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Research Article

Thermal Study of a Newly Synthesized Cu(II) Complex Binding to Bovine β-Lactoglobulin

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We have investigated the interactions between β-lactoglobulin, BLG, and new synthesized Cu(II) complex (2,2′-dibipyridine Cu(II) chloride) using isothermal titration calorimetry (ITC) methods at different temperatures of 298 and 310 K. The heats of BLG + Cu(II) interactions are reported and analyzed in terms of the extended solvation theory for calculation of binding and thermodynamic parameters of the interaction. The results suggested that binding of Cu(II) complex on BLG resulted in significant changes on the tertiary structure and conformation of protein via increasing of hydrophobicity and inducing partially unfolded structure in BLG which has a good agreement with the solvation parameters recovered by the extended solvation model suggesting destabilization of the protein.

1. Introduction

Milk proteins consist of caseins (78.3%), whey proteins (19%), and miscellaneous proteins (2.7%) [1]. Whey proteins are widely used in formulated foods because they have high nutritional value and excellent functional properties. Whey proteins, which include serum albumin, immunoglobulins, α-lactalbumin, and β-lactoglobulin (BLG), are more reactive because they dissolve in the serum. BLG, the major protein in whey, is responsible for most of the bioactive properties of whey proteins. Due to their emulsifying, interfacial, and gelation properties, tryptic peptides from whey proteins are of great interest to the food industry. BLG composed of 162 amino acid residues and two disulfide bonds (Cys66-Cys160 and Cys106-Cys119) belongs to the lipocalin protein family [1, 2]. At physiological pH, BLG is mostly found as dimers, and at pH values below 3.5 and above 7.5, the protein tends to be monomeric [3]. BLG solutions form gels in various conditions, when the native structure is sufficiently destabilised to allow aggregation. As milk is a known allergen, manufacturers need to prove the presence or absence of β-lactoglobulin to ensure that their labelling satisfies the requirements of the aforementioned directive. Food testing laboratories can use ELISA (enzyme-linked immunosorbent assay) methods to identify and quantify β-lactoglobulin in food products [4–11].

Copper is an essential trace element for all biological organisms, from bacterial cells to humans. There are eight negative charges on the surface of BLG in neutral pH conditions, which may be sites for binding positively charged metal ions [12, 13].

Since BLG is one of the most important milk carrier proteins and has great ability for binding to different drugs, specially cancer chemotherapy drugs, then it may has a central role in the molecular pharmacology of drugs used in cancer chemotherapy [14]. Then, in the present study, we have decided to investigate the binding of a new synthesized Cu(II) complex (2,2′-dibipyridine Cu(II) chloride), with milk carrier protein of BLG at different temperatures of 298 (room temperature) and 310 K (physiologic temperature) (upper fever temperature) using calorimetric (ITC) methods of the extended solvation theory.

2. Materials and Method

The isothermal titration calorimetric experiments were carried out on a VP-ITC ultrasensitive titration calorimeter (MicroCal, LLC, Northampton, MA). The microcalorimeter consists of a reference cell and a sample cell of 1.8 mL in volume, with both cells insulated by an adiabatic shield. All solutions were thoroughly degassed before use by stirring under vacuum. The sample cell was loaded with BLG solution (47 μM), and the reference cell contained NaCl solution. The solution in the cell was stirred at 307 rpm by the syringe (equipped with micropropeller) filled with Cu(II) complex solution (1.5 mM) to ensure rapid mixing. The titration of protein with Cu(II) complex solution involved 30 consecutive injections of the copper solution, the first being 5 μL, and the remaining ones of 10 μL. In all cases, each injection was done in 6 s at 3-minute intervals. To correct the thermal effects due to Cu(II) complex dilution, control experiments were done in which identical aliquots were injected into the NaCl solution in the absence of BLG. The measurements were performed at constant temperatures of 298.00 and 310.00 ± 0.02 K, and the temperature was controlled using a Poly-Science water bath. The data were collected automatically, and the heat of interaction between BLG + Cu(II) complex was subtracted from heat of Cu(II) complex dilution. The measured heats of binding have been shown graphically in Figure 1.

3. Results and Discussion

It has been reported previously [15–23] that the heats of interactions of biopolymers with ligands (BLG-A + Cu(II) complex in this case) in the aqueous solvent (Cu(II) complex + water in the present case) mixtures can be reproduced via the following equation:

$$q = q_{\max} x_B' - \delta_A^\theta \left(x_A' L_A + x_B' L_B\right) - \left(\delta_B^\theta - \delta_A^\theta\right)\left(x_A' L_A + x_B' L_B\right) x_B'. \quad (1)$$

The parameters δ_A^θ and δ_B^θ are the indexes of the BLG stability as a result of interaction with Cu(II) complex in the low and high Cu(II) complex concentrations, respectively. If the binding of ligand at one site increases the affinity for ligand at another site, the macromolecule exhibits positive cooperativity. Conversely, if the binding of ligand at one site lowers the affinity for ligand at another site, the protein exhibits negative cooperativity. If the ligand binds at each site independently, the binding is noncooperative. $p < 1$ or $p > 1$ indicates positive or negative cooperativity of macromolecule for binding with ligand, respectively; $p = 1$ indicates that the binding is noncooperative. x_B' can be expressed as follows:

$$x_B' = \frac{p x_B}{x_A + p x_B}. \quad (2)$$

x_B' is the fraction of the bound Cu(II) complex to the binding sites, and $x_A' = 1 - x_B'$ is the fraction of unbound Cu(II) complex. We can express x_B fractions as the total Cu(II) complex concentrations divided by the maximum concentration of the Cu(II) complex upon saturation of all BLG-A as follows:

$$x_B = \frac{[\text{Cu (II)}]}{[\text{Cu (II)}]_{\max}}, \qquad x_A = 1 - x_B. \quad (3)$$

[Cu(II)] is the concentration of Cu(II) complex, and $[\text{complex}]_{\max}$ is the maximum concentration of the Cu(II) complex upon saturation of all BLG. L_A and L_B are the relative contributions of unbound and bound Cu(II) complex to the heats of dilution in the absence of BLG and can be calculated from the heats of dilution of Cu(II) complex in buffer, q_{dilut}, as follows:

$$L_A = q_{\text{dilut}} + x_B \left(\frac{\partial q_{\text{dilut}}}{\partial x_B}\right), \qquad L_B = q_{\text{dilut}} - x_A \left(\frac{\partial q_{\text{dilut}}}{\partial x_B}\right). \quad (4)$$

The heats of Cu(II) complex + BLG interactions, q, were fitted to (1) over the whole Cu(II) complex compositions. In the procedure, the adjustable parameter (p) was changed until the best agreement between the experimental and calculated data was approached (Figure 1). The optimized δ_A^θ and δ_B^θ values are recovered from the coefficients of the second and third terms of (1). The binding parameters for Cu(II) complex + BLG interactions recovered from (1) were listed in Table 1. The agreement between the calculated and experimental results (Figure 1) is striking and gives considerable support to the use of (1).

P values were calculated to be one, at different temperatures, which indicates that there are not any cooperativity in four binding sites of BLG (Table 1).

δ_A^θ values (Table 1) for BLG + Cu(II) complex at 298 and 310 K are −3.49 and −9.95, respectively, indicating that

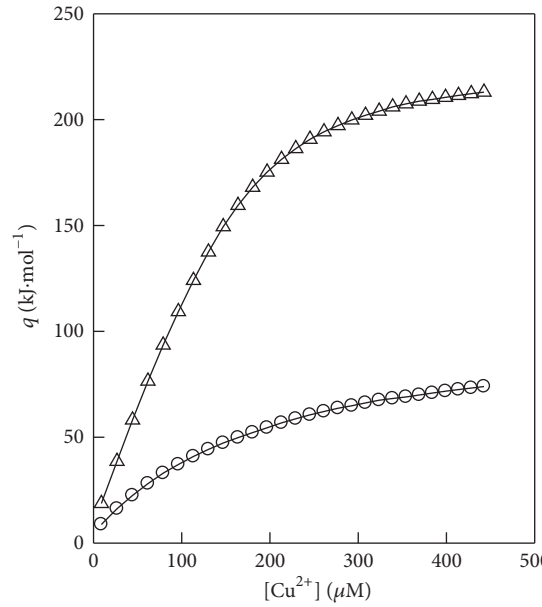

Figure 1: Comparison between experimental heats of interactions between BLG and copper ion at 25 (○) and 37°C (Δ) and calculated heats obtained from (1).

Table 1: Binding parameters for BLG + Cu(II) complex interaction recovered from (1) and (2) at different temperatures of 298 and 310 K. $p = 1$ indicates that the binding is noncooperative in the four binding sites. The negative values of δ_A^θ or δ_B^θ indicate that the low and high concentrations of Cu(II) complex destabilize the BLG structure. The interaction is strong as indicated by equilibrium association constants. The interaction is entropy driven, indicating that the hydrophobic forces are dominant in this interaction.

Parameters	$T = 298$ K	$T = 310$ K
K_a/L mol^{-1}	$3.92 \times 10^4 \pm 42$	$1.07 \times 10^5 \pm 42$
g	4	4
p	1.00 ± 0.01	1.00 ± 0.01
δ_A^θ	-3.49 ± 0.11	-9.95 ± 0.15
δ_B^θ	-5.47 ± 0.11	-22.02 ± 0.16
ΔH/kJ mol^{-1}	19.23 ± 0.05	53.82 ± 0.25
ΔG/kJ mol^{-1}	-26.38 ± 0.04	-28.88 ± 0.08
ΔS/kJ mol^{-1} K^{-1}	0.15 ± 0.01	0.27 ± 0.02

in the low concentrations of Cu(II) complex, BLG structure has been destabilized to allow aggregation. Negative values for δ_A^θ and δ_B^θ at both of temperatures indicate that Cu(II) complex destabilizes BLG structures and is a good support for significant structural changes of BLG due to interaction with Cu(II) complex, predicted by the extended solvation model.

For a set of identical and independent binding sites to provide the number of binding sites (g) and the dissociation binding constant (K_d), a plot of $(\Delta q/q_{\max})M_0$ versus $(\Delta q/q)L_0$ should be a linear plot by a slope of $1/g$ and the vertical intercept of $(-k_d/g)$:

$$\frac{\Delta q}{q_{\max}} M_0 = \frac{\Delta q}{q} L_0 \frac{1}{g} - \frac{K_d}{g}. \quad (5)$$

M_0 and L_0 are the concentrations of BLG and Cu(II), respectively. While q represents the heat value at a certain L_0 and $q_{\max}$ indicates the heat value upon saturation of all BLG, $\Delta q = q_{\max} - q$.

The linearity of the plot has been examined by different estimated values for $q_{\max}$ to find the best value for the correlation coefficient. The small relative standard coefficient errors (±0.001) and the high r^2 values (0.99999) indicate the best linear plot of $(\Delta q/q_{\max})M_0$ against $(\Delta q/q)L_0$. If the optimized $q_{\max}$ is calculated per mole of BLG, then the standard molar enthalpy of binding for each binding site will be $\Delta H^\circ = q_{\max}/g$.

The change of the standard Gibbs free energy of binding (ΔG°) is determined by using the association binding constant (K_a), obtained from the inverse of K_d value, in (6), where R is the gas constant and T is the absolute temperature:

$$\Delta G^\circ = -RT \ln k_a. \quad (6)$$

The change in standard entropy (ΔS°) of this binding can be calculated as (7)

$$\Delta S^\circ = \frac{\Delta H^\circ - \Delta G^\circ}{T}. \quad (7)$$

All calculated thermodynamic parameters are reported in Table 1.

4. Conclusion

Negative values of δ_A^θ and δ_B^θ calculated from the extended solvation theory show that low and high concentrations of Cu(II) complex induced some structural changes in β-lactoglobulin which lead to destabilization of the protein. Previous reports represent that gelation of globular proteins results from an aggregation process, which is generally triggered by a conformational change of the protein induced by a modification of solvent conditions. In the aggregation reactions, either or both covalent (inter- and intramolecular disulphide bonds) and noncovalent bonds (hydrophobic, hydrogen, and ionic interactions) are involved. Since thermal denaturation of BLG is characterized by changes in the secondary and tertiary structures, exposing hydrophobic residues to the solvent and aggregation process due to hydrophobic interactions may subsequently take place [24–30].

The above interpretations are in agreement with the solvation parameters recovered from the extended solvation model (Table 1), suggesting destabilization of BLG upon interaction with Cu(II) complex.

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