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Physicochemical Properties of Vegetable Proteins: Part V—Complexes of Vegetable Proteins with Zn(II), Co(II) & Ni(II)

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The binding of Zn^{2+}, Co^{2+} and Ni^{2+} ions to a few vegetable protein sols stabilized at *p*H 7·5 has been investigated using the equilibrium dialysis technique. The vegetable proteins used are whole Punjab soybean No. 1 protein, Punjab soybean No. 1 glycinin, whole Bragg soybean protein, Bragg soybean glycinin, peanut arachin, peanut conarachin, and whole gliadin. The number of moles of the metal cations bound per 10^5 g of protein increases with the increase in [metal cation] in the solution at equilibrium and attains a maximum value which is very close to the number of imidazole groups in the proteins. The intrinsic binding constants (k) as well as the standard free energy (ΔF°_1) and entropy (ΔS°_1) changes of the process have been calculated.

THE biological significance of the natural metallo-proteins have led several workers[1-7] to study the complex formation of various proteins with different metal cations under different conditions. Klotz *et al.*[4-7], while working with a few animal proteins, estimated the number of binding sites and found that the association constants vary significantly with the nature of metal cation and the protein. An examination of the literature shows that similar investigations on some of the common vegetable proteins have not been reported. The experiments based on flocculation studies of the sols of a few such proteins by the action of electrolytes[8], however, indicate that such proteins can also form complexes with certain transition metal cations. This paper reports the results of such a study.

Materials and Methods

Proteins and their major fractions isolated from the two varieties of soybeans (Punjab soybean No. 1 and Bragg variety) and peanuts (C 501 variety of Punjab Agricultural University) together with gliadin fraction of wheat protein (gluten, BDH) were used in the present investigations. The methods used for the isolation of the proteins and their fractions have been described elsewhere[9]. Sols of 1% concentration at *p*H 7·5 were prepared by suspending the required amounts of proteins in 20 ml of CO_2-free distilled water, adding enough of NaOH solution gradually with continuous stirring to dissolve the proteins and raise the *p*H to ~8·0. The excess of alkali was removed by dialysis till the *p*H was brought down to 7·5. The electrolytes used were the chlorides of zinc, cobalt and nickel of AR (BDH) grade. The solutions were prepared in triply distilled water.

Procedure — Aliquots (5 ml each) of the sols were taken in cellophane bags and placed in contact with aq. solutions of increasing concentrations of a given electrolyte. The amount of cation taken up by the sol, on the attainment of equilibrium at 30° (which required usually 36 hr), was estimated by noting the fall in [cation] in the surrounding solution by the usual analytical methods. Solutions of the electrolytes were prepared in 0·15M $NaNO_3$ to avoid Donnan membrane effect.

Results and Discussion

The number of moles of various metal cations (Zn^{2+}, Co^{2+} and Ni^{2+}) bound by the various protein sols were plotted against log [cation] in solution at equilibrium (Fig. 1). It is seen that the number of moles of any of the ions bound to a protein increases with the increase [free ions] in solution and, ultimately, tends to attain a maximum value. The maximum values of various cations bound to 10^5 g of each protein are given in Table 1 and are seen to be fairly close to one another as well as to the number of imidazole groups contained in each protein as obtained from their respective titration curves against sodium hydroxide[10]. This shows that only imidazole groups are involved in the binding of Zn^{2+}, Co^{2+} and Ni^{2+} ions. In the case of most of the animal proteins it has been reported[2,11,12] that while at *p*H 7 Zn^{2+} ions are bound only to imidazole groups, Co^{2+} and Ni^{2+} ions are bound to carboxyl groups also, as the values are much higher in the latter cases[12]. The vegetable proteins studied presently contain large number of carboxyl groups, varying from 39 in wheat gliadin to 162 per 10^5 g in conarachin fraction of peanut protein, which ionize at *p*H 7·5, but there appears to be no evidence for the involvement of these groups with any of the three cations studied as the values remain quite low.

Fiess and Klotz[13], applying the law of mass action, have shown that if all the binding sites have the same intrinsic binding constant k then,

$$\text{Lim}_{[\text{Free ion}]\to 0}\ r/[\text{Free ion}] = nk \qquad \ldots(1)$$

where r is the number of moles of the metal ion bound per 10^5 g of protein and n is the number of

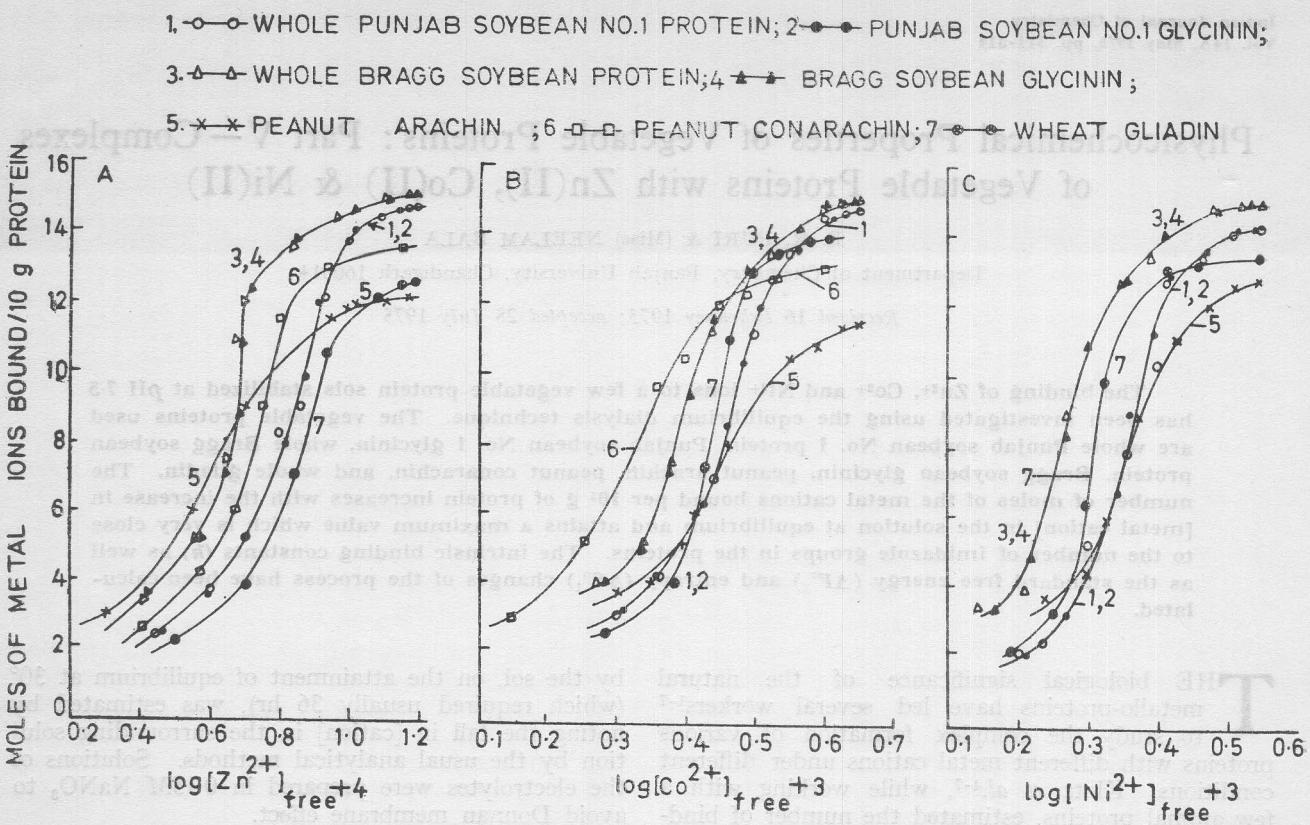

Fig. 1 — Effect of concentration of free metal cations on their binding with various vegetable proteins at 30° [(A) Zn^{2+}; (B) Co^{2+}; (C) Ni^{2+}]

TABLE 1 — MAXIMUM NUMBER OF MOLES OF Zn^{2+}, Co^{2+} AND Ni^{2+} IONS BOUND TO VARIOUS VEGETABLE PROTEINS AT *p*H 7·5

No. of moles of metal cations bound per 10^5 g protein			No. of imidazole groups/ 10^5 g protein	No. of Carboxyl groups/10^5 g protein
Zn^{2+}	Co^{2+}	Ni^{2+}		
Whole Punjab soybean No. 1 protein				
14·73	14·75	14·40	12	113
Punjab soybean No. 1 glycinin				
14·73	14·75	14·40	12	134
Whole Bragg soybean protein				
15·10	15·00	15·08	16	132
Bragg soybean glycinin				
15·10	15·00	15·08	12	144
Peanut arachin				
12·15	11·50	12·90	12	137
Peanut conarachin				
13·55	13·05	—	10	162
Wheat gliadin				
12·60	13·90	13·40	13	39

available sites, viz. the number of groups (imidazole+carboxyl) ionizing at *p*H 7·5. Further, for a single set of binding sites, $nk = k_1$, where k_1 is the equilibrium constant for the first metal ion complexed with the protein. Evidently, the equilibrium constant (k_1) can be evaluated by plotting r/[free ion] against [free ion] and noting the intercept at [free ion] = 0. Such plots for the binding of Zn^{2+} ions to various proteins are shown in Fig. 2. The values of k_1 obtained by extrapolating the above linear plots to zero concentration for the various proteins are given in Table 2.

The intrinsic binding constant (k) can also be calculated by the simplified form of Scatchard[14] Eq. (2),

$$\ln r/(n-r)[\text{free ion}] = \ln k \qquad \ldots(2)$$

which has been used by a number of investigators[7,13] in the case of proteins. The data relating to the pependence of the extent of binding on [free metal ions] plotted in Fig. 1 were used for calculating k and hence k_1. The values of log k (Table 3) so calculated were found to be fairly constant. Thus Scatchard equation was quite applicable to the complex formation of metal cations with various vegetable proteins. It is significant to note that the values of equilibrium constant (k_1) obtained from Eqs. (1) and (2) are in fair agreement with each other (Table 2).

The standard free energy change ($\Delta F_1°$) for the complex formation of first metal ion were calculated employing the well known relationship (3), and the values, recorded in Table 2, are seen to be of the same order. However, the values for the binding

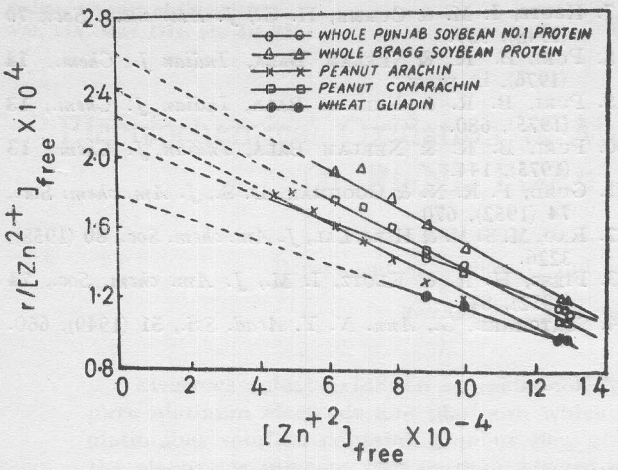

Fig. 2 — Plots of r/[free ion] versus [free ion] for the binding of Zn^{2+} ions with various vegetable proteins

Table 2 — Values of Equilibrium Constant (k_1), Free Energies ($-\Delta F_1^0$) and Entropies (ΔS_1^0) of Binding of Various Cations to Vegetable Proteins

Cation	$k_1 \times 10^{-4}$	$-\Delta F_1^0$ (kcal mole^{-1})	ΔS_1^0 (cal mole^{-1} deg^{-1})
Whole Punjab soybean No. 1 protein			
Zn^{2+}	2·10 (1·55)	6·05	19·97
Co^{2+}	0·53 (0·38)	5·23	17·25
Ni^{2+}	0·69 (0·50)	5·40	17·79
Whole Bragg soybean protein			
Zn^{2+}	2·59 (1·90)	6·15	20·31
Co^{2+}	0·59 (0·45)	5·29	17·46
Ni^{2+}	0·82 (0·53)	5·49	18·10
Pebnut arachin			
Zn^{2+}	2·24 (1·47)	6·11	20·17
Co^{2+}	0·37 (0·30)	5·02	16·56
Ni^{2+}	0·59 (0·43)	5·27	17·41
Peanut conarachin			
Zn^{2+}	2·39 (1·64)	6·13	20·22
Co^{2+}	0·62 (0·41)	5·32	17·56
Ni^{2+}	—	—	—
Wheat gliadin			
Zn^{2+}	1·78 (1·32)	5·93	19·57
Co^{2+}	0·63 (0·48)	5·34	17·62
Ni^{2+}	0·79 (0·64)	5·46	18·04

Values in parentheses were calculated using Scatchard equation.

of Zn^{2+} ions are slightly higher in magnitude than those for other metal cations.

$$-\Delta F_1^\circ = RT \ln k_1 \qquad ...(3)$$

The standard entropy change (ΔS_1°) for the complex formation of first metal cation was calculated using Eq. (4) (Table 2).

$$\Delta S_1^\circ = -\Delta F_1^\circ / T \qquad ...(4)$$

The standard enthalpy changes (ΔH_1°) as calculated

Table 3 — Log k Values for the Binding of Various Cations at Different Concentrations with the Vegetable Proteins as Obtained from Scatchard Equation

$[Zn^{2+}] \times 10^4$ (M)	log k for Zn^{2+}	$[Co^{2+}]$ or $[Ni^{2+}] \times 10^4$ (M)	log k	
			Co^{2+}	Ni^{2+}
Whole Punjab soybean No. 1 protein				
6·31	2·000	28·18	1·361	1·623
7·94	2·087	31·62	1·499	1·623
8·91	2·108	35·48	1·525	1·568
10·00	2·095	39·81	1·509	—
Whole Bragg soybean protein				
6·31	2·196	28·18	1·484	1·580
7·94	2·117	31·62	1·487	1·556
8·91	2·080	35·48	1·502	1·506
10·00	2·040	39·81	1·442	—
Peanut arachin				
6·31	2·065	28·18	1·267	1·477
7·94	2·010	31·62	1·320	1·462
8·91	1·973	35·48	1·325	1·431
10·00	1·929	39·81	1·303	—
Peanut conarachin				
6·31	2·033	28·18	1·425	—
7·94	2·004	31·62	1·397	—
8·91	1·964	35·48	1·362	—
10·00	1·919	39·81	1·312	—
Wheat gliadin				
6·31	2·281	28·18	1·908	2·086
7·94	2·422	31·62	2·021	2·040
8·91	2·464	35·48	1·963	2·130
10·00	2·450	39·81	1·959	—

using Eq. (5) for the complex formation of various metal cations to proteins are found to be negligibly small[13]. The results were checked by repeating the experiments at two other temperatures (25° and 35°) when almost identical values were obtained as at 30°.

$$\Delta F_1^\circ = \Delta H_1^\circ - T\Delta S_1^\circ \qquad ...(5)$$

It is seen from the data in Table 2 that the process in each case is accompanied by an increase in entropy. This appears to be due to extensive hydration of the protein micelles as well as of metal cations. Some of the polarized molecules of water attached to them are released during the complex formation reaction. This results in an increase in the number of molecular species and hence the entropy of the reacting system.

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