 and 9 had V_{50} values indicative of slightly greater degrees of intermolecular repulsion and hence decreased sensitivity to desolvation. The behaviour of the A-type gelatins is more complex, since acid-processed gelatins have IEP values ranging between 6.0 and 9.4.[2] However, the overall behaviour of the gelatin solutions can be interpreted in the same manner as for B-type gelatins.

Table 1
Effect of pH on addition of ethanol to B75 and A175 gelatin solutions

Experiment Conditions	**V_{50} (mL, mean ± SEM, n = 3)**
B75 gelatin	
pH 5	47.4 ± 0.7
pH 7	58.2 ± 0.4
pH 9	65.0 ± 0.3
A175 gelatin	
pH 5	46.2 ± 0.6
pH 7	44.2 ± 0.2
pH 9	53.3 ± 0.3

The effect of added NaCl dramatically altered the behaviour of gelatin solutions towards ethanol. For both B75 and A175 gelatins, the gelatin solutions adjusted to pH's 5, 7 and 9 all became progressively less sensitive to increasing ethanol concentration with increasing ionic strength of the system, as exhibited by the increasing V_{50} values (F_{B75}=21.32, p<0.01; F_{A175}=22.61, p<0.01). However, in the presence of added salt, the sensitivity to increasing ethanol concentration was no longer dependent on the solution pH (F_{B75}=0.719, p>0.05; F_{A175}=0.811, p>0.05) or the gelatin type (r=0.9424, p<0.01). In terms of the DLVO theory, the addition of salt to the gelatin solutions where the molecules carried a net charge caused a reduction of the electrical double layer thickness, decreasing the energy barrier to aggregation. However, in solutions carrying little or no net charge, the added salt reduced the tendency towards aggregation, aiding the solubility of gelatin in ethanol. This may be explained by noting that in spite of the absence of a net charge, charged moieties still exist in regions along the molecule, resulting in intramolecular forces responsible for protein folding. Addition of salt could shield these forces, resulting in a more soluble entity.

Table 2
Effect of added salt and pH on addition of ethanol to B75 and A175 gelatin solutions

Experiment Conditions	**V_{50} (mL, mean ± SEM, n = 3)**		
	0.1% w/v NaCl	**0.5% w/v NaCl**	**0.9% w/v NaCl**
B75 gelatin			
pH 5	55.2 ± 0.3	63.1 ± 0.2	63.8 ± 0.1
pH 7	56.9 ± 0.1	61.4 ± 0.1	63.5 ± 0.0
pH 9	58.8 ± 0.2	62.1 ± 0.1	63.5 ± 0.1
A175 gelatin			
pH 5	55.0 ± 0.3	62.2 ± 0.3	63.6 ± 0.0
pH 7	54.5 ± 0.2	62.7 ± 0.1	69.4 ± 33.4
pH 9	55.5 ± 0.2	62.7 ± 0.1	63.8 ± 0.1

Conclusion

It appears that in the absence of salt, the solution pH and gelatin type affect the net charge of the protein, altering interchain interactions and the response of the protein to non-solvent. The presence of salt affects the ionic environment surrounding localised charges and alters the response of the protein to a greater extent; this effect is superimposed on changes in precipitability caused by solution pH or gelatin type.

References

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