Journal of Thermal Analysis and Calorimetry, Vol. 96 (2009) 2, 631–635

A HIGH PERFORMANCE METHOD FOR THERMODYNAMIC STUDY ON THE BINDING OF COPPER ION AND GLYCINE WITH ALZHEIMER'S AMYLOID β PEPTIDE

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The interaction of Cu^{2+} to the first 16 residues of the Alzheimer's amyloid β peptide, *A*β(1–16) was studied by isothermal titration calorimetry at pH 7.2 and 37°C in aqueous solution. The Gholamreza Rezaei Behbehani (GRB) solvation model was used to reproduce the enthalpies of interactions of *A*β(1–16) with glycine, Gly+*A*β(1–16), and Cu^{2+} ions, Cu^{2+}+*A*β(1–16), over the whole range of Cu^{2+} concentrations. The binding parameters recovered from the solvation model were attributed to the structural change of *A*β(1–16) due to the glycine and Cu^{2+} interactions. It was found that there is a set of two identical binding sites for Cu^{2+} ions. p=2 indicates that the binding has positive cooperativity in the two binding sites. *A*β(1–16) structure is destabilized greatly as a result of binding to Cu^{2+} ions.

Keywords: *Alzheimer's amyloid* β *peptide, binding parameters, isothermal titration calorimetry*

Introduction

Thermodynamic of biomacromolecule-ligand interaction is very important to understand the structure function relationship in proteins. One of the most powerful techniques useful to obtain additional information about the structure of proteins in biophysical chemistry field is isothermal titration calorimetry (ITC) [1–4]. ITC gives invaluable information about biomacromolecule-ligand interaction [5–7], protein denaturation [8–10]. During the last 6 years we attempt to study the metal ion binding study on different proteins [1–13]. We have previously developed a theory to account for the solvation of solutes in mixed solvent systems. The extended solvation model satisfactorily reproduces all the experimental enthalpies transfer of the solutes from pure solvents into mixed solvent systems across the whole range of solvent compositions [11–13]. Although copper, zinc and iron are essential for brain development and function, an imbalance of these metals may play a role in the development of brain plaques associated with Alzheimer's disease. Inhibition of neocortical β-amyloid accumulation may be essential in an effective therapeutic intervention for Alzheimer's disease [14]. Cu^{2+} and Zn^{2+} are enriched in β-amyloid deposits in Alzheimer's disease, which are solubilized by Cu^{2+}/Zn^{2+}-selective chelators in vitro. There are some reports that transgenic mice treated orally with clioquinol, an antibiotic and bioavailable Cu^{2+}/Zn^{2+} chelator. The results suggest that β-amyloid metabolism is extraordinarily sensitive to small changes in copper concentrations that might be transduced across the blood–brain barrier [14–17]. Clioquinol is a chelator that crosses the blood–brain barrier and has greater affinity for zinc and copper ions than for calcium and magnesium ions. Clinical ratings showed slight improvement after 3 weeks treatment of 20 patients with clioquinol [17]. Organ meats, such as liver, and shellfish are the foods with the highest copper levels, followed by nuts, seeds, legumes, whole grains, potatoes, chocolate and some fruits. Copper pipes may also add trace amounts of the metal to drinking water.

As a clear understanding of operational stability constitutes an important goal in protein technology, our efforts aimed at elucidation of the structure-stability using the extended solvation model. This model is able to correlate the solvation parameters to the effect of ligands on the stability of a protein in a very simple way. The present paper reports some interesting experimental data for the enthalpies of Cu^{2+}+*A*β(1–16) interactions and analyses of these data using GRB solvation theory.

Experimental

Materials

$CuCl_2 \cdot H_2O$, glycine and N-(2-hydroxyethyl) piperazine-N'(2-ethanesulfonic acid), HEPES, were

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1388–6150/$20.00

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purchased from Sigma-Aldrich. All reagents were 99% pure. The peptide amyloid (1-16), *A*β(1–16), was purchased as a custom synthetic peptide, 95% purity, from Biosynthesis, Inc. (Lewis-ville, TX). All solutions were made with nanopure water (>18 MΩ, resistance).

Method

ITC measurements were carried out on a VP-ITC ultrasensitive microcalorimeter (MicroCal, Northampton, MA). All the solutions were degassed before titrations were performed. This procedure was carried out as follows:

Glycine (2.8 mM), $CuCl_2$ (0.7 mM) and *A*β(1–6) (75 μM) solutions were prepared in HEPES buffer (20, 150 mM NaCl, pH 7.2, at 37°C). During the titration, 8 μL of the $CuCl_2$ or glycine solution was injected in 5 min intervals into 1.41 mL of the peptide solution in the reaction cell. The cell was stirred at 307 rpm. The titration was conducted at 37°C. The peptide concentrations were estimated by the BCA Assay (Pierce Biotechnology). Injection of the ligands solutions into the vessel was repeated 30 times, with 8 μL per injection. The above procedure was repeated for the injection of $CuCl_2$ solution (0.7 mM Cu^{2+}) in 1.41 mL of the peptide solution. The calorimetric signal was measured by a digital voltmeter that was part of a computerized recording system. The heat of each injection was calculated by the 'Thermometric Digitam 3' software program. The heats of dilution of $CuCl_2$ or glycine were measured as described above except *A*β(1–16) was excluded. The enthalpies of dilution of the ligands solutions were subtracted from the enthalpies of Gly+*A*β(1–16) and Cu^{2+}+*A*β(1–16) interactions. The enthalpies of dilution of *A*β(1–16) are negligible. The microcalorimeter was frequently calibrated electrically during the course of the study. The enthalpies of Gly+*A*β(1–16) and Cu^{2+}+*A*β(1–16) interactions have been calculated in kJ mol^{-1} and were analyzed with GRB solvation model using a non-linear least square method and were shown graphically in Fig. 1.

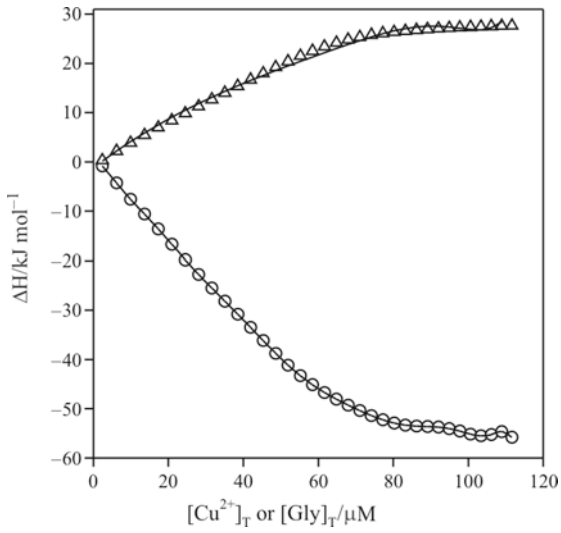

Fig. 1 Comparison between the experimental enthalpies, Δ*H*, for O – Cu^{2+}+*A*β(1–16) and △ – Gly+*A*β(1–16) interactions and calculated data (lines) via Eq. (1). Subscript *T* means total concentrations

Results and discussion

The enthalpies of the ligands+biopolymer interactions in the aqueous solvent systems, can be accounted for quantitatively in terms of three factors: cooperative binding by the ligands, weakening or strengthening of solvent-solvent bonds by *A*β(1–16) and the change in the enthalpy of the ligands+*A*β(1–16) interactions [18–31]. This treatment leads to:

$$\Delta H = \Delta H_{max} x_B^{'} - \delta_A^{\theta}(x_A^{'} L_A + x_B^{'} L_B) - \\ -(\delta_B^{\theta} - \delta_A^{\theta})(x_A^{'} L_A + x_B^{'} L_B) x_B^{'} \quad (1)$$

The parameters $\delta_A^{\theta} = (\alpha n + \beta N)_A^{\theta}$ and $\delta_B^{\theta} = (\alpha n + \beta N)_B^{\theta}$ are the indexes of *A*β(1–16) structural changes due to its interaction with ligands in the low and high ligand concentrations respectively, with αn resulting from the formation of a cavity wherein *n* solvent molecules become the nearest neighbours of the solute and β*N* reflecting the enthalpy change from strengthening or weakening of solvent–solvent bonds of *N* solvent molecules (*N*>*n*) around the cavity (β<0 indicates a net strengthening of solvent–solvent bonds). The constants α and β represent the fraction of the enthalpy of water+Cu^{2+} interaction associated with the cavity formation or restructuring, respectively. The superscript θ in all cases refers to the quantities in infinite dilution of the solute. Cooperative binding requires that the macromolecule have more than one binding site, since cooperativity results from the interactions between binding sites. 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 non-cooperative. *p*<1 or *p*>1 indicate positive or negative cooperativity of macromolecule for binding with ligand respectively; *p*=1 indicates that the binding is non-cooperative. $x_B^{'}$ can be expressed as follow:

$$x_B^{'} = \frac{p x_B}{x_A + p x_B} = \frac{\nu}{g} \quad (2)$$

$x_B^{'}$ is the fraction of the ligand needed for saturation of the binding sites, and x_A=1–x_B is the fraction of unbounded ligand. Now the model is a simple mass

action treatment, with ligands replacing water molecules, at the binding sites in the present case. We can express x_B fractions, as the total Cu^{2+} or glycine concentrations divided by the maximum concentration of the Cu^{2+} or glycine upon saturation of all *A*β(1–16) as follow:

$$x_B = \frac{[Cu^{2+}]_T}{[Cu^{2+}]_{max}} \quad x_A = 1 - x_B \tag{3}$$

$[Cu^{2+}]_T$ is the total concentration of surfactant and $[Cu^{2+}]_{max}$ is the maximum concentration of the Cu^{2+} upon saturation of all *A*β(1–16). In general, there will be 'g' sites for binding of Cu^{2+} per *A*β(1–16) molecule and ν is defined as the average moles of bound Cu^{2+} per mole of total *A*β(1–16). L_A and L_B are the relative unbounded and bounded Cu^{2+} contributions to the enthalpies of dilution in the absence of *A*β(1–16) and can be calculated from the enthalpies of dilution of Cu^{2+} in buffer, ΔH_{dilut}, as follow:

$$\begin{aligned} L_A &= \Delta H_{dilut} + x_B \left(\frac{\partial \Delta H_{dilut}}{\partial x_B} \right), \\ L_B &= \Delta H_{dilut} - x_A \left(\frac{\partial \Delta H_{dilut}}{\partial x_B} \right) \end{aligned} \tag{4}$$

The enthalpies of Cu^{2+}+*A*β(1–16) interactions, ΔH, were fitted to Eq. (1) over the whole Cu^{2+} compositions. In the procedure the only adjustable parameter (*p*) was changed until the best agreement between the experimental and calculated data was approached (Fig. 1). δ_A^θ and δ_B^θ parameters have been also optimized to fit the data. The optimized δ_A^θ and δ_B^θ values are recovered from the coefficients of the second and third terms of Eq. (1). The small relative standard coefficient errors and the high r^2 values (0.99999) support the method. The binding parameters for Gly+*A*β(1–16) and Cu^{2+}+*A*β(1–16) interactions recovered from Eq. (1) were listed in Table 2.

Φ is the fraction of *A*β(1–16) molecule undergoing complexation with Cu^{2+} which can be expressed as follow:

$$\Phi = \frac{\Delta H}{\Delta H_{max}} \tag{5}$$

ΔH_{max} represents the heat value upon saturation of all *A*β(1–16). The appearance association equilibrium constant values, K_a, as a function of Cu^{2+} concentration can be calculated as follow:

$$K_a = \frac{\Phi}{(1-\Phi)[Cu^{2+}]_F} \tag{6}$$

where $[Cu^{2+}]_F$ is the unbounded or the free Cu^{2+} ion concentration. The variable Φ represents the fraction of binding sites that are occupied on the peptide molecule. Therefore, 1–Φ represents the fraction of binding sites that are not occupied. The appearance association equilibrium constants, K_a, for successive replacement of the Cu^{2+} ions by water molecules are as follow:

$$K_a = x_A^g - \sum_{i=1}^{g} K_i \frac{x_B^i}{x_A^{i-g}} \tag{7}$$

where the K_i's are the extrinsic equilibrium constants for any individual binding site. In fact appearance association equilibrium values K_a, are the equilibrium constants for the equilibria:

$$A\beta(1\text{–}16) + gCu^{2+} \Leftrightarrow A\beta(1\text{–}16)(Cu^{2+})_g \tag{E1}$$

K_a values obtained from Eq. (6), have been fitted to Eq. (7) using a computer program for non-linear least-square fitting. Therefore, we can approach to '*g*' value and the extrinsic equilibrium constants in the first, K_1, and the second, K_2, binding site (Table 2). Finally, ν values can be calculated at any concentration of Cu^{2+} via Eq. (4). The binding parameters obtained form this method are listed in the Tables 1–2. The Gibbs energies as a function of Cu^{2+} concentrations can be obtained as follow:

$$\Delta G = -RTLnK_a \tag{8}$$

Gibbs energies calculated from Eq. (8) have shown graphically in Fig. 2. ΔS values were calculated using ΔG values and the enthalpies of Cu^{2+}+*A*β(1–16) and Gly+*A*β(1–16) interactions and have shown in Fig. 3. Therefore for the first time, we managed to calculate ΔS values with using one set of experimental data in one temperature. Binding parameters for Cu^{2+}+*A*β(1–16) and Gly+*A*β(1–16) interactions using the GRB solvation model are listed in Tables 1 and 2.

Table 1 The binding parameters for Cu^{2+}+*A*β(1–16) and Gly+*A*β(1–16) interactions recovered from Eq. (1)

Cu^{2+}+*A*β(1–16)	*p*	1
	δ_A^θ	–0.170
	δ_B^θ	–29.967
	K_1/μM^{-1}	1.765
	K_2/μM^{-1}	1.677
	ΔH_{max}/kJ mol^{-1}	–55.789
	g	2
Gly+*A*β(1–16)	*p*	2
	δ_A^θ	–0.010
	δ_B^θ	0.0013
	K_1/μM^{-1}	0.889
	K_2/μM^{-1}	2.527
	ΔH_{max}/kJ mol^{-1}	31.495
	g	2

Table 2 K_a and ν values *vs*. Cu^{2+} or glycine concentrations for Cu^{2+}+*A*β(1–16) and Gly+*A*β(1–16) interactions. The precisions are ±0.005 or better

$[Cu^{2+}]$/μM	$K_a(Cu^{2+})/\mu M^{-1}$	K_a(Gly)/μM^{-1}	ν
2.3891	5.0818e–3	0.00313	0.0423
6.1779	0.0148	0.0047	0.1093
9.9257	0.0179	0.0051	0.1757
13.6333	0.0204	0.0056	0.2413
17.3013	0.0229	0.0062	0.3062
20.9302	0.0256	0.0068	0.3704
24.5208	0.0287	0.0075	0.4340
28.0736	0.0325	0.0084	0.4969
31.5893	0.0370	0.0096	0.5591
35.0683	0.0425	0.0109	0.6207
38.5113	0.0493	0.01267	0.6816
41.9189	0.0578	0.0148	0.7419
45.2915	0.0683	0.0175	0.8016
48.6297	0.0819	0.0209	0.8607
51.9341	0.0999	0.0255	0.9192
55.2050	0.1236	0.0316	0.9771
58.4432	0.1551	0.0396	1.0344
61.6490	0.1966	0.0502	1.0911
64.8229	0.2512	0.0642	1.1473
67.9654	0.3223	0.0824	1.2029
71.0769	0.4138	0.1060	1.2580
74.1580	0.5353	0.1373	1.3125
77.2090	0.6833	0.1757	1.3665
80.2304	0.8719	0.2249	1.4200
83.2227	1.1135	0.2882	1.4730
86.1862	1.4705	0.3824	1.5254
89.1213	1.9788	0.5176	1.5774
92.0286	2.6732	0.7046	1.6288
94.9082	3.7282	0.9930	1.6798
97.7608	5.4466	1.4720	1.7303
100.5865	8.6471	2.3881	1.7803
103.3859	14.6113	4.1801	1.8298

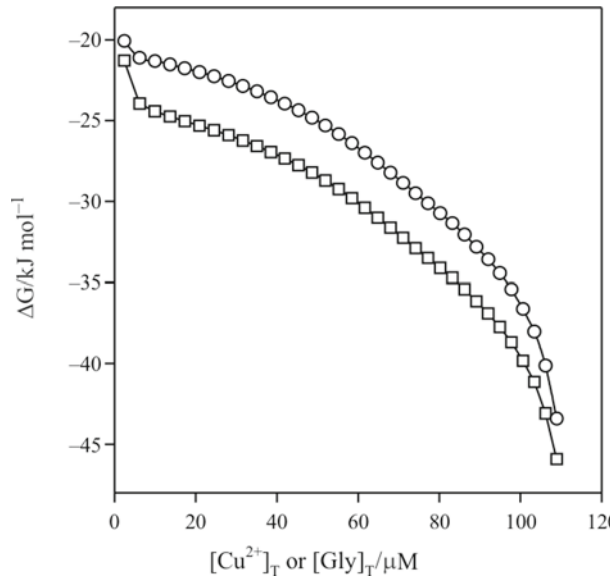

Fig. 2 Δ*G* for □ – Cu^{2+}+*A*β(1–16) and ○ – Gly+*A*β(1–16) interactions *vs*. the Cu^{2+} or glycine concentrations (in μM)

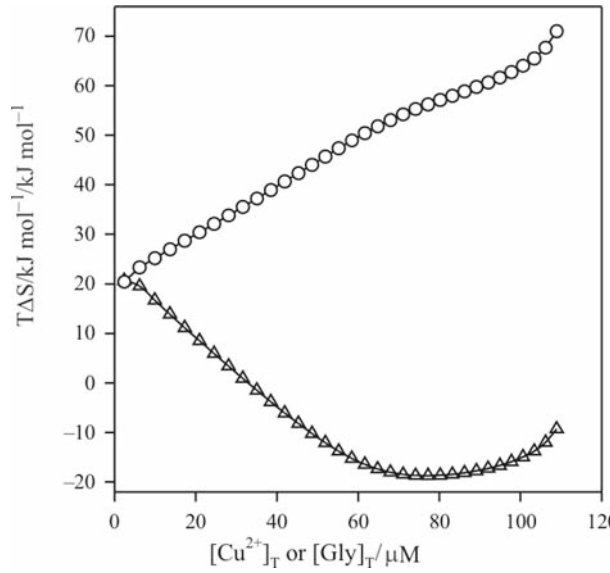

Fig. 3 *T*Δ*S* for △ – Cu^{2+}+*A*β(1–16) and ○ – Gly+*A*β(1–16) interactions *vs*. the Cu^{2+} or glycine concentrations

The δ_A^θ and δ_B^θ values reflect to the hydrophobic hydration of *A*β(1–16) and give a measure of relative enhancement of water structure. The greater the extent of this enhancement, the greater will be the stabilization of the *A*β(1–16) structure and the greater the values of δ_A^θ and δ_B^θ and vice versa. In the high Cu^{2+} concentration region, there is a decrease in the δ_B^θ value, indicating that *A*β(1–16) structure is destabilized. Cu^{2+}+*A*β(1–16) interaction results to a decrease in the hydrophobic property of the *A*β(1–16) as evidenced by the negative δ_B^θ value (–29.967 in Table 2). The greater the δ_A^θ and δ_B^θ values, the greater the biological activity of *A*β(1–16) and vice versa. These results ($\delta_B^\theta < \delta_A^\theta$) indicate that Cu^{2+} ions are able to destabilize the *A*β(1–16) greatly. We all have some amyloid beta in the brain normally. When we age, amyloid beta accumulates a little bit more in the brain naturally. But the process is greatly accelerated in people with Alzheimer's disease. Elucidation of the effects of transition-metal ions on the *A*β(1–16) structure stability is important to drug design for the Alzheimer's disease treatment. *A*β(1–16) structure is destabilized greatly as a result of binding to Cu^{2+} ions as evi-

denced by –29.967 value of δ_B^θ. Therefore, it is possible to conclude that Cu^{2+} ions can be used as an Alzheimer's disease treatment.

The low K_a values (Table 1) in the low amounts of bound Cu^{2+} ions reflect to the lower affinity of *A*β(1–16) in this domain; however, as the amount of bound ligand increases, the protein comes to prefer the higher affinity which is in agreement with the higher K_a values in the higher amounts of bound Cu^{2+} ions. The structural studies have supported the Gholamreza–Rezaei–Behbehani (GRB) solvation model [29–31]. The more negative Gibbs free energies in the high Cu^{2+} concentrations (Fig. 2) also indicate higher affinity in this region. p=1 indicates that the binding is non-cooperative in two identical and non interacting binding sites. The above interpretations are in agreement with the previous reports of Alzheimer's disease treatment with Cu^{2+}-selective chelators [17]. Glycine has minor effect on the stability of *A*β(1–16) as evidenced by small δ_A^θ and δ_B^θ values. p=2 indicates that the binding has positive cooperativity in the two binding sites. The less negative Gibbs free energies for Gly+*A*β(1–16) interactions (Fig. 2) indicate that glycine has lower affinity to *A*β(1–16) than Cu^{2+}.

Conclusions

The new extended solvation model was used to reproduce the enthalpies for the interaction of glycine and Cu^{2+} to the first 16 residues of the Alzheimer's amyloid β peptide. We have calculated all thermodynamic functions, cooperativity parameters, equilibrium constants and stability prediction as a result of ligand interaction with a biopolymer, just using Eqs (1) and (7) and it is a revolution in the ligand+macromolecule interactions.

Acknowledgements

Financial support from the Universities of Imam Khomeini (Qazvin) and Tehran and the Iranian National Science Foundation (INSF) are gratefully acknowledged.

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Received: May 28, 2008
Accepted: October 6, 2008
Online First: January 12, 2009

DOI: 10.1007/s10973-008-9311-9