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Study of calcium binding to different preparations of yeast (*Saccharomyces cerevisiae*) protein by using an ion selective electrode

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Abstract

The calcium binding capacity of a native brewer's yeast protein (PL) was compared to protein from the same source, after treatment with 3% sodium trimetaphosphate (PLP). Calcium titration was done by the use of an ion-selective electrode. For measurements made at pH 6.5, the number of calcium binding sites per 10^5g protein was 29 for PL and 37 for PLP. The association constant K was 3.2×10^{-3} and 2.7×10^{-3} for PL and PLP, respectively. Dialysis of sample PLP against 0.01 M EDTA increased the number of binding sites to 49. The higher number of binding sites in PLP is explained by the presence of phosphate moieties associated with the protein molecules and also by possible conformational changes occurring in the protein molecules as a result of STMP treatment, exposing a larger number of amino acid side chains capable of combining with Ca^{2+}.

1. Introduction

Proteins in general can interact with and bind small ions. The binding capacity of a protein is dependent on its chemical and structural properties. In addition, pH, size, charge and ion concentration are also influencing factors (Carr, 1953). Large multivalent ions are more strongly bound than small monovalent ions. Normally proteins bind ions of opposite charge at a certain pH due to deprotonation of side chain amino acid residues (Carr, 1953).

Binding of Ca^{2+} ions increases with increasing pH due to deprotonation of carboxyl and hydroxyl groups and supression of the ionisation of amino groups as well as conformational changes that might accompany the charge alterations. Measurements of quantity and intensity of bound ions as a function of pH offer information which permits the calculation of the equilibrium constant and the number of binding sites per a certain mass of protein (Edsall & Wyman, 1966).

Binding of ions by proteins may be of nutritional interest because ions can be transported and absorbed in a bound state. In recent decades, consumption of protein isolates has increased, in the form of sodium proteinate, due to alkaline conditions used for solubilization of these isolates. This may contribute to a high sodium intake and hypertension in humans.

Wallace and Sattelee (1977) studied the calcium binding capacity of soy and bovine blood protein isolate, alfalfa leaf and gluten protein concentrates in the pH range 7 to 11 and temperatures of 25, 35 and 45°C. They found that all protein sources studied could bind Ca^{2+} and this increased with increasing pH and temperature above 35°C. The authors argue that the calcium bound to these protein concentrates and isolates should be considered as a new source of calcium in human diets.

Appurao and Rao (1975) studied calcium binding, at pH 7.8, to the 11S soybean globulin. The main amino acid residue participating in this binding was histidine, through its imidazolic group. It was found that the presence of phytic acid in some vegetable proteins contributed to Ca^{2+} binding and the binding increases proportionally to the concentration of phosphorus compounds (Saio, Koyama, & Watanabe, 1968). Addition of phosphate groups to casein and phosphorylated β-lactoglobulin contributed to an increase in Ca^{2+} binding capacity. Viscosity of the phosphorylated β-lactoglobulin was

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double that of non-phosphorylated protein (Woo & Richardson, 1983).

Results from the author's laboratory showed that treatment with sodium trimetaphosphate (STMP) yielded yeast protein concentrates with much lower RNA contents. It seems that STMP substantially changes the physicochemical and structural properties of native yeast protein with positive effects on its functionality (Pacheco, Caballero-Córdoba, & Sgarbieri, 1997; Pacheco & Sgarbieri, 1998). The objective of the present work was to compare the Ca^{2+} binding capacity of a native brewer's yeast protein concentrate with a concentrate that had been treated with sodium trimetaphosphate.

2. Materials and methods

2.1. Materials

Protein concentrates were obtained from spent brewer's yeast which had been cleaned, debittered and the cell walls broken mechanically by abrasion in a Dynomill, according to Caballero-Córdoba, Pacheco, and Sgarbieri (1997).

2.2. Methods

Protein (Nx 5.58) and lipids were determined by procedures described by the AOAC (Horwitz, 1990). Phosphorus was determined by the molybdovanadate colorimetric method, procedures 22.042–2045 (AOAC, 1990).

Calcium ion was determined by the use of an Orion ion-selective membrane, 93-20, coupled to a model 90-01 simple junction reference electrode. The electrodes were connected to an ionic concentration specific reader (model EA-920), with communication with a pH electrode and temperature monitoring device. Bound Ca^{2+} (blank) and free Ca^{2+} in the presence of the protein were quantified after 2 min homogenization. Measurements were done at 0.5 and 2% (w/v) yeast protein dispersion in 20 mM KCl solution at pH 5 and 8 and Ca^{2+} concentrations of 10^{-5} to 10^{-1} M.

The influence of ion concentration on protein binding can be treated quantitatively, according to Edsall and Wyman (1966) by use of the equation:

$$\bar{\nu}/[A] = k(N - \bar{\nu})$$

The plot of $\bar{\nu}/[A]$ vs $\bar{\nu}$ should generate a straight line in which k, the association constant, is calculated from the slope of the straight line and the independent term N, calculated by the intercept of the lines with the ordinate, and represents the estimate of the number of binding sites per a certain mass of protein. This graphical representation is known as the Scatchard plot (Scatchard, Wu, & Shen, 1959).

3. Results and discussion

The interaction of Ca^{2+} with yeast protein was performed in a concentrate obtained by precipitation, at the pI (PL), of yeast protein concentrate obtained by precipitation at pI, after treatment of the extract with 3% STMP (PLP) at pH 11 in 35°C temperature for 3 h. The concentrate, PLP, was extensively dialysed against 0.01 M EDTA (PLPd). Details of the treatments used for preparation of the concentrates have been reported elsewhere (Pacheco et al., 1997). The preparations PL and PLP contained, respectively, 66 and 74% true protein, 15 and 3.7% RNA, 5.6 and 4% total lipids. PLP presented the highest content of phosphorus (26.5 mg/g sample), followed by PLPd (21.9 mg/g), and PL (16.9 mg/g).

This study was conducted with the whole concentrate and assumed that Ca^{2+} complexation was mainly due to the protein component, since most of the carbohydrate in the concentrates is in the form of glycoproteins. Precipitation of the proteins from water dispersion with 10% TCA and determination of carbohydrate in the supernatant revealed that only 2% of total carbohydrate of the sample remained in the supernatant.

The Scatchard plots of the experimental data are shown in Fig. 1, where comparisons can be made for the preparations PL, PLP and PLPd. The plotted lines permitted calculations of the number of binding sites (N) from the intersection of the lines with the ordinate X and of the association constant (K) by using the slopes of the straight lines. The association constant represents a measure of the binding force and an indication of the affinity of Ca^{2+} for the binding sites in the protein molecule. For the Scatchard plot, measurements are made at low Ca^{2+} concentration (10^{-5} to 10^{-3} M of Ca^{2+}) to avoid electrostatic interactions and interactions between binding sites. When this occurs, the Scatchard plot does not lead to straight lines making extrapolations and calculation of N and K more difficult (Edsall & Wyman, 1966).

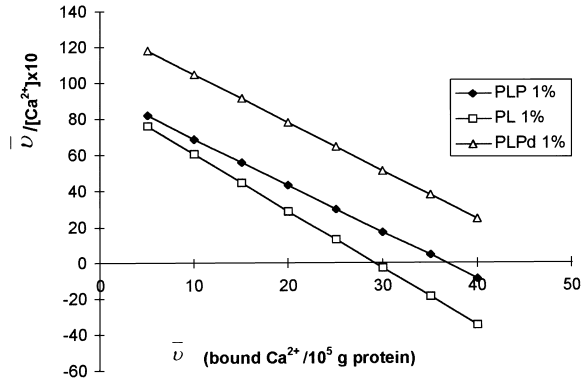

Fig. 1. Scatchard plot for binding of Ca^{2+} to yeast nucleoproteins (PL), yeast protein treated with sodium trimetaphosphate (PLP), and treated with trimetaphosphate and dialysed (PLPd), concentration 1% (w/v), pH 6.5.

The slopes of the straight lines showed a slightly higher value of K for the preparation PL and indicate that, although the number of binding sites were larger for PLP and PLPd, the interactions of Ca^{2+} with phosphate groups may be less strong than the interaction with functional groups of amino acid side chains at pH 6.5. The numerical values of N and K are presented in Table 1 to facilitate comparison among preparations. The values of the association constants for PLP and PLPd were essentially the same, confirming the influence of the phosphate groups in the binding phenomenon.

Although dialysis against EDTA of the PLP preparation increased the number of binding sites, the association constant was essentially the same for PLP and PLPd, which may indicate that Ca^{2+} had been bound to the same side chain functional groups in both preparations.

The sample obtained by precipitation of the yeast protein at the pI (PL) exhibited 20 binding sites less than the concentrate PLP. This may be attributed to the absence of phosphate groups bound to serine residues interacting with Ca^{2+} as well as to the more compact stereochemical configuration of the nucleic acid-protein complex.

According to the literature, in unicellular microorganisms RNA may amount to about one third of the cell protein content. RNA and protein may form nucleoprotein complexes through non-covalent bonds, such as electrostatic and hydrophobic interactions and hydrogen bonds. Ionic binding occurs predominantly among anionic phosphate groups of the nucleic acids and basic amino groups of the proteins (Kinsella, 1987).

Evidence suggests that, in the three-dimensional structure of the yeast nucleoprotein complex, RNA is localized in the center, with ribosomal proteins on the outside. The highly hydrophobic nature of these proteins suggests a potential for hydrophobic interactions among the proteins and protein-RNA, forming an apolar moiety in the center of the complex. The formation of hydrogen bonds and electrostatic interactions in this region of low dielectric potential may generate a high stabilization energy for the complex (Damodaran & Kinsella, 1984; Shetty & Kinsella, 1982).

In studies on native soy protein, utilizing a selective membrane electrode, Kroll (1984) found 45 binding sites for 10^5g protein and a K value of 2.07×10^{-3} at pH 7 and 8, similar to the values reported in this paper for yeast protein treated with sodium trimetaphosphate.

Varying the protein concentration from 0.5% to 1.0 and 2.0% (Table 1) and maintaining the same conditions of pH, ionic strength and Ca^{2+} concentration, one would expect similar results for the number of binding sites, since protein concentration was extrapolated to 10^5 g. However, PLP at 0.5% concentration presented a larger number of binding sites compared to higher concentrations. The lower protein concentration may have permitted a more effective interaction of solute-solvent, causing the exposure of a larger number of side chain functional groups.

Quantitative results for binding at pH 6.5, 0.02 M KCl, and different Ca^{2+} concentrations are shown in Table 2. For all samples studied, PL, PLP and PLPd, binding of Ca^{2+} increased as Ca^{2+} concentration increased. Binding capacity was highest for PLPd, intermediate for PLP and lowest for PL.

Binding of Ca^{2+} to PLPd was four-fold that of PL and twice that of PLP at both Ca^{2+} concentrations studied. The increased capacity of PLP for binding Ca^{2+}, compared to PL, may be attributed mainly to bound phosphate groups of serine residues. According to Pacheco et al. (1997), 30% of serine residues reacted with phosphate groups from STMP in the PLP sample.

Studies conducted with soybean protein, β-lactoglobulin and casein showed an increase in the number of Ca^{2+} binding sites as a result of reaction and/or complexation of these proteins with phosphate (Kroll, 1984; Woo & Richardson, 1983; Yoshikawa, Sasaki, & Chiba, 1981).

Table 1
Results of N and K obtained from the Scatchard plot at pH 6.5, 0.02 M KCl as a function of protein concentration

Protein sample	Number of binding sites (N)	Association constant of binding ($K \times 10^3$)
PL[a] 1.0%	29	3.16
PLPd[b] 1.0%	49	2.68
PLP[c] 0.5%	44	2.64
PLP 1.0%	37	2.58
PLP 2.0%	36	2.68

[a] PL, protein concentrate obtained at pI (pH 4.2).
[b] PLPd, concentrate PLP submitted to dialysis against EDTA solution.
[c] PLP, protein precipitated at pI after treatment with sodium trimetaphosphate.

Table 2
Influence of calcium concentration on Ca^{2+} binding to the protein concentrates PL, PLP, PLPd at pH 6.5

Protein sample	Active Ca^{2+} (mmoles/liter)	Bound Ca^{2+} (mmoles/liter)	Bound Ca^{2+} (moles/10^5 g protein)
PL[a]	4.50	0.60	14.9
PL	0.42	0.26	6.45
PLP[b]	4.50	1.30	37.4
PLP	0.50	0.46	12.0
PLPd[c]	5.00	2.20	63.2
PLPd	0.50	0.49	14.0

[a] PL, protein concentrate obtained at pI (4.0 g/liter).
[b] PLP, protein concentrate treated with STMP (3.5 g/liter).
[c] PLPd, PLP dialyzed against EDTA (3.5 g/liter).

Table 3
Values of N and K calculated from the Scatchard plot as a function of pH for the PLP at 1% (w/v) concentration

pH	N[a]	$K \times 10^3$ [b]
5.5	33	2.91
6.5	37	2.58
7.5	36	3.22
7.8	39	1.76

[a] N, number of binding sites/10^5 g protein.
[b] K, association constant.

Protein conformation should influence determination of the number of binding sites, since Ca^{2+} binding will depend on the degree of electrovalent dissociation of amino acid side chain residues as well as of conformational changes which may accompany the dissociations (Edsall & Wyman, 1966). As the sample PL exhibited a high RNA content, probably as protein–RNA complexes of high stability, the conformational changes involved may have inhibited a more effective binding of Ca^{2+}. Treatment with STMP may have caused destabilization of the complex, exposing negative charges on the protein, and bringing about a loosening of the structure, permitting higher interactions of Ca^{2+} with the protein molecules (Huang & Kinsella, 1987).

Table 3 shows values of N and K determined for the PLP sample as a function of pH. The number of binding sites increased with increase in pH, as expected, since it is directly proportional to the number of negative charges on the protein. It was not possible to perform measurements for pH values above 7.8 because of insolubilization and precipitation of Ca^{2+}, probably in the form of calcium hydroxide.

The highest value of the association constant was measured at pH 7.5, the carboxylic, imidazolic and phosphate groups are all deprotonized and are more active toward complexation in this form.

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