

The Effects of Salts on the Rheological Properties of Plasma Protein Gels

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(Received 7 October 1988; revised version received and accepted 14 March 1989)

ABSTRACT

Added salts influenced the rheological properties of heat-induced plasma protein gels. Gel strength increased in the presence of 0.01M added CaCl₂ and 0.02M added MgCl₂. However, gel strength was reduced at higher concentrations of these salts. The ability of plasma protein gels to recover following deformation was lower than for the control (no added salt) in the presence of 0.02–0.1M added CaCl₂ and MgCl₂, but gel cohesiveness was markedly higher. The strength of plasma protein gels formed in the presence of added potassium salts was in the order F⁻ > SO₄²⁻ > Cl⁻ > I⁻ > SCN⁻. These salts had similar effects on gel cohesiveness and gumminess. KF and K₂SO₄ decreased gel recoverability following deformation; KI and KSCN increased recoverability, while KCl had little effect.

The study suggests that hydrophobic interactions and electrostatic forces play an important role in the formation and stabilisation of plasma protein gels.

INTRODUCTION

Protein gels are defined as structures in which polymer–polymer and polymer–solvent interactions occur in an ordered manner, resulting in the immobilisation of large amounts of solvent by a small amount of protein (Ferry, 1948; Schmidt, 1981). The ability of proteins to form heat-induced gels depends upon the protein, its amino acid composition, molecular weight

and net hydrophobicity (Shimada & Matsushita, 1981), heating and cooling rates and the critical balance between attractive and repulsive forces (Ferry, 1948; Egelanddal, 1980; Schmidt, 1981; Babajimopoulos *et al.*, 1983). Available data suggest that disulphide crossbridges (Tombs, 1970; Hillier *et al.*, 1980; Schmidt, 1981), hydrophobic associations (Shimada & Matsushita, 1980*a,b*; Utsumi & Kinsella, 1985) and ionic interactions (Kalab & Emmons, 1972; Johns & Ennis, 1981; Hayakawa & Nakai, 1985) are involved in stabilising protein gels.

Blood plasma proteins by virtue of their gel forming ability are considered potentially useful functional ingredients in the manufacture of processed foods and meats. It has been postulated that disulphide bonds are primarily involved in plasma protein gel formation, with hydrogen bonding and hydrophobic interactions also involved (Howell & Lawrie, 1985). Salts and pH are known to have a dramatic effect on plasma protein gelation, which suggests that electrostatic forces, which provide coulombic cross-links, also contribute to the gel formation (Fretheim & Gumpen, 1978; Hickson *et al.*, 1980; Hermansson, 1982*a,b*).

In an attempt to characterise further the forces involved in the formation of plasma protein gel structures, the effects of various salts on plasma protein gelation were determined in this study. This approach was followed because at low concentrations (<0.15M) salts affect electrostatic interactions (Damodaran & Kinsella, 1982), while at high concentrations they also affect hydrophobic interactions between non-polar residues of proteins (von Hippel & Schleich, 1969; Damodaran & Kinsella, 1981), by altering both the degree of hydration and the orientation of water molecules around non-polar side chains. In this respect salts such as F^- and SO_4^{2-} , which enhance the hydrogen bonding structure of water, promote protein-protein hydrophobic interactions and conversely I^- and SCN^- salts disrupt water structure resulting in decreased protein-protein hydrophobic associations (Hatefi & Hanstein, 1969; von Hippel & Schleich, 1969; Damodaran & Kinsella, 1981).

If either electrostatic or hydrophobic interactions are involved in the formation of plasma protein gel structure, then it is reasonable to assume that salts may selectively affect these forces and help reveal their relative contribution to the formation and maintenance of the gel structure.

MATERIALS AND METHODS

Preparation of plasma protein

Lyophilised, dialysed plasma protein was prepared as described by O'Riordan *et al.* (1988*a*).

Preparation of plasma protein gels

A stock solution of plasma protein (20% w/v, 0.55M NaCl) was prepared by solubilising the lyophilised protein in 0.1M imidazole-HCl buffer, pH 9.0. To prepare the gelling solutions, the stock solution was diluted in a 1:1 volume ratio with distilled water or solutions of CaCl₂, MgCl₂, K₂SO₄, KF, KI, KCl or KSCN to give the desired salt concentration, and the pH was readjusted to 9.0 using 1M HCl or NaOH as required. Aliquots (1 ml) of the gelling solutions were transferred to glass tubes (inside diameter 6.0 mm) and centrifuged at low speed to remove air bubbles. The tubes were then heat-sealed and placed in a waterbath at 90°C for 30 min to induce gelation. The tubes were removed from the waterbath, cooled in ice water and held at 4°C overnight.

Measurement of gel rheological properties

The gels were carefully removed from the glass tubes and sections 5 mm high were sliced with a razor edge cutting device. The rheological properties of these sections were determined as described by O'Riordan *et al.* (1988b).

The following rheological parameters, as described by Mulvihill and Kinsella (1988), were determined:

- Stress at 20% compression ($\sigma_{0.2}$), units = N/m².
- Apparent modulus of deformation (AMD), units = N/m².
- Degree of elasticity (DE_{0.2}) (the elastic deformation divided by the total deformation during the first 20% compression cycle), units = ratio = dimensionless.
- Mechanical hysteresis loss (MHL), units = %.
- Stress at 70% compression ($\sigma_{0.7}$), units = N/m².
- Cohesiveness (C_{0.7}) (the ratio of the positive stress energy during the second and first 70% compression cycles), units = ratio = dimensionless.
- Gumminess (G_{0.7}), units = N/m².

RESULTS AND DISCUSSION

The effects of added CaCl₂ and MgCl₂ on the rheological properties of plasma protein gels

Gel strength, as evidenced by $\sigma_{0.2}$, $\sigma_{0.7}$ and AMD (Fig. 1), increased slightly in the presence of 0.01M added CaCl₂ or 0.02M added MgCl₂ in comparison to the control (no added salt). This suggests that, on addition of low levels of

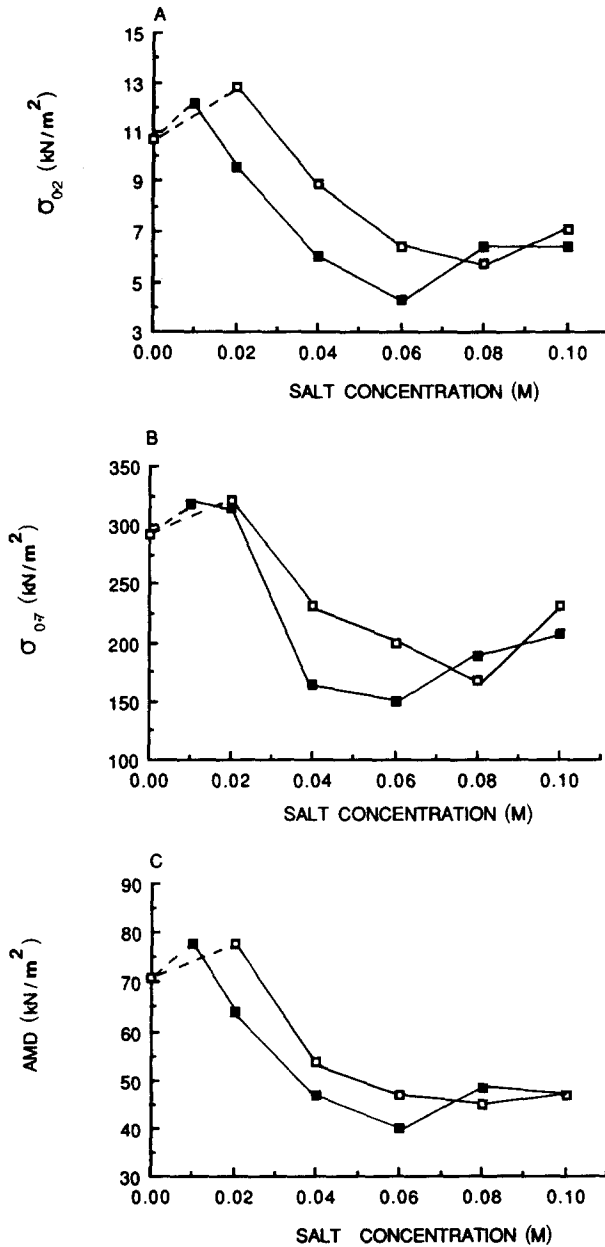


Fig. 1. The effects of concentration of CaCl₂ (■) or MgCl₂ (□) on (A) stress at 20% compression, $\sigma_{0.2}$ (kN/m²) (B) stress at 70% compression, $\sigma_{0.7}$ (kN/m²) and (C) apparent modulus of deformation, AMD (kN/m²), of plasma protein gels.

these salts, the critical balance between electrostatic attraction and repulsion is altered in such a manner as to confer maximum strength on plasma protein gels. However, the reduced gel strength and the presence of some unbound water at higher concentrations (up to 0.1M) of these salts, suggests that electrostatic interactions are increased to such an extent that the protein network throughout the gel is no longer evenly dispersed but that protein aggregates are formed, with large areas of unbound water. Added CaCl_2 (≥ 15 mM) had similar effects on heat-induced β -lactoglobulin gels (Mulvihill & Kinsella, 1988). The greater effectiveness of CaCl_2 in reducing gel strength, in comparison to MgCl_2 , suggests that plasma proteins have a greater affinity for Ca^{2+} than Mg^{2+} . The principal plasma protein, blood serum albumin, is reported to isomerise at alkaline pH and this results in increased binding of calcium ions (Peters, 1985).

The ability of plasma protein gels to recover following deformation was lower than the control (no added salt) in the presence of 0.02M–0.1M added

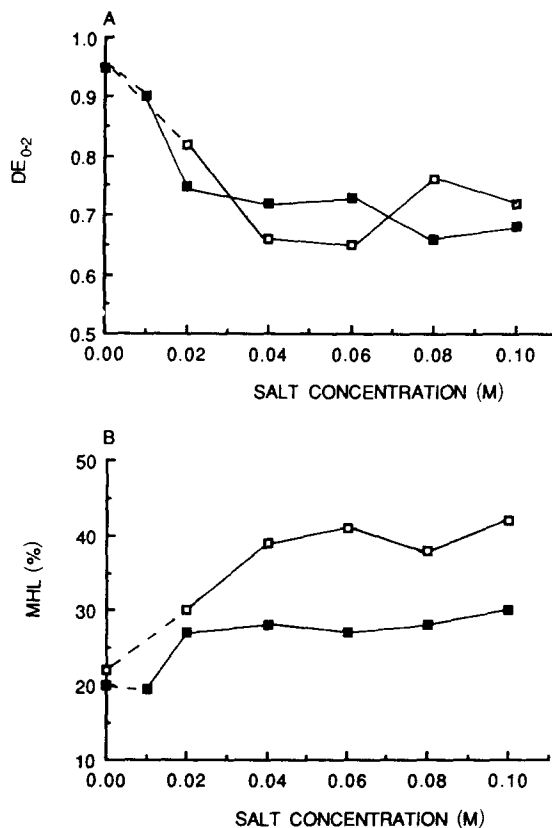


Fig. 2. The effects of concentration of CaCl_2 (■) or MgCl_2 (□) on (A) degree of elasticity, $DE_{0.2}$, and (B) mechanical hysteresis loss, MHL (%), of plasma protein gels.

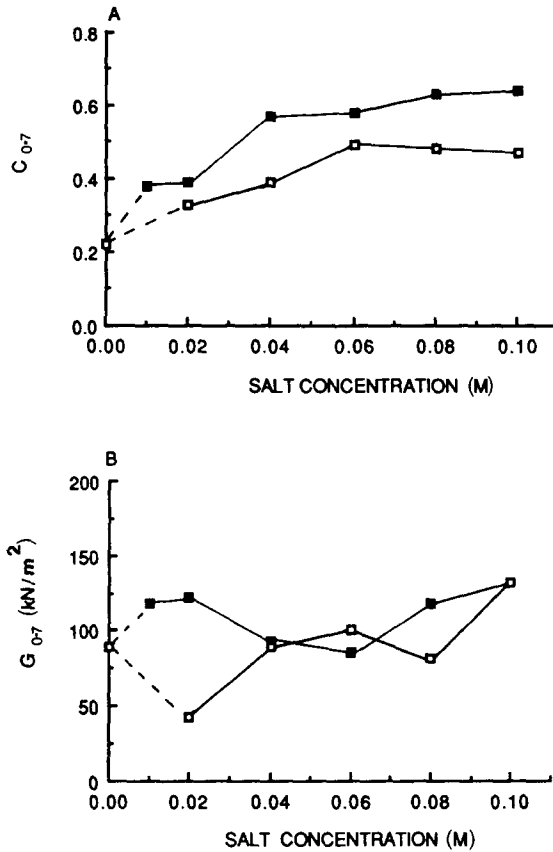


Fig. 3. The effects of concentration of CaCl_2 (■) or MgCl_2 (□) on (A) cohesiveness, $C_{0.7}$ and (B) gumminess, $G_{0.7}$ (kN/m^2), of plasma protein gels.

CaCl_2 or MgCl_2 , as indicated by a decrease in $\text{DE}_{0.2}$ and an increase in MHL values (Fig. 2). The cohesiveness ($C_{0.7}$) of gels formed in the presence of added CaCl_2 or MgCl_2 was higher than gels with no added salt (Fig. 3(a)). It is likely that these salts resulted in increased electrostatic interactions and led to the formation of localised aggregates, which increased the cohesiveness of the gel, but did not contribute to the gel strength or its ability to recover following deformation.

Overall, the effects of low concentrations ($\leq 0.1\text{M}$) of added CaCl_2 or MgCl_2 on the rheological properties of plasma protein gels clearly indicate that gels with optimum rheological properties occur when there is a critical balance between electrostatic attraction and repulsion. The importance of electrostatic forces in the formation of plasma protein gels was also shown by the marked effect of pH on the rheological properties of these gels (Fretheim & Gumpen, 1978; Hermansson, 1982a,b; O'Riordan *et al.*, 1988b).

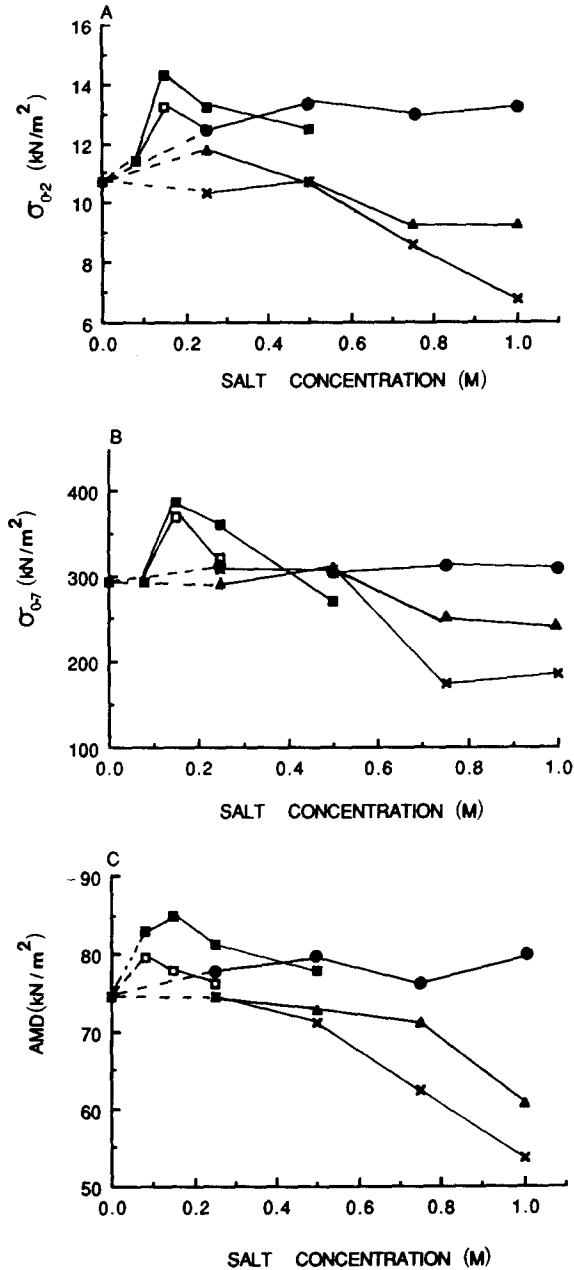


Fig. 4. The effects of concentration of KF (■), K₂SO₄ (□), KCl (●), KI (▲) or KSCN (×) on (A) stress at 20% compression, $\sigma_{0.2}$ (kN/m²) (B) stress at 70% compression, $\sigma_{0.7}$ (kN/m²) and (C) apparent modulus of deformation, AMD (kN/m²), of plasma protein gels.

The effects of anions (added in the form of potassium salts) on the rheological properties of plasma protein gels

The rheological parameters relating to gel strength ($\sigma_{0.2}$, $\sigma_{0.7}$ and AMD) were lower than the control (no added salt) in the presence of $>0.5\text{M}$ added KI and KSCN and higher than the control in the presence of added K_2SO_4 , KF or KCl (Fig. 4). Overall the strength of plasma protein gels formed in the presence of anions (in the form of potassium salts) was in the order $\text{F}^- > \text{SO}_4^{2-} > \text{Cl}^- > \text{I}^- > \text{SCN}^-$, which is the lyotropic series of added anions. The anions had a similar effect on gel cohesiveness (Fig. 5(a)) and gumminess (Fig. 5(b)). These results are consistent with those of Shimada and Matsushita (1981), who reported that salts promoted thermo-coagulation of BSA according to the lyotropic series.

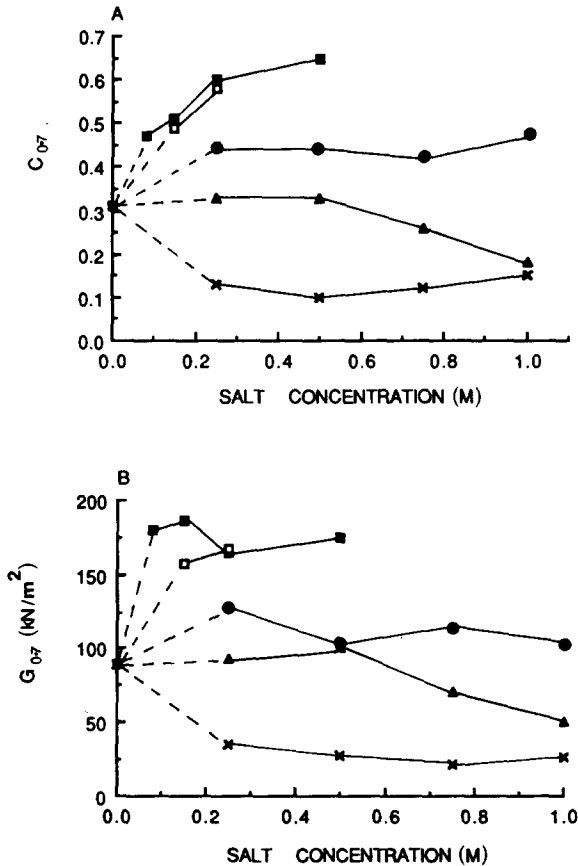


Fig. 5. The effects of concentration of KF (■), K_2SO_4 (□), KCl (●), KI (▲) or KSCN (×) on (A) cohesiveness, $C_{0.7}$ and (B) gumminess, $G_{0.7}$ (kN/m^2), of plasma protein gels.

Both SCN^- and I^- are large ions with low charge densities and tend to break the hydrogen bonded structure of water. Hence, the driving force for hydrophobic interaction between protein molecules is decreased in their presence. The decreased plasma protein gel strength and cohesiveness in the presence of KSCN and KI suggests a reduction in hydrophobic associations. In contrast, KCl , KF and K_2SO_4 are stabilisers of hydrophobic associations. Hence, the positive effects of these ions on gel firmness, cohesiveness and gumminess suggest the involvement of hydrophobic associations in plasma protein gels.

The ability of plasma gels to recover following deformation, as evidenced by $\text{DE}_{0.2}$ and MHL values, was lower in the presence of KF and K_2SO_4 , and higher in the presence of KI and KSCN (0.25–1.0M) than in the absence of these salts (Fig. 6). KCl did not affect gel recoverability. The negative effect of K_2SO_4 and KF and the positive effect of KI and KSCN on gel elasticity

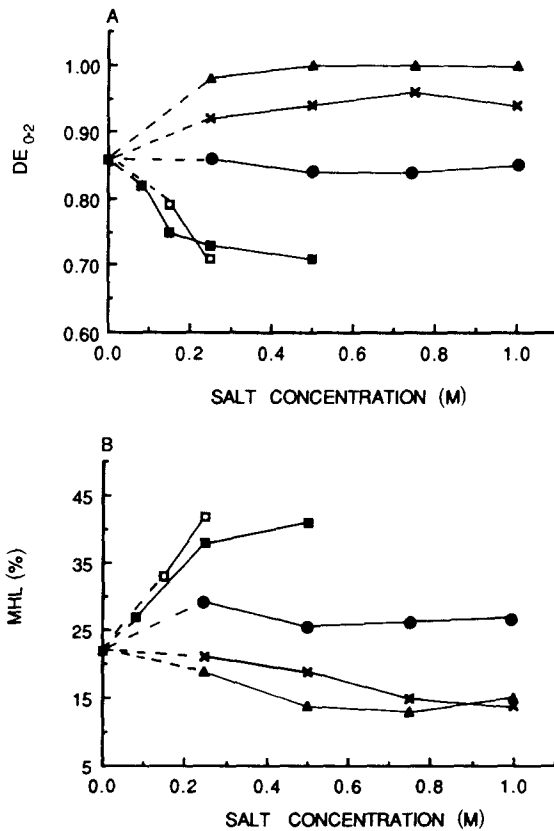


Fig. 6. The effects of concentration of KF (■), K_2SO_4 (□), KCl (●), KI (▲) or KSCN (×) on (A) degree of elasticity, $\text{DE}_{0.2}$ and (B) mechanical hysteresis loss, MHL (%), of plasma protein gels.

suggest that a high degree of protein-protein hydrophobic interaction inhibits gel recovery following compression, while fewer such crosslinks result in a gel structure with superior recovery characteristics.

The effects of the lyotropic series of anions on plasma protein gelation in this study suggest that hydrophobic interactions may be significant in plasma protein gel network formation. This is consistent with the work of Howell and Lawrie (1985), who found that addition of sodium dodecyl sulphate reduced plasma gel strength and concluded that hydrophobic interactions were involved in plasma protein gelation. Hydrophobic interactions have also been implicated in BSA gelation (Clark *et al.*, 1981).

Overall, hydrophobic interactions may be the dynamic driving force in plasma protein gelation, and the formation of a plasma protein network is controlled by a balance of attractive forces between hydrophobic segments of protein molecules generated by thermal unfolding, and repulsive forces due to protein charge. Covalent bonding and hydrogen bonding may also be involved in the plasma protein gel network, and the contribution of these bonds to the gel matrix will be the basis of subsequent studies.

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