Food Sci. Technol. Res., **8** (1), 1–3, 2002

Technical paper

Effect of Copper, Iron, Zinc and Magnesium Ions on Bovine Serum Albumin Gelation

Zahur Z. HAQUE and Kayanush J. ARYANA

Department of Food Science and Technology, Southeast Dairy Foods Research Center, MAFES, Mississippi State, University, MS 39762.

Received April 18, 2001; Accepted October 30, 2001

This study was conducted to observe the effect of trace amounts of various ions $CuSO_4$, $FeSO_4$, $ZnSO_4$ and $MgSO_4$, on the microstructure of bovine serum albumin (BSA) gels. Microstructure was studied using transmission and scanning electron microscopy. Protein dispersions (5% w/v) were made in 0.1 M NaCl containing 5 mM of each of the divalent cations. The pH was adjusted to pH 7 using 0.1 N NaOH. Addition of $CuSO_4$ markedly changed the microstructure of BSA gels; significantly larger water entrapping void spaces were seen and the gel matrix comprised of larger aggregates. An opposite effect was seen for $MgSO_4$ which gave compact gels with significantly smaller gel matrix forming protein aggregates and void spaces. Trace amount of minerals impacting microstructure of gels as observed in this study has dramatic functional implications. This is because the aggregate size determines whether the gel is opaque or transparent and the degree of water entrapment effects texture.

Keywords: microstructure, trace-metals, gelation

Gelation property is one of numerous desirable functional attributes of food proteins. The process involves initial denaturation, i.e. conformational alteration, of native protein followed by an aggregation step to allow denatured protein molecules to orient themselves and interact at specific points, resulting in a three dimensional matrix (Ferry, 1948; Hermansson, 1979). Gel formation induced either by divalent cations or action of heat or both is dependant on type and concentration of protein, processing conditions used to induce gel formation and pH and ionic environment (Hermansson, 1979; Schmidt, 1981). Water is structured by the anions PO_4^{3-} and SO_4^{2-}, i.e., they decrease the entropy of the surrounding water (Verall, 1973), hence, the thermodynamic advantage of hydrophobic interactions is reduced (Tanford, 1980; Haque & Khalifa, 1992). Mulvahill and Kinsella (1988) looked at the effect of calcium and sodium chloride on β-lactoglobulin gelation. They concluded that at concentrations higher than 10 mM, calcium chloride enhanced coagulation rather than gelation. Haque *et al*. (1994) observed change in BSA functionality as a result of these divalent cations. Haque *et al*. (1994) further observed decreased emulsifying activity index in the presence of 3 mM Fe^{2+} Cu^{2+} and Zn^{2+} in various concentrations of BSA (0.2, 0.4, 0.8, 1 and 2 mM). Emulsion stability increased was reported in 1,2 and 3 mM concentrations of $FeSO_4$, $CuSO_4$ and 1 mM of $MgSO_4$ and $ZnSO_4$. On the other hand, interfacial thickness index increased at 3 mM of $MgSO_4$ $FeSO_4$, $CuSO_4$, $ZnSO_4$ in 0.2 mM BSA. Decrease in solubility was noted at 1 mM in BSA. They also noticed increase in gelation properties due to Cu^{2+} and Zn^{2+}. However, effects of trace levels of ions on BSA gel microstructure is not clearly understood.

This study was conducted to study the effect of trace amounts of various ions on the microstructure of BSA gels.

Approved as journal article # 9836 of the Miss. Agric. & For. Exp. Sta., Mississippi State, MS 39762. Funded by MAFES Project #3153
E-mail: haque@ra.msstate.edu

Materials and Methods

Preparation of gels Protein contents of samples were determined using the Kjeldahl method (AOAC 930.29), total solids and moisture content of the samples were determined according to AOAC 926.08 (Association of the Official Analytical Chemists, 1990). Protein gels were made using a slight modification of the method of Lee *et al*. (1997). Bovine serum albumin was hydrated at (5% w/v) in 0.1 N NaCl with 5 mM $CuSO_4$/$MgSO_4$/$FeSO_4$/$ZnSO_4$. The pH was adjusted to 7.00 with 0.1 N NaOH. Dispersions were stirred for an hour at moderate speed with minimum air incorporation and degassing was carried out *in vacuo*. Two 15 cm long glass tubes (22 mmO.D., 19 mm I.D.×1.5 mm thick) were sprayed with Sigmacote (Sigma, St. Louis, MO) or cooking oil-based spray and brushed with a test tube brush inside the tube for complete coverage. The degassed protein dispersion was stirred for uniformity before pouring in the tubes whose bottom ends had been closed with a cap (7/8 inch (2.22 cm) heat resistant plastic or rubber) which was obtained from a local hardware store. Top end was closed with a cap that was punctured to make a (1.5 mm dia) vent. Tubes were placed vertically in holding racks in a water bath such that water level was slightly above the sample line and heated at 90˚C for 15 min. Tubes were then immediately cooled in cold water (10±1˚C) for 20 min and left overnight in a refrigerator (4±1˚C).

Transmission (TEM) and scanning electron microscopy (SEM) Samples were prepared for transmission and scanning electron microscopy according to Aryana and Haque (2001). Length of maximum void spaces were calculated from representative micrographs from three replications by a ruler.

Experimental design and statistical analyses Experiment was conducted as a completely randomized design. The treatments were 5 mM concentrations of $CuSO_4$, $MgSO_4$, $FeSO_4$, $ZnSO_4$. Samples were examined in triplicates. Averages were analyzed according to SAS (SAS, 1990). Differences between

means were determined using least significant difference ($p<=$ 0.05).

Results and Discussion

Control gel (BSA 5% w/v) had a compact matrix (Fig. 1A). With the incorporation of $CuSO_4$, $FeSO_4$ and $ZnSO_4$, the gel matrix became less compact compared to control (Figs 1B,C,D) On addition of $MgSO_4$, the matrix became more compact (Fig. 1E) than the control. This is because Mg^{++} specifically binds to heat denatured whey proteins and forms aggregates (Varunsatian *et al*., 1983). Tang and McCarthy (1994) reported that the cations they tested all increased G' (gel stiffness) of whey protein isolate gels in the order of $Mg^{2+}> Fe^{3+}>Ca^{2+}>K^{+}>Na^{+}$. Size of individual aggregates that formed the matrices were significantly larger (Table 1) with the incorporation of $CuSO_4$ (Fig. 1B) compared to the control (Fig. 1A). Gels with $CuSO_4$, $FeSO_4$, $ZnSO_4$ had aggregates that appeared clustered and more fused together than the control. On the other hand, gel with $MgSO_4$ had aggregates that were more dense (Fig. 1E) with distinct boundaries, less fused together and this was because Mg^{++} destabilizes whey proteins (Varunsatian *et al*., 1983). Arntfield (1996) reported magnesium ions improved firmness of ovalbumin gels due to increased crosslinking between proteins. The most open to most compact matrix was seen in case of $CuSO_4$, $ZnSO_4$, $FeSO_4$, control, and $MgSO_4$ in that order. Different ions have different hydrodynamic radii and different binding sites. As heat denaturation of proteins takes place the hydrophobic residues are exposed which favors structuring of water around it (clathrate structures). The gels were made at pH 7.00. As the pH increases, the net charge on the proteins become increasingly negative. As the cations bind to it, charge masking takes place which effect hydrophobic interactions that are pre-requisite for gelation.

Gel strength is expected to increase with the compactness of the matrix. Matsudomi *et al*. (1991) reported that hardness of BSA gels was at a maximum with 5 mM $CaCl_2$ but was not affected by NaCl. They also reported dithiothreitol, at levels higher than 5 mM, significantly decreased BSA gel hardness indicating the importance of disulfide bonds. Green (1980) reported that gelling capacity of different whey protein powders was approximately proportional to the sulfhydryl content of the powder. They observed that when the sulfhydryl groups were partially reacted by titration with Hg^{2+}, the gelling capacity reduced proportionally. They further observed that the gel structure was disrupted by treatment with dithiothreitol and 2-mercaptoethanol, which breaks disulfide bonds, indicating that the gel was stabilized by disulfide linkages. Schmidt (1981) reported maximum gel strength on addition of 11.1 mM $CaCl_2$ to a 10% (w/v) WPC which had been dialyzed to remove lactose and salts. Thereafter, gel strength was decreased on further addition of $CaCl_2$. They thought this was perhaps due to excessive cross-linking and aggregation. They further reported that addition of cysteine increased gel strength up to 9.7 mM and at higher levels gel strength was dramatically reduced. Johns and Ennis (1981) studied gelation of WPC prepared by ultrafiltration of acid whey from which various levels of calcium had been removed and replaced by sodium. They observed that gel hardness increased

Table 1. Average aggregate size and maximum length of void space of BSA gels with various ions over three replications.

Ions	Mean aggregate size (μm)	Length of maximum void space (μm)
Control	0.1^{b}	3.2^{d}
Cu^{2+}	0.5^{a}	40.0^{a}
Fe^{2+}	0.1^{b}	7.6^{c}
Zn^{2+}	0.1^{b}	10.8^{b}
Mg^{2+}	0.05^{c}	0.7^{e}

Different letters indicate significant differences (α=0.05)

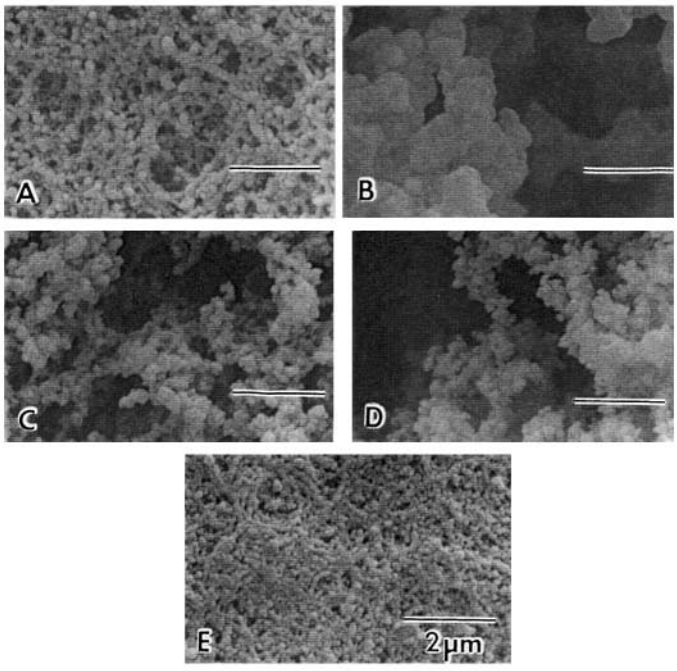

Fig. 1. Scanning electron micrograph of A) control BSA (5% w/v) gel, B) BSA (5% w/v) gel with $CuSO_4$, C) BSA (5% w/v) gel with $FeSO_4$, D) BSA (5% w/v) gel with $ZnSO_4$, E) BSA (5% w/v) gel with $MgSO_4$. Bars = 2 μm.

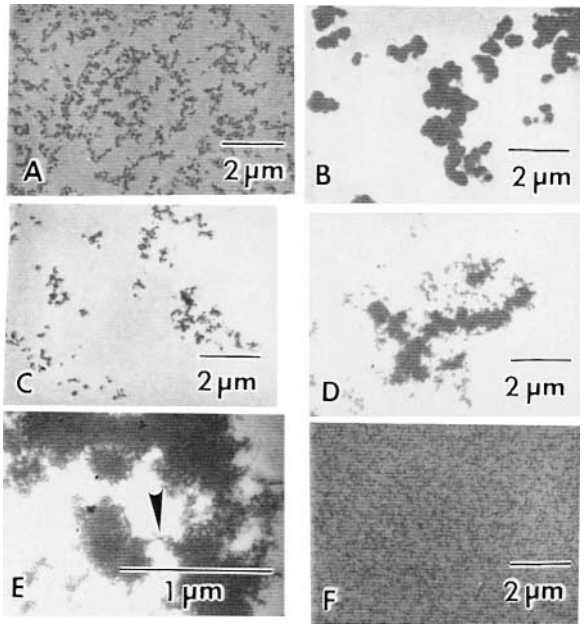

Fig. 2. Transmission electron micrograph of A) control BSA (5% w/v) gel, B) BSA (5% w/v) gel with $CuSO_4$, C) BSA (5% w/v) gel with $FeSO_4$, D) BSA (5% w/v) gel with $ZnSO_4$, E) BSA (5% w/v) gel with $ZnSO_4$ at higher magnification, F) BSA (5% w/v) gel with $MgSO_4$.

from ~3 to ~8 kg on replacement of 100% calcium, while gumminess, springiness, cohesiveness and chewiness also increased as calcium was replaced.

The TEM studies corroborated the SEM related observations. The control gel (BSA 5%w/v) was relatively compact (Fig. 2A), but gelation in presence of $CuSO_4$ (Fig. 2B), $FeSO_4$ (Fig. 2C) and $ZnSO_4$ (Fig. 2D) resulted in open matrices. However an opposite trend was seen with addition of $MgSO_4$, which resulted in a more compact matrix (Fig. 2F). Gels with $CuSO_4$, $FeSO_4$ and $ZnSO_4$ had aggregates (also as seen by SEM) that appeared clustered and more fused together than control. Xiong *et al.* (1993) reported that β-lactoglobulin in diluted water exhibited a single transition (> or =76°C) in protein-protein interaction (aggregation). Addition of $CaCl_2$ (0.005 to 0.2 M) and NaCl (0.02 to 1.0 M) promoted the transition. Karleskind *et al.* (1995) reported that WPC gel formation is affected by calcium, sodium, lactose and lipids. Foegeding *et al.* (1992) reported that β-lactoglobulin gels containing 20 mM $CaCl_2$ were more deformed than gels containing 100 mM NaCl. The β-lactoglobulin solutions containing 20 mM $CaCl_2$ gelled more rapidly and had lower gel points than similar solutions containing 100 mM NaCl. After gelation at 80°C and cooling to 25°C gels containing 100 mM NaCl had greater values G' (storage modulus) than those containing 20 mM $CaCl_2$.

Gels with $ZnSO_4$ showed presence of appendages (Fig. 2D) and these aggregates were not as fused as in case of gels with $CuSO_4$. Gels with $CuSO_4$ indicated large bead like outer boundaries of aggregates. Okabe and Hokaze (1993) reported significant influence of Cu^{2+} ions on thyroxine binding to BSA. Quing *et al.* (1996) used Hummel-Dreyer gel permeation technique to investigate the binding of BSA with Zn^{2+} and Cd^{2+} and have found two different specific binding sites on the BSA molecule. The various trace ions had varying effects on the microstructure of BSA gels.

References

Arntfield, S.D. (1996). "Macromolecular interactions in food technology." ed. by N, Parris, A, Kato, L.K, Creamer, J. Pearce, 82–92 ACS: Washington, DC.

Aryana, K.J. and Haque, Z.U. (2001). Effect of commercial fat replacers on the Microstructure of low-fat Cheddar cheese. *Int. J. Food Sci. Tech.*, **36**, 169–177.

Association of the Official Analytical Chemists. (1990). Official methods of analysis. Vol. 2, 15th ed., ed. by K. Helrich, AOAC. Washington, D.C.

Ferry, J.D. (1948). Advances in Protein Chemistry, Academic Press, **4**, 1–77.

Foegeding, E.A., Kuhn, P.R. and Hardin, C.C. (1992). Specific divalent cation-induced changes during gelation of β-lactoglobulin. *J. Agric. Food Chem.*, **40**, 2092–2097.

Green, M.L. (1980). The formation and structure of milk protein gels. *Food Chem.*, **6**, 41-49.

Haque, Z.U. and Khalifa, M.Y. (1992). Kappa-casein heterogeneity and mild heating effects on susceptibility to chymosin action. *J. Food Sci.*, **57**, 49–54.

Haque, Z.U., Rantamaki, P. and He, Z. (1994). Effect of trace amounts of ions on some functional properties of bovine serum albumin. In book of Abstracts Annual Meeting of the IFT, Atlanta, GA, June 28, 1994. Abs#S67.

Hermansson, A.M. (1979). Functionality and protein structure, ACS Symp. Ser. 92, ed. by A. Pour-El, ACS Washington, DC 81–103.

Johns, J.E.M. and Ennis, B.M. (1981). The effect of replacing calcium with sodium ions in acid whey on the functional property of whey protein concentrates. *N.Z. J. Dairy Technol.*, **15**, 79–86.

Karleskind, D., Laye, I., Nei, F.I. and Moor, C.V. (1995). Gelation properties of lipid reduced and calcium reduced whey protein concentrates. *J. Food Sci.*, **60**, 727–737.

Lee, C.M., Filpi, I., Xiong, Y., Smith, D., Regenstein, J., Damodaran, S., Ma, C.Y. and Haque, Z.U. (1997). Standardized failure compression test of protein gels from a collaborative study. *J. Food Sci.*, **62**, 1163–1166.

Matsudomi, N., Rector, D. and Kinsella, J.E. (1991). Gelation of bovine serum albumin and beta-lactoglobulin, effects of pH, salts and thiol reagents. *Food Chem.*, **40**, 55–69.

Mulvahill, D.M. and Kinsella, J.E. (1988). Gelation of β-lactoglobulin: Effects of sodium chloride and calcium chloride on the rheological and structural properties of gels. *J. Food Sci.*, **53**, 231–236.

Okabe, N. and Hokaze, M. (1993). Effect of divalent metal ions on the binding of thyroxine to bovine serum albumin as measured by fluorescence. *Biol. Pharm. Bull.*, **16**, 719–721.

Quing, H., Yanlin, H., Fenlin, S. and Zuyi, T. (1996). Effect of pH and metal ions on the conformation of bovine serum albumin in aqueous solution. An attenuated total reflection (ATR) FTIR spectroscopic study. *Spectrochimica Acta*, Part A. **52**, 1795–1800.

SAS® User's Guide: Statistics, Version 6.0 Edition. (1990). SAS Inst., Inc., Cary, NC.

Schmidt, R.H. (1981). Protein Functionality in Foods. ACS Symp. Ser. 147, ed. by J.P. Cherry, ACS Washington, DC 131–147.

Tang, Q. and McCarthy, O.J. (1994). Oscillatory rheological comparison of the gelling characteristics of egg white, whey protein concentrate, whey protein isolate and β-lactoglobulin. *J. Agric. Food Chem.*, **42**, 2126–2130.

Tanford, C. (1980). The hydrophobic effect: Formation of micelles and biological membranes, 60–78. 2nd ed., John Wiley and Sons, New York, NY.

Varunsatian, S.,Watanabe, K., Hayakawa, S. and Nakamura, R. (1983). Effects of Ca^{++} , Mg^{++} and Na^{+} on heat aggregation of whey protein concentrates. *J. Food Sci.*, **48**, 42–46, 70.

Verall, R.E. (1973). Water: A Comprehensive Treatise. Aqueous solutions of simple electrolytes. ed. by F. Franks Plenum press, NY 3: 211.

Xiong, Y.L., Dawson, K.A. and Wan, L. (1993). Thermal aggregation of β-lactoglobulin. Effect of pH, ionic environment, and thiol reagent. *J. Dairy Sci.*, **76**, 70–77.