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Zinc bioavailability from whey. Enthalpy-entropy compensation in protein binding

Ning Tang, Leif H. Skibsted *

Food Chemistry, Department of Food Science, University of Copenhagen, Rolighedsvej 30, DK 1958 Frederiksberg C, Denmark

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ABSTRACT

Zinc amino acid or peptide complexes improve zinc absorption, however, the thermodynamics behind the interaction between zinc ion and these potential ligands is not well characterized. Therefore, binding of zinc to amino acids, peptides and whey proteins were investigated by isothermal titration calorimetry to provide useful information for improving zinc bioavailability which is lowered by many food components like phytate. Zinc binding to lactoferrin and to bovine serum albumin was found exothermic with $\Delta H = -100$ kJ/mol and -30 kJ/mol, respectively, in aqueous 0.16 M NaCl of pH 7.4 at 25 °C by isothermal titration calorimetry, while binding to α-lactalbumin and β-lactoglobulin was slightly endothermic. Still the binding constant was for all four proteins found to have a value around 2×10^5 L/mol indicating enthalpy-entropy compensation as also confirmed for zinc binding to amino acids with cysteine being enthalpically favored, while serine < histidine < glutamate < phosphorylated serine increasingly were found entropically favored. The enthalpy-entropy compensation makes whey proteins more homogeneous as zinc vehicles counteracting concentration fluctuation and precipitation of zinc.

1. Introduction

The content of zinc in the body is estimated to be around 2 g, >95% of which is intracellular (Livingstone, 2015). As an essential trace element, zinc is involved in a large number of processes in cellular metabolism, especially being required for the growth and for the immune system (Maret & Sandstead, 2006). Bioinformatic studies have shown that up to 3000 proteins corresponding to ~10% of all encoded proteins participate in zinc binding (Andreini, Banci, Bertini, & Rosato, 2006). Moreover, many enzymatic catalysis reactions, structure organizations and function regulations involve zinc (Kochanczyk, Drozd, & Krezel, 2015). Although zinc is abundant in the body, there is no specialized zinc storage, and a daily intake of zinc is required to maintain a steady state. Among foods, oyster contains more zinc than most other foods and zinc binding peptides were identified from oyster protein fractions (Chen et al., 2013). Around 2.2 billion people worldwide are affected by zinc deficiency especially infants, pregnant and lactating women, and older adults and most frequent in developing countries (Prasad, 2012). The most common cause of zinc deficiency is an inadequate intake, although zinc deficiency is often non-recognized as it is rather difficult to measure zinc nutritional status adequately using laboratory tests due to the distribution of zinc throughout the body (Vanwouwe, 1995). Zinc absorption takes place in the duodenum and proximal jejunum, and zinc is transported into enterocytes and expressed in the apical membrane (Turnlund, King, Keyes, Gong, & Michel, 1984). High dietary intake of zinc enriched foods and zinc supplements are recognized to prevent zinc deficiency. Unfortunately, absorption of dietary zinc is inhibited by phytate, polyphenols, fibers and saponins which can form insoluble zinc complexes that cannot be digested or absorbed in the gastrointestinal tract. In addition, zinc supplements like zinc sulphate can cause gastrointestinal tract irritation, and are not suitable for long-term intake (Cousins, 1985; Kenny, Sriram, & Hammond, 1989). On the other hand, zinc absorption is enhanced by soluble organic compounds such as amino acids, peptides and citric acid that could act as zinc binding ligand to improve the bioavailability (Carbonell-Capella, Buniowska, Barba, Esteve, & Frigola, 2014).

Isothermal titration calorimetry (ITC) is a widely used nondestructive method for determining the thermodynamics of biochemical interactions. Currently, it is recognized to be the most accurate thermochemical method with higher sensitivity and good reproducibility for determination of thermodynamic parameters through measurement of heat changes (Behbehani, Saboury, Mohebbian, & Ghammamy, 2010; Wilcox, 2008). In recent years, ITC has also become a valuable and powerful tool in food science/technology to study various phenomena, but still the use in food science is rather limited. Among physicochemical properties of zinc complexes, the measurement of zinc binding affinity is critical for understanding how zinc mediates their function and for zinc bioavailability from food (Kochanczyk et al., 2015). Therefore, the interactions between zinc ion and food components with focus on whey proteins were investigated in the present study. Moreover, binding of zinc to most amino acids has not been investigated by ITC yet. Accordingly, in the present study, the thermodynamic parameters of zinc binding to amino acids, peptides, and whey

* Corresponding author.
E-mail address: ls@food.ku.dk (L.H. Skibsted).

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proteins were determined by ITC for a discussion of mechanisms behind zinc binding to food components in relation to bioavailability.

2. Materials and methods

2.1. Chemicals

Zinc chloride, 2-amino-2-hydroxymethyl-propane-1,3-diol, sodium chloride and sodium hydroxide were all from Merck (Darmstadt, Germany). L-cysteine (purity ≥97%), DL-homocysteine (purity ≥95%), L-cysteine oxidized (purity ≥98%), L-histidine (purity ≥99%), L-aspartic acid sodium salt monohydrate (purity ≥98%), L-glutamic acid monosodium salt hydrate (purity ≥99%), glycine (purity ≥99%), L-serine (purity ≥99%), O-phospho-L-serine of analytical grade, Cys-Gly (purity ≥85%), Cys-Gly oxidized (purity ≥95%), glutathione (purity ≥98%), glutathione oxidized (purity ≥98%), bovine serum albumin (purity ≥96%), lactoferrin from bovine milk (purity ≥85%), α-lactalbumin from bovine milk (purity ≥85%) and β-lactoglobulin from bovine milk (purity ≥90%) were all purchased from Sigma-Aldrich (Steinheim, Germany). Gly-His and Gly-Gly-His of analytical grade were from Bachem (Bubendorf, Switzerland).

2.2. pH measurement

The pH of the solution was determined using a 713 pH meter with a combined Metrosensor glass electrode (Metrohm, Denmark). Before the measurement, the pH meter was standardized by pH standard solution with pH 4.0, 7.0 and 9.0. The pH of all aqueous solutions with 100 mM Tris-HCl buffer (ionic strength 0.16 M) was adjusted to 7.4 by a dropwise addition of 0.5 M NaOH or 0.5 M HCl.

2.3. Isothermal titration calorimetry

Isothermal titration calorimetric measurements were performed at 25 °C using a Nano-ITC titration calorimeter with a gold sample cell (TA Instruments, New Castle, USA). Generally, zinc (titrant) and amino acid, peptide and protein (macromolecule) stock solutions were diluted with the same Tris-HCl buffer (pH 7.4, 0.16) to a final concentration required during the titration experiment according to the heat changes. All solutions were degassed for 10 min before the titration experiment using a degassing station (TA Instruments, New Castle, USA). The solutions in the cell were stirred at 300 rpm by the syringe to ensure the rapid mixing. Typically, 10 μL of titrant was injected to the sample cell over 25 s with a 300 s interval between the injections to allow complete equilibration. For the background titration, the 10 μL of titrant was injected to the sample cell consisting of only buffer solution to be subtracted from the titration results as the heat of dilution. The data were analyzed using NanoAnalyze (TA Instruments, New Castle, USA) by one-site binding model. The enthalpy changes (ΔH), binding constant (K_a) and stoichiometry (n) were calculated from the isothermal titration curve.

3. Results and discussion

Zinc binding to food components such as amino acids, peptides and proteins can be monitored by isothermal titration calorimetry through direct measurement of heat changes during the binding reaction. From the heat changes during the titration of a sample with a fixed amount of zinc for increasing amino acids or peptides concentration or for a fixed amount of protein for increasing zinc concentration, observed enthalpy (ΔH_{obs}) and binding constant (K_a), are directly obtained, allowing the calculation of Gibbs free energy of binding (ΔG) and entropy of binding (ΔS) based on the following equation:

$$\Delta G = -RT\ln K_a = \Delta H_{obs} - T\Delta S \quad (1)$$

For the calculation of the thermodynamic parameters from the calorimetric data the following simple equilibrium is considered:

$$Zn^{2+} + L^{-} \rightleftharpoons ZnL^{+} \quad (2)$$

The fitting algorithm was derived according to the equations below:

$$C_{Zn}{}^{2+} = \left[Zn^{2+}\right] + \left[ZnL^{+}\right] \quad (3)$$

$$C_{L}{}^{-} = [L^{-}] + \left[ZnL^{+}\right] \quad (4)$$

$$K_a(\mathrm{ITC}) = \frac{[ZnL^{+}]}{[ZnL^{+}][L^{-}]} \quad (5)$$

where L^{-} is the ligand like an amino acid, a peptide, or a protein; C_{Zn}^{2+} is the total concentration of zinc and C_{L}^{-} is the total concentration of ligand. According to Eqs. (3), (4) and (5), the second order equation for $[ZnL^{+}]$ was obtained:

$$n_{[ZnL^{+}]}^{2} - \left(\frac{V_i}{K_a} + n_{[Zn^{2+}]}^{0} - in_{[L^{-}]}^{inj}\right) n_{[ZnL^{+}]} + n_{[Zn^{2+}]}^{0} in_{[L^{-}]}^{inj} = 0 \quad (6)$$

where $n_{[ZnL^{+}]}$ is the amount of zinc complex; $n_{[Zn^{2+}]}{}^{0}$ is initial amount of zinc ion in the cell; $n_{[L^{-}]}{}^{inj}$ is the amount of ligand injected in each injection; and V_i is the volume in the cell after the injection i. Standard method for solving Eq. (6) gives the value of $n_{[ZnL^{+}]}$ as following:

$$n_{[ZnL^{+}]} = \frac{\left(\frac{V_i}{K_a} + n_{[Zn^{2+}]}^{0} - in_{[L^{-}]}^{inj}\right) \pm \sqrt{\left(\frac{V_i}{K_a} + n_{[Zn^{2+}]}^{0} - in_{[L^{-}]}^{inj}\right)^{2} - 4n_{[Zn^{2+}]}^{0} in_{[L^{-}]}^{inj}}}{2} \quad (7)$$

Then the heat generated from each injection can be calculated from the following equation:

$$Q_i = \left(n_{[ZnL^{+}]}^{i} - n_{[ZnL^{+}]}^{i-1}\right)\Delta H \quad (8)$$

The fitting of above equations resulting in the thermodynamic parameters for zinc ion binding to the amino acids as presented in Table 1. A comparison of titrations of zinc ion into amino acid solutions and titrations of amino acids into zinc solutions, shown that the parameters can be fitted more accurately for the latter experiment. Moreover, the addition of ligand into the zinc solution ($L^{-} \rightarrow Zn^{2+}$) also prevented zinc hydrolysis in aqueous solution for higher zinc concentrations. Fig. 1 shows the ITC data for titration of a zinc solution with cysteine, and for a titration of zinc with histidine in 100 mM Tris-HCl at pH 7.4 and 25 °C, both of which were exothermic, and an excellent fit of the

Table 1
Thermodynamic parameters of zinc binding to amino acids in aqueous 0.16 M NaCl of pH 7.4 at 25 °C measured by isothermal titration calorimetry (ITC).

Amino acids	Stoichiometry (n)	Binding constant (L/mol)	ΔG (kJ/mol)	ΔH (kJ/mol)	ΔS (J/mol K)
L-cysteine	2.2	6.5×10^4	−27.5	−32.9	−18.0
DL-homocysteine	1.5	4.3×10^3	−20.8	−29.1	−28.1
L-cysteine oxidized	1.4	3.1×10^4	−25.6	−6.6	63.9
L-histidine	1.6	6.4×10^3	−21.7	−21.0	2.5
L-aspartic acid	1.1	1.4×10^3	−18.1	−9.2	29.8
L-glutamic acid	1.0	1.8×10^2	−12.9	−9.9	10.3
Glycine	1.0	8.0×10^2	−16.6	−49.7	−111
L-serine	1.0	2.3×10^2	−13.5	−13.0	1.5
L-O-phosphoserine	1.0	2.4×10^3	−19.4	−3.5	53.3

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