Harmon, B. G., Becker, D. E., Jensen, A. H., Baker, D. H., *J. Anim. Sci.* **28**, 459 (1969).
Klotz, I. M., Hunston, D., *Biochemistry*, **10**, 3065 (1971).
Koshiyama, I., *Int. J. Pept. Protein Res.* **4**, 167 (1972).
Malik, W. V., Singh, M. V., *J. Indian Chem. Soc.* **46**, 618 (1969).
Moffitt, W., Yang, J. T., *Proc. Natl. Acad. Sci. U.S.A.* 42, 596 (1956).
Scatchard, G., *Ann. N.Y. Acad. Sci.* **51**, 660 (1949).
Schachman, H. K., "Ultracentrifugation in Biochemistry", Academic Press, New York, N.Y., 1959.
Shvarts, V. S., Vaintraub, I. A., *Biochemistry (Engl. Transl.)* **32**, 135 (1967).
Urnes, P., Doty, P., *Adv. Protein Chem.* **16**, 401 (1961).
Wolf, W. J., in "Soybeans: Chemistry and Technology", Smith, A. K., and Circle, S. J., Ed., Avi Publishing Co., Westport, Conn., 1970.
Wolf, W. J. Babcock, G. E., Smith, A. K., *Arch. Biochem. Biophys.* **99**, 265 (1962).
Wolf, W. J., Briggs, D. R., *Arch. Biochem. Biophys.* **76**, 377 (1958).
Wolf, W. J., Briggs, D. R., *Arch. Biochem. Biophys.* **85**, 186 (1959).
Wolf, W. J., Tamura, T., *Cereal Chem.* **46**, 331 (1969).

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Binding of Ca(II), Mg(II), and Zn(II) by 7S Fraction of Soybean Proteins

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Binding of Ca(II) or Mg(II) by 7S protein at pH 7.8 appears to occur at the imidazole groups of the histidine residues of the protein molecule. Zn(II) binding at pH 6.5 also occurs at imidazole groups. The 7S protein binds more Ca(II) or Mg(II) in borate buffer than in Tris-HCl buffer of the same pH. Rate of proteolysis, fluorescence, optical rotatory dispersion, and circular dichroism measurements do not indicate any conformational change in the protein due to metal ion binding. Ca(II), Mg(II), or Zn(II) increases the heat coagulation of 7S protein. At room temperature the protein is precipitated to an extent of 40% by Ca(II), 10% by Mg(II), and 90% by Zn(II). NaCl (0.5 M) suppresses precipitation by Ca(II) or Mg(II) and decreases only slightly precipitation by Zn(II).

Precipitation of soybean proteins by Ca(II) or Mg(II) is used in the isolation of 7S fraction (Koshiyama, 1965; Appu Rao and Narasinga Rao, 1976a). These cations precipitate the 11S protein, the other major protein in soybeans; they are bound by the protein also (Appu Rao and Narasinga Rao, 1975a,b). Of the various soybean proteins, 11S and 7S proteins have been isolated in homogeneous form and considerable information is available on their chemical and physico-chemical properties (Wolf, 1970). The interaction of Ca(II), Mg(II), or Zn(II) with 11S protein has been reported (Appu Rao and Narasinga Rao, 1975a,b, 1976b). In this paper we describe the interaction of Ca(II), Mg(II), and Zn(II) with 7S protein and compare it with that of 11S protein.

EXPERIMENTAL SECTION

Improved Pelican variety soybean was used in this investigation. Ca-45 and Zn-65 were purchased from Bhabha Atomic Research Center, Bombay, India, and α-chymotrypsin from Worthington Biochemicals. All the reagents were of analytical or equivalent grade. Solutions were prepared with deionized water.

Preparation of 7S Fraction. From the water extract of soybean proteins, 15S and 11S proteins were precipitated with Ca(II) (Koshiyama, 1965). The 7S and 2S proteins which remained in the supernatant were precipitated by full $(NH_4)_2SO_4$ saturation. The precipitate was dissolved in 0.05 M phosphate buffer of pH 7.8 containing 0.35 M NaCl and passed through a column of Sephadex G-100 which had been equilibrated with the same buffer. Fractions corresponding to the peak emerging immediately after the void volume of the column were collected. To the pooled fraction EDTA was added to a concentration of 5×10^{-3} M to remove any bound Ca(II); the solution was then dialyzed extensively against the buffer. The homogeneity of the preparation was determined by ultracentrifugation and gel electrophoresis at pH 7.8. It contained 5–10% of 11S protein.

Protein Concentration. This was determined by absorbance measurements at 280 nm. A value of $E_{1\ \mathrm{cm}}^{1\%}$ = 6.0 was used for converting absorbance to concentration. This value was determined by making absorbance measurements with a series of solutions of known protein concentration which had been determined by Kjeldahl nitrogen estimation. A value of 6.25 was used to convert nitrogen content to protein content.

Equilibrium Dialysis. The details of the method are the same as described earlier (Appu Rao and Narasinga Rao, 1975a,b, 1976b).

Estimation of Metal Ions. Ca(II), Mg(II), and Zn(II) were estimated by the methods described earlier (Appu Rao and Narasinga Rao, 1975a,b, 1976b).

Rate of Proteolysis by α-Chymotrypsin. Ten milliliters of 2% protein solution in 0.05 M Tris-HCl buffer of pH 7.8 containing varying amounts of metal ions was incubated at 37 °C. After preincubation, 10 ml of α-chymotrypsin (20 μg/ml) was added and the solutions were mixed well. At the end of each time interval, 2 ml of the reaction mixture was withdrawn and the reaction was "stopped" by the addition of 2 ml of 20% Cl_3CCOOH. After centrifugation, absorbance of the supernatant was read at 280 nm. Time intervals from 2 to 30 min were used.

Optical Rotatory Dispersion (ORD) and Circular Dichroism (CD). A JASCO-J-20 automatic spectropolarimeter fitted with xenon lamp photomultiplier was used for ORD and CD measurements using a quartz cell of 5-mm optical path. Protein solutions (0.04%) were used. The optical rotation was converted to mean residue ro-

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tation, $[m]$, and CD data were converted to mean residue ellipticity, $[\theta]$. A value of 115 for mean residue weight was used (Fukushima, 1968).

Fluorescence Spectra. The measurements were made with a Perkin-Elmer fluorescence spectrophotometer 203 at room temperature (~25 °C). Protein solutions (0.01%) were used for measurements.

Heat Coagulation. Five milliliters of 0.5% protein solution in buffer containing metal ion was heated for 15 min at various temperatures from 30 to 97 °C. For each temperature a separate aliquot was used. At the end of 15 min the solution was cooled to room temperature. In the case of Ca(II) and Mg(II), the turbidity that developed on heating could not be sedimented by centrifugation at 18000 rpm. Therefore, the turbidity was measured to determine the extent of coagulation. Turbidity was determined by measuring the transmittance at 540 nm; as turbidity increased transmittance decreased. From the measurement, a quantity $[(T_0 - T)/T_0] \times 100$ was calculated, where T_0 is the transmittance of the unheated solution and T that of the heated solution. This quantity was taken as a measure of coagulation. In the case of Zn(II), the precipitate formed on heating was sedimented at 6000 rpm and the absorbance of the supernatant was read at 280 nm from which the extent of precipitation was calculated.

Precipitation of Proteins with Metal Ions at 30 °C. To 2.0 ml of 2% protein solution in buffer, varying amounts of metal ion were added and the final volume made to 5.0 ml. The solution was maintained at 30 °C for 6 h in a constant temperature water bath with shaking. At the end of the period the resulting precipitate was removed by centrifugation at 6000 rpm for 20 min. The absorbance of the supernatants, after dilution wherever necessary, was read at 280 nm. From the absorbance of the original solution and that of the supernatant, the percentage of protein precipitated was calculated.

RESULTS AND DISCUSSION

For the isolation of 7S fraction from soybean proteins Ca(II) was used to precipitate the 11S protein. It was necessary, therefore, to remove Ca(II) ions that might have been already bound by the 7S protein. The bound ions were removed by treating the protein with EDTA and dialyzing extensively. The following experiment was made to determine the efficiency of this method to remove the bound Ca(II) ions. Ca-45 was mixed with cold Ca(II) and this mixture was used in the preparation of the 7S protein. The radioactivity of the 7S protein was measured both before and after EDTA treatment. The value before EDTA treatment was 17355 cpm/10 mg of precipitate and after EDTA treatment it was 50 cpm/10 mg suggesting that Ca(II) had been removed almost completely.

Binding of Ca(II). The binding data were determined by equilibrium dialysis at pH 7.8 in 0.1 M borate buffer and 0.05 M Tris-HCl buffer. The latter buffer system was used since 7S protein is a glycoprotein and the carbohydrate residue may form a negatively charged carbohydrate–borate complex (Davidson, 1967) and this complex may itself bind Ca(II). The binding data at pH 5.5 were collected in 0.1 M acetate buffer containing 0.5 M NaCl; the addition of salt to the buffer was necessary to obtain required protein concentration and the solubility of the protein in NaCl-free buffer solution was poor. The binding of Ca(II) by 7S protein is shown in Figure 1A. The data have been expressed as ν, the number of ions bound per 100000 g of protein plotted against the logarithm of free equilibrium metal ion concentraton, A. Binding at any Ca(II) concentration less than saturation was higher in

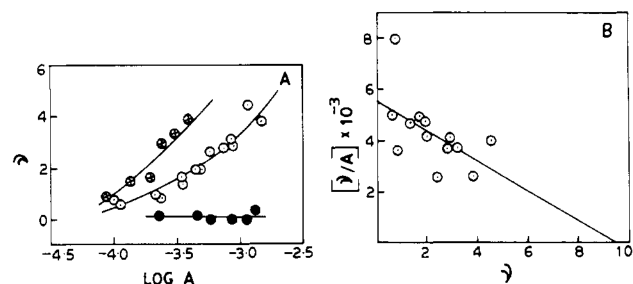

Figure 1. Binding of Ca(II) by 7S protein. (A) (⊙) Binding of Ca(II) by 7S protein in 0.05 M Tris-HCl buffer of pH 7.8. (⊕) Binding of Ca(II) by 7S protein in 0.1 M borate buffer of pH 7.8. (•) Binding of Ca(II) by 7S protein in 0.1 M acetate buffer of pH 5.5. Solid line in each case is a calculated curve. (B) Scatchard plot of binding of Ca(II) by 7S protein in 0.05 M Tris-HCl buffer of pH 7.8. The line drawn is a least-squares line.

Table I. Values of n and k of 7S Protein for the Binding of Different Metal Ions

Metal	Buffer	k	n
Ca(II)	0.1 M borate (pH 7.8)	1.4×10^3	10
Ca(II)	0.05 M Tris-HCl (pH 7.8)	5.5×10^2	10
Mg(II)	0.1 M borate buffer (pH 7.8)	1.2×10^3	10
Mg(II)	0.05 M Tris-HCl (pH 7.8)	5.0×10^2	10
Zn(II)	0.2 M acetate (pH 6.5) + 0.1 M NaCl	3.75×10^3	8
Zn(II)	0.2 M acetate (pH 6.5) + 0.5 M NaCl	1.0×10^3	8

borate buffer than in Tris-HCl buffer. At pH 5.5 the binding was negligible.

The binding data were analyzed with the Scatchard equation (Scatchard, 1949) to determine the association constant, k, and the maximum number of ions bound, n. The Scatchard plot of the binding data in Tris-HCl buffer is given in Figure 1B. The binding data could be fitted to a single straight line. The values of k and n are given in Table I. Using these values the binding curve was calculated with eq 1. The calculated curve is shown as

$$v = knA/(1 + kA) \tag{1}$$

the solid line in Figure 1A. There was a good fit between the calculated curve and the experimental data.

The maximum number of Ca(II) ions bound by 7S protein in Tris-HCl buffer was 10. This value agrees well with the histidine content of 7S protein (Koshiyama, 1968). At pH 5.5 there was practically no binding (Figure 1). This could also be due to the suppressing effect of NaCl on metal binding. The observation that the maximum number of ions bound (n) agreed with the histidine content of the protein would suggest that the imidazole groups of the histidine residues are the probable binding sites. It has been reported earlier that the probable binding sites in 11S protein for Ca(II) are the imidazole groups of the histidine residues (Appu Rao and Narasinga Rao, 1975a). A comparison of k values for Ca(II) binding by 7S and 11S proteins suggests that 11S protein binds Ca(II) more avidly than the 7S fraction. There is more than a twofold increase in affinity.

It has been mentioned earlier that the 7S protein preparation contained 5–10% 11S protein impurity. Correction of the binding data for binding of Ca(II) by 11S protein did not materially affect the conclusions drawn above.

Binding of Mg(II). The binding of Mg(II) by 7S protein is shown in Figure 2A. The binding data were determined at pH 7.8 in both 0.1 M borate buffer and 0.05 M Tris-HCl buffer. Here again, binding was higher in

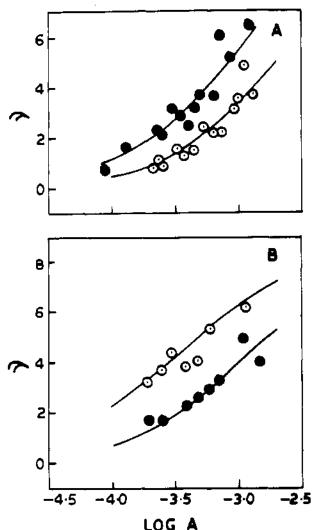

Figure 2. Binding of Mg(II) and Zn(II) by 7S protein. (A) (◎) Binding of Mg(II) by 7S protein in 0.05 M Tris-HCl buffer. (●) Binding of Mg(II) by 7S protein in 0.1 M borate buffer. (B) (◎) Binding of Zn(II) by 7S protein in 0.2 M acetate buffer of pH 6.5 containing 0.1 M NaCl. (●) Binding of Zn(II) by 7S protein in 0.2 M acetate buffer containing 0.5 M NaCl. Solid line in each case is a calculated curve.

borate buffer than in Tris-HCl buffer at concentrations less than saturation. The binding data gave a linear Scatchard plot. The n value was the same in both the buffers but the k value was higher in borate buffer. The n value obtained agreed well with the histidine content.

The affinity of 7S protein for Ca(II) and Mg(II) was higher in borate buffer than in Tris-HCl buffer. The fact that the n value was the same in both the buffer systems excludes the possibility that the negatively charged borate complex itself binds metal ions. The increase in k value could be due to increased negative charge on the protein molecule at the same pH in borate buffer.

Binding of Zn(II) by 7S Protein. Zn(II) binding studies with 7S protein were made at pH 6.5 in 0.2 M acetate buffer containing 0.1 M NaCl. Zn(II) was bound by the protein under the experimental conditions (Figure 2B). The binding was decreased by the addition of 0.5 M NaCl.

The Scatchard plot of the binding data consisted of a single straight line in both cases. The values of k and n are given in Table I. The solid line given in Figure 2B is a curve computed with these values. The value of n was the same in both cases (0.1 and 0.5 M NaCl) but k decreased considerably by the addition of NaCl. The n value suggests that here again probably the imidazole groups bind the metal ion.

The effect of binding of Ca(II), Mg(II), or Zn(II) on the physico-chemical properties of the protein was measured and is discussed below.

Rate of Proteolysis. The effect of binding of Ca(II) or Mg(II) on the rate of proteolysis of 7S protein by α-chymotrypsin was measured in 0.05 M Tris-HCl buffer of pH 7.8 (Figure 3). The addition of Ca(II) and Mg(II) had no effect on the rate of proteolysis of 7S protein. It is known that the rate of proteolysis of proteins is influenced by their conformation (Rupley, 1967). In the case of 11S protein a correlation has been reported between the extent of hydrolysis and the extent of denaturation of the protein (Fukushima, 1968). Therefore, the observation that the addition of Ca(II) or Mg(II) to the 7S fraction did not alter the rate of proteolysis would suggest that no marked changes in the conformation of the protein had occurred.

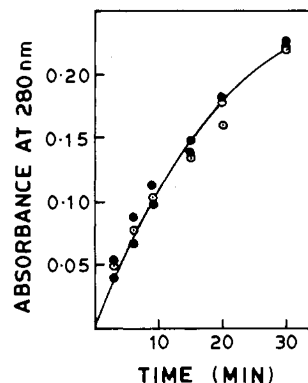

Figure 3. Rate of proteolysis of 7S protein by α-chymotrypsin in 0.05 M Tris-HCl buffer of pH 7.8 in the presence of Ca(II) or Mg(II): (◎) 7S protein; (⊕) 7S protein in the presence of 5.0×10^{-4} M Ca(II); (●) 7S protein in the presence of 5×10^{-4} M Mg(II).

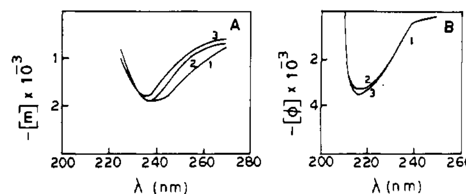

Figure 4. ORD and CD spectra of 7S protein in the presence of Ca(II) or Mg(II). (A) ORD of 7S protein in 0.05 M Tris-HCl buffer of pH 7.8: (1) 7S protein; (2) 7S protein in the presence of 5.0×10^{-4} M Ca(II); (3) 7S protein in the presence of 5×10^{-4} Mg(II). (B) CD of 7S protein in 0.05 M Tris-HCl buffer of pH 7.8: (1) 7S protein; (2) 7S protein in the presence of 5×10^{-4} M Ca(II); (3) 7S protein in the presence of 5×10^{-4} M Mg(II). Curves 1 and 2 are superposed.

Optical Rotatory Dispersion (ORD). The ORD of 7S protein measured in the uv region from 270 to 225 nm is given in Figure 4A. It exhibited a trough around 235 nm with $[m] = -1900$ deg cm^2/dmol. The wavelength and magnitude of the trough agreed well with the reported values (Fukushima, 1968). The protein contains predominantly the β structure (Fukushima, 1968). The magnitude of the trough was followed as a function of added metal ion concentration. It had no effect on either the position of the trough or its magnitude. Apparently no discernible conformational changes occurred in the protein due to the binding of Ca(II) or Mg(II).

Circular Dichroism (CD). The circular dichroism spectra of 7S protein exhibited a trough around 215 nm with $[\theta] = -3500$ deg cm^2/dmol (Figure 4B). Koshiyama and Fukushima (1973) have reported that the CD spectrum of 7S protein exhibits a trough around 210 nm with $[\theta] = -5700$ deg cm^2/dmol. The reason for the discrepancy in the trough position and magnitude of the peak is not clear. Polypeptides with β structure exhibit a characteristic trough around 215 nm (Greenfield and Fasman, 1969). The addition of Ca(II) or Mg(II) at the 5.0×10^{-4} M level did not cause any noticeable change in either the $[\theta]$ value or the peak position.

Fluorescence Spectrum. The fluorescence emission spectrum of 7S protein showed a maximum at 330 nm; the excitation maximum was at 280 nm (Figure 5A,B). The fluorescence was essentially due to tyrosine excitation and tryptophan emission. The 7S protein has 20 tyrosine and 2 tryptophan residues per 100000 g of protein (Koshiyama, 1968). Though the tryptophan content is low, the emission spectrum of 7S has the characteristics of tryptophan emission (Chen et al., 1969). The addition of Ca(II) or Mg(II) at 5.0×10^{-4} M concentration had no effect on

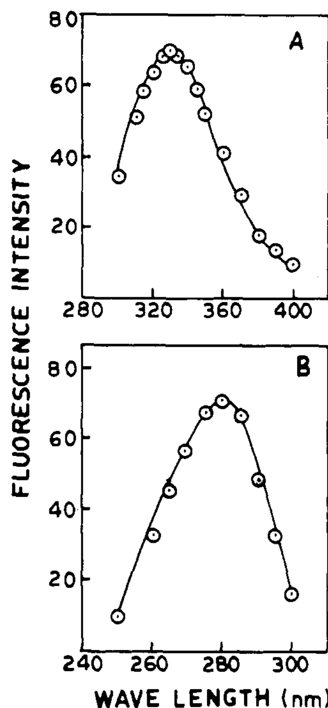

Figure 5. Fluorescence spectra of 7S protein in 0.05 M Tris-HCl buffer of pH 7.8: (A) emission spectrum of 7S protein; (B) excitation spectrum of 7S protein.

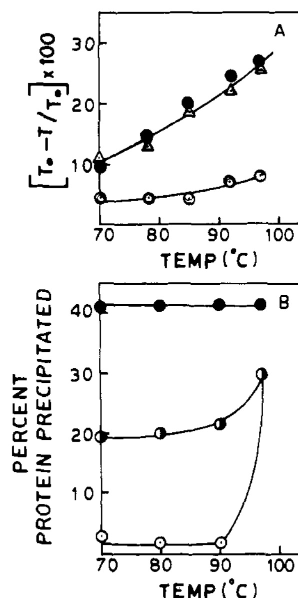

Figure 6. Heat coagulation of 7S protein in the presence of Ca(II), Mg(II), or Zn(II). (A) Heat coagulation of 7S protein in 0.05 M Tris-HCl buffer of pH 7.8: (◎) 7S protein; (●) 7S protein in the presence of 5 × 10^{-4} M Ca(II); (△) 7S protein in the presence of 5 × 10^{-4} M Mg(II). (B) Heat coagulation of 7S protein in 0.2 M acetate buffer of pH 6.5 containing 0.5 M NaCl: (◎) 7S protein; (◑) 7S protein in the presence of 4 × 10^{-4} M Zn(II); (●) 7S protein in the presence of 8 × 10^{-4} M Zn(II).

either the fluorescence emission intensity or position of emission maximum. The conformation around tyrosine or tryptophan did not appear to be affected. The results of experiments on proteolysis, ORD, CD, and fluorescence spectrum did not indicate any conformational change in the protein due to metal ion binding.

Heat Coagulation. The heat coagulation behavior of 7S protein was studied in the presence and absence of added metal ions. The effect of Ca(II) or Mg(II) on the heat coagulation of 7S protein is shown in Figure 6A. At pH 7.8, in the absence of added metal ion, the coagulation of 7S protein by heat was low as indicated by low turbidity. The addition of Ca(II) or Mg(II) at 5.0 × 10^{-4} M enhanced the aggregation of 7S protein. Both the metal ions caused the same amount of change. The effect was, however, smaller than in the case of 11S protein (Appu Rao and Narasinga Rao, 1975a,b).

The effect of Zn(II) on heat coagulation of 7S protein at pH 6.5 is shown in Figure 6B. The 7S protein precipitated to 30% at 97 °C. The addition of Zn(II) at 4 × 10^{-4} M enhanced the extent of precipitation at lower temperature but it was the same at 97 °C. At 8.0 × 10^{-4} M Zn(II) the precipitation increased from an initial value of 23% at 30 °C to 43% at 70 °C and then remained constant. However, no complete precipitation of 7S was obtained in the presence of Zn(II). This contrasted with the behavior of 11S protein where complete precipitation occurred in the presence of Zn(II) (Appu Rao and Narasinga Rao, 1976b).

Precipitation of 7S Protein by Metal Ions at 30 °C. The ability of Ca(II), Mg(II), and Zn(II) to precipitate the 7S protein is shown in Figure 7. The protein was precipitated to an extent of 40% by Ca(II) at a concentration of 1.0 × 10^{-2} M. Even at this high concentration of the metal ion the protein was not completely precipitated. The precipitation increased up to a Ca(II) concentration of 1.0 × 10^{-2} M and then decreased. At 5.0 × 10^{-2} M there was only 10% precipitation. The mechanism of precipitation is not clear. Addition of NaCl at 0.5 M concentration completely suppressed precipitation. In the case of Mg(II),

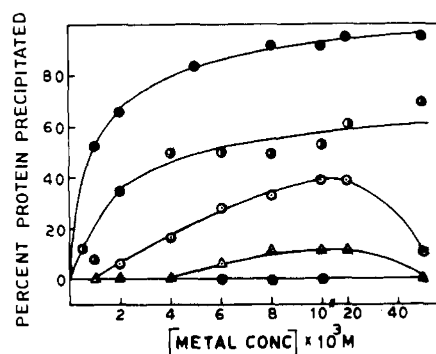

Figure 7. Precipitation of 7S protein by Ca(II), Mg(II), or Zn(II): (◎) Ca(II) at pH 7.8 in 0.05 M Tris-HCl buffer; (⊕) Ca(II) at pH 7.8 in 0.05 M Tris-HCl buffer containing 0.5 M NaCl; (△) Mg(II) at pH 7.8 in 0.05 M Tris-HCl buffer; (▲) Mg(II) at pH 7.8 in 0.05 M Tris-HCl buffer containing 0.5 M NaCl; (●) Zn(II) at pH 6.5 in 0.2 M acetate buffer containing 0.1 M NaCl; (◑) Zn(II) at pH 6.5 in 0.2 M acetate buffer containing 0.5 M NaCl.

there was very little precipitation up to 5.0 × 10^{-3} M; in the region 5.0 × 10^{-3} to 1.0 × 10^{-2} M it was about 10% which decreased at 5 × 10^{-2} M Mg(II). Both Ca(II) and Mg(II) were good precipitants only at low concentrations and at higher concentrations the protein was again solubilized. Though Ca(II) and Mg(II) were bound to the same extent, their ability to precipitate 7S protein differed. In the case of Zn(II) the extent of precipitation increased with metal ion concentration up to 5.0 × 10^{-3} M concentration. The 7S protein was almost quantitatively precipitated by Zn(II) at 5.0 × 10^{-2} M. Thus, Zn(II) was a better precipitant of this protein than either Ca(II) or Mg(II). It can be recalled here that the protein binds Zn(II) more avidly than it does either Ca(II) or Mg(II). The addition of NaCl decreased the precipitation of 7S by Zn(II), but it was not completely suppressed. At 5.0 × 10^{-2} M, about 60–65% protein was precipitated.

Comparison of the Binding of Metal Ions by the 11S and 7S Proteins. The measurements with 11S and 7S proteins indicated the following similarities and differences. Both the proteins bind Ca(II) and Mg(II) at pH 7.8 possibly at the imidazole groups of histidine residues. They bind Zn(II) at pH 6.5 also at the imidazole groups. A change in buffers (borate or Tris-HCl) had no effect on the binding of Ca(II) by 11S protein. However, the binding of Ca(II) or Mg(II) by 7S protein was higher in borate buffer than in Tris-HCl buffer. This is attributed to the increased negative charge on the protein due to the formation of a borate–carbohydrate complex; 7S protein is a glycoprotein. The Scatchard plot of Ca(II) binding by the 11S protein consisted of a single straight line. On the other hand the plot of Mg(II) or Zn(II) binding consisted of two straight lines suggesting nonequivalence of binding sites. In the case of 7S protein the binding of all the three metals gave Scatchard plots which consisted of single straight line. The addition of NaCl to the buffer had no effect on Zn(II) binding by 11S protein; it decreased Ca(II) binding and totally suppressed Mg(II) binding. In the case of Zn(II) binding by 7S, this was decreased by the addition of NaCl.

The addition of Ca(II) or Mg(II) to the EDTA-treated 11S protein caused changes in the sedimentation velocity pattern of protein which suggested reaggregation of the protein. In the case of 7S protein no such effect was observed. The addition of metal ions to 11S protein or 7S protein did not affect the rate of hydrolysis of protein by α-chymotrypsin. ORD and CD spectra suggested minor conformational changes in 11S protein due to the binding of Ca(II) or Mg(II). However, no such effect was observed in the case of 7S protein. The fluorescence spectrum of both the proteins was unaffected by the addition of metal ions. Ca(II) and Mg(II) enhanced the turbidity caused by heat coagulation of 11S and 7S proteins and Zn(II) enhanced precipitation. At room temperature addition of Ca(II), Mg(II), and Zn(II) at higher concentrations precipitated the 11S protein quantitatively. On the other hand, Ca(II) precipitated the 7S protein to 40%, Mg(II) 10%, and Zn(II) 90%. In all the cases addition of NaCl reduced precipitation.

LITERATURE CITED

Appu Rao, A. G., Narasinga Rao, M. S., *Cereal Chem.* **52**, 21 (1975a).
Appu Rao, A. G., Narasinga Rao, M. S., *J. Agric. Food Chem.* **23**, 657 (1975b).
Appu Rao, A. G., Narasinga Rao, M. S. (1976a), *J. Agric. Food Chem.*, in press.
Appu Rao, A. G., Narasinga Rao, M. S. (1976b), *J. Agric. Food Chem.*, preceding paper in this issue.
Chen, R. F., Edelhoch, H., Steiner, R. F., in "Physical Principles and Techniques of Protein Chemistry", Part A, Leach, S. J., Ed., Academic Press, New York, N.Y., 1969, p 171.
Davidson, E. A., "Carbohydrate Chemistry", Holt, Rinehart and Winston, New York, N.Y., 1967, p 111.
Fukushima, D., *Cereal Chem.* **45**, 203 (1968).
Greenfield, N., Fasman, G. D., *Biochemistry* **8**, 4108 (1969).
Koshiyama, I., *Agric. Biol. Chem. (Tokyo)* **29**, 885 (1965).
Koshiyama, I., *Cereal Chem.* **45**, 394 (1968).
Koshiyama, I., Fukushima, D., *Cereal Chem.* **50**, 114 (1973).
Rupley, J. A., *Methods Enzymol.* **11**, 905 (1967).
Scatchard, G., *Ann. N.Y. Acad Sci.* **51**, 660 (1949).
Wolf, W. J., in "Soybeans: Chemistry and Technology", Smith, A. K., and Circle, S. J., Ed., Avi Publishing Co., Westport, Conn., 1970.

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Metmyoglobin and Nonheme Iron as Prooxidants in Egg-Yolk Phospholipid Dispersions and Cooked Meat

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The influence of adding either metmyoglobin (MetMb) or ferrous, nonheme iron (Fe^{2+}) upon oxidation of egg-yolk phosphatidylethanolamine (PE) was studied at pH 5.5 and 7.0. Control (untreated) PE dispersions had greater oxygen uptake at pH 7.0 than at pH 5.5, whereas addition of either MetMb or Fe^{2+} caused little further oxidation at pH 7.0. On the other hand, both Fe^{2+} and MetMb accelerated oxidation of PE by three- to fourfold at pH 5.5. MetMb increased the production of fluorescent materials from PE at both pH 5.5 and 7.0, while Fe^{2+} accelerated production of fluorescent compounds only at pH 5.5 and inhibited fluorescence at pH 7.0. Tentative identification of n-hexanal in the headspace of oxidizing PE dispersions and of cooked meat by GLC (gas–liquid chromatography) suggested that it is one of the principal products of oxidation. Addition of 5 ppm of Fe^{2+} to water-extracted and heated meat produced an n-hexanal peak over twice the area of the control, whereas addition of tripolyphosphates decreased the quantity of hexanal by one-half. Results indicate that both Fe^{2+} and MetMb may act as prooxidants under certain conditions.

Phospholipids undergo rapid oxidation in many food products, including cooked meat, which results in serious flavor problems (Keller and Kinsella, 1973; Younathan and Watts, 1960). The speed of oxidation for phospholipids in cooked meat is at least partially due to their high content of unsaturated fatty acids (Luddy et al., 1970; O'Keefe et al., 1968; Giam and Dugan, 1965; Hornstein et al., 1961). The nature of the nitrogenous component bound in ester linkage to the phosphoric acid moiety may also influence the oxidation of the unsaturated fatty acids in the phospholipid molecule (Corliss and Dugan, 1970; Tsai and Smith, 1971).

Tsai and Smith (1971) studied the effects of ethanolamine, choline, and serine on the oxidation of methyl

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