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Application of Hydrogen Ion Equilibria Studies to the Binding of Metals with Protein : Part I. Binding of Zn (II), Mg (II) and Mn(II) with Soybean Protein by Equilibrium Dialysis Technique

Wahid U. Malik and M. V. Singh

Hydrogen ion equilibria studies have been of great importance in ascertaining the different ionizable groups of the protein. Several workers namely, Linderstrom-Lang[1], Klotz[2], Cannon[3] and Tanford[4] *et. al.*, have done work in this direction. Malik and coworkers[5-7] have used these studies to determine the ionizable groups of transfusion gelatin and its binding with a number of metallic cations. Recently these studies have been extended to soybean protein. The results of these investigations have been applied to determine the binding of Zn(II), Mg(II) and Mn(II) with soybean protein employing equilibrium dialysis technique.

EXPERIMENTAL

Soybean protein used in the present studies was prepared by the method of Kunihiko Suminokura[8]. The soybeans (B.D.H.) were dehulled, powdered and then defatted with cold petroleum ether. The defatted material was treated with eight times as much as of 0·05*N* KOH. The extraction of the protein was repeated four times. The extracted protein was precipitated at *p*H 4·90 with dil. HCl. The protein thus obtained was redissolved and reprecipitated twice at the same *p*H. The above sample was washed with doubly distilled water until free from chloride ions.

Soybean protein is a mixture of three components, namely glycinin, gluletin and phaseolin. Glycinin is the major component of soybean protein and precipitation of the protein at *p*H 4·90 yields only glycinin fraction.

Reagents and solutions : Solution of soybean protein was prepared at *p*H 7·0 using dil. KOH. Estimation of the protein was done by applying the correction for potassium content.

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2. I. M. Klotz, "The Protein", H. Neurath and K. Bailey (ed.), Vol. I, Pt. B, Acemedic Press, New York, 1953, 727.
3. R. K. Cannon, A. C. Kibrick and A. H. Palmer, *Ann. N.Y. Acad. Sci.*, 1941, **41**, 243.
4. C. Tanford "Hydrogen Ion Titration Curves of Proteins" in Shedlovsky (ed.), Electrochemistry in Biology and Medicine, John Wiley and Sons, New York, 1955, 248.
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8. Kunihiko Suminokura, *J. Agr. Chem. Soc., Japan*, 1939, **15A**, 133.

A.R. samples of zinc sulphate, magnesium and manganese chlorides were dissolved in doubly distilled water and the metal contents of the stock solutions were estimated by complexometric titrations[9] using EDTA as titrant and Erichrome Black T as indicator.

Acetate buffer of *p*H 5·57 was prepared by mixing sodium acetate and acetic acid (each of 0·2*M*). Ammonia-ammonium chloride buffer of *p*H 7·5 was prepared by mixing 1 molar solution of each.

The hydrogen ion equilibria studies[10] of soybean protein have provided the following information regarding the different ionizable groups of the protein.

Ionizable Groups in Soybean Protein

Groups	Reasonable analytical values	Observed
Side chain carboxyl	--	94
Imidazole	18	18
Side chain amino	45	46
Guanidinium	42	41
Tyrosine/Tryp.	3.18	3.14
Total cationic	105	102

Equilibrium dialysis sets : 5·0 ml. of protein solution (conc. 1·5%) was placed on Visking Nojax Sausage bag and equilibrated against 5·0 ml. of metal solution in each case. A series of such equilibration tubes were prepared containing varying amounts of metal ions. With mechanical shaking the time to attain the equilibria was found approximately 40 hr. After the required period, the dialysis bags were withdrawn from the tubes and then external solution was analysed for metal contents. Blanks were also run to determine the amount of metal ions bound to the material of the dialysis bags but the amount of the metal bound was found to be extremely small. All the measurements were carried out at temp. $25 \pm 1°$.

RESULTS AND DISCUSSIONS

Equilibrium dialysis studies provide enough evidence for the interaction of these bivalent metal cations with the carboxyl as well as imidazole groups of the protein.

Two different *p*H levels were chosen for the present study. At *p*H 5·5 the carboxyl groups of the protein are completely deprotonated and thus available for interaction and at *p*H 7·5 imidazole groups lose their positive charge. A series of observations were made at *p*H 5·5 and 7·5 over a wide range of metal concentration. Fig. 1 represents the extent of

9. Schwarzenbach, G. and H. Irvin, "Complexometric Titration" (Interscience, New York, 1957).
10. Ph.D. thesis, submitted to the U.O.R. Roorkee in 1967.

binding with increase of metal ion concentration. The curves provide evidence for the binding. The Fig. 1A reveals that eight zinc ions are bound to the protein at *p*H 5·5. After this saturation limit is attained. Since at *p*H 5·5 only carboxyl groups are deprotonated[11],

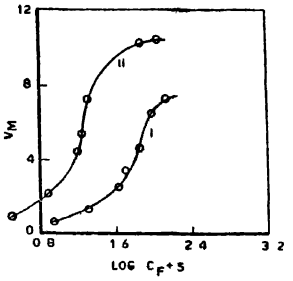

FIG 1 A - BINDING OF Zn(II) WITH SOYBEAN PROTEIN I, PH 5 5, II, PH 7 5

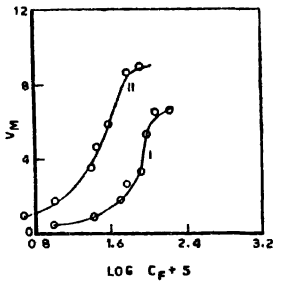

FIG 1 B - BINDING OF Mg (II) WITH SOYBEAN PROTEIN I, PH 5 5, II, PH 7 5

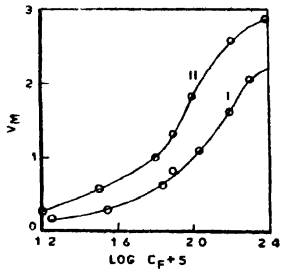

FIG 1 C - BINDING OF Mn(II) WITH SOYBEAN PROTEIN I, PH 5 5, II, PH 7 5

hence binding takes place only with carboxyl groups. At *p*H 7·5, the binding is with imidazole group. The data to obtain the intrinsic association constant K, were calculated by applying the Scatchard's equation,

$$K = \frac{V^{-}M}{(n - V_{\bar{H}} - V_{\bar{M}})C_F} \qquad (1)$$

where $V^{-}M + V^{-}H$ are the active sites covered by metal ions and hydrogen ions. C_F is the free metal concentration at equilibrium and n is the number of intrinsically identical groups. The values of n and V_H[10] were taken from literature.

The log K values of zinc-carboxyl system (calculated from eq. 1) have been found to be constant over a wide range of metal ion concentration This fact further supports that carboxyl groups of the protein are taking part in the reaction. As the *p*H increases from 5·5 to 7·5, V_M also increases, indicating that imidazole groups are also involved in the reaction.

11. Wahid U. Malik and Salahuddin, *J. Electroanal Chem.*, 1963, **5**, 68.

A similar explanation may also be offered for the data on the magnesium-soybean protein system. Manganese shows very little binding with the carboxyl and imidazole groups.

The log K values for the binding of zinc to carboxyl and imidazole groups of soybean protein resemble the log of the first association constant of zinc-acetate and zinc imidazole system (Table 1).

TABLE 1

Comparative data on the binding of metal ions with different proteins.

Ligand	Method	log K (carboxyl group)			log K (imidazole group)		
		Zn^{+2}	Mg^{+2}	Mn^{+2}	Zn^{+2}	Mg^{+2}	Mn^{+2}
Soybean protein	Equilibrium[a]	1.86	1.66	0.98	2.86	2.63	1.55
,,	*p*H-metric	1 90	—	1.00	2.77	—	1.76
Serum albumin	Polarographic[b]	—	—	—	2.90	—	—
,,	Equilibrium[c]	—	—	—	2.82	—	—
Acetate		1.03	1.05[d]	—	—	—	—
Imidazole		—	—	—	2.58[e]	—	—

a. Present study, *b.* Reference 12, *c.* Reference 13, *d.* Reference 14, *e.* Reference 15.

The ability of metal ions to interact with the carboxyl and imidazole groups of the protein follows the order,

$$Zn(II) > Mg(II) > Mn(II)$$

A comparison of the log K values for different systems is given in Table 1.

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Department of Chemistry,
University of Roorkee,
Roorkee.

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