Journal of Thermal Analysis and Calorimetry, Vol. 93 (2008) 2, 479–483

APPLICATION OF AN EXTENDED SOLVATION THEORY TO STUDY ON THE BINDING OF MAGNESIUM ION WITH MYELIN BASIC PROTEIN

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Binding properties of myelin basic protein (MBP) from bovine central nervous system due to the interaction by divalent magnesium ion (Mg^{2+}) was investigated at 27°C in aqueous solution using isothermal titration calorimetry (ITC) technique. An extended solvation model was used to reproduce the enthalpies of Mg^{2+}–MBP interaction over the whole Mg^{2+} concentrations. It was found that there is a set of two identical and noninteracting binding sites for Mg^{2+} ions. The dissociation equilibrium constant is K_d=45.5 μM. The molar enthalpy of binding site is identical for both sites; ΔH= –15.24 kJ mol^{-1}.The solvation parameters recovered from the solvation model were attributed to the structural change of MBP due to the metal ion interaction.

Keywords: *isothermal titration calorimetry, magnesium, myelin basic protein, solvation parameters*

Introduction

Calorimetry, the principal source of thermodynamic information, is of the most powerful tools for expanding knowledge in biosciences. A principal calorimetric technique that has contributed is isothermal titration calorimetry (ITC). The method of ITC is now widely used to obtain thermodynamics information about biochemical binding processes at constant temperature to obtain additional information on biomacromolecule structure function relationship [1–10]. During the last decade years we attempt to study the metal ion binding study on different proteins [10–20]. They will change the conformational stability and formation of aggregates.

Myelin basic protein (MBP) is one of the most important proteins of the myelin sheath [21]. Various aspects of MBP (*MW*=18.500), including its immunological properties have been summarized in several reviews [22–24]. The structure of MBP, interaction of this protein with other molecules, particularly lipids, the influence of other molecules on the structure of the protein and the nature of its association with the myelin membrane have been reviewed by Smith [25]. In aqueous solution, the thermodynamically stable state of MBP is a flexible coil in which the protein contains about 20% β-sheet secondary structure [26, 27]. MBP lacks both the disulfide bonds and a little secondary structure [25]. Some metal ions (Zn^{2+}, Co^{2+}, Cu^{2+}) inhibits dissociation of MBP from the membrane [28]. Binding of Cd, Co, Cu, Hg, Mn, Pb, Zn, Ca and Mg ions by isolated MBP of bovine central nervous system (CNS) have been assessed by centrifugal equilibrium dialysis [29]. These metal ions were bound in the order of Hg>Cu>Zn> Mg>Cd>Co, exempting Mn, Pb and Ca. A complete study on thermodynamics of binding zinc [30] and copper [10, 31] ions has been reported before by our group. 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 metals on the stability of a protein in a very simple way. The present paper reports some interesting experimental data for the heats of interaction of Mg^{2+} ions with MBP and analyses these using the extended solvation theory. Studies within our group are aimed at developing an understanding of how the metal ions and other ligands binding proteins affect on the stability of the biomolecules. One of the unique aspects of our approach is studying the stability of proteins by using the extended solvation model. The extended solvation model enables us to determine the dissociation constants for the interactions of metal ions with MBP directly and accurately.

Experimental

MBP from bovine central nervous systems (CNS) obtained from Sigma Chemical Co. Magnesium nitrate was purchased from Merck Co. All other materials and reagents were of analytical grade, and solutions were made in double-distilled water.

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1388–6150/$20.00

Akadémiai Kiadó, Budapest, Hungary
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The isothermal titration microcalorimetric experiments were performed with the four channel commercial microcalorimetric system, Thermal Activity Monitor 2277, Thermometric, Sweden. The titration vessel was made from stainless steel. Magnesium nitrate solution (500 μM) was injected by use of a Hamilton syringe into the calorimetric titration vessel, which contained 1.8 mL MBP (13.5 μM). Thin (0.15 mm inner diameter) stainless steel hypodermic needles, permanently fixed to the syringe, reached directly into the calorimetric vessel. Injection of magnesium solution into the perfusion vessel was repeated 30 times, with 30 μL per injection. 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 heat of dilution of the magnesium solution was measured as described above except MBP was excluded. The enthalpies of dilution of the magnesium solutions were subtracted from the enthalpy of Mg^{2+}–MBP interaction. The enthalpies of dilution of MBP are negligible. The microcalorimeter was frequently calibrated electrically during the course of the study. The molecular mass of MBP was taken to be 18500 Da. The heats of Mg^{2+}–MBP interactions have been calculated in kJ mol^{-1} and reported in Table 1.

Results and discussion

It has been suggested that solvation of a solute in binary solvent mixtures is analogous to complexation, with the better solvent taking the role of the ligand.

Table 1 Enthalpies of Mg^{2+}–MBP interactions at 300 K in kJ mol^{-1} Q_{dilut} is the enthalpies of dilution of MBP with water. Precision is ±0.005 kJ or better

MBP_T/μM	Mg^{2+}/μM	Q	Q_{dilut}
13.279	8.197	–3.419	–3.94
12.857	23.809	–8.704	–3.551
12.656	38.460	–12.428	–3.005
12.089	52.239	–15.098	–2.589
11.739	65.217	–17.069	–2.271
11.408	77.465	–18.564	–2.014
11.096	89.040	–19.728	–1.809
10.800	100.000	–20.654	–1.639
10.519	110.389	–21.411	–1.498
10.253	120.253	–22.033	–1.378
10.000	129.626	–22.559	–1.276
9.759	138.554	–23.004	–1.189
9.529	147.059	–23.39	–1.112
9.310	155.172	–23.724	–1.044
9.000	166.667	–24.152	–0.956

The model used to analyze the enthalpies of transfer of a solute from a pure solvent into a mixed solvent system has been presented in detail previously [32–41]. This takes account of preferential solvation by the components of a mixed solvent, the extent to which the solute disrupts or enhances solvent–solvent bonding and the interaction of the solute with the surrounding solvent molecules. This treatment leads to:

$$\Delta H_t^\theta = \overset{A\to B}{\Delta} H_t^\theta x_B' - (\alpha n + \beta N)(x_A' L_A + x_B' L_B) \quad (1)$$

where $\overset{A\to B}{\Delta} H_t^\theta$ is the enthalpy of transfer from pure solvent A to pure solvent B. x_A' and x_B' are the local mole fractions of the components A and B in the solvation sphere, where the solvent molecules are the nearest neighbours of the solute, which can be expressed as follow:

$$x_B' = \frac{p x_B}{x_A + p x_B} = \frac{n_B}{n}, \quad x_A' = 1 - x_B' \quad (2)$$

ΔH_t^θ is the enthalpy of transfer of the solutes from solvent A to the mixture of solvent A and B. x_A and x_B represent the bulk mole fractions of the components A and B in the binary mixtures. L_A and L_B are the relative partial molar enthalpies of A and B in the mixed solvent. The parameter (αn+βN) reflects the net effect of the solute on the solvent–solvent bonding, with αn resulting from the formation of a cavity wherein n solvent molecules become the nearest neighbours of the solute and βN reflecting the enthalpy change from strengthening or weakening of solvent–solvent bonds of N solvent molecules ($N \geq n$) around the cavity ($\beta<0$ indicates a net strengthening of solvent–solvent bonds). The constants α and β represent the fraction of the enthalpy of solvent–solvent bonding associated with the cavity formation or restructuring, respectively. The superscript θ in all cases refers to the quantities in infinite dilution of the solute. $p<1$ or $p>1$ indicate a preference for solvent A or B, respectively; $p=1$ indicates random solvation. As the parameters, β, n, N and (αn+βN) are not constant during the solvent compositions; thereby the net effect of the solute on solvent–solvent bonds in mixture $(\alpha n+\beta N)^{mix}=\delta^{mix}$, is changed over the solvent compositions and we can express this parameter as follow:

$$\delta^{mix} = \delta_A^\theta x_A' + \delta_B^\theta x_B' = \delta_A^\theta + (\delta_B^\theta - \delta_A^\theta) x_B' \quad (3)$$

x_A' and x_B' are the local mole fractions of the components A and B in the vicinity of the solute or solvation sphere. δ_A^θ and δ_B^θ are the net effects of the solute on solvent-solvent bonds in water-rich domain and cosolvent-rich region respectively. Therefore, Eq. (1) changes to:

$$\Delta H_t^\theta = \overset{A\to B}{\Delta} H_t^\theta x_B' - \delta^{mix}(x_A' L_A + x_B' L_B) \quad (4)$$

Substituting δ^{mix} from Eq. (3) into Eq. (4), leads to:

$$\Delta H_t^\theta = \overset{A\to B}{\Delta} H_t^\theta x_B' - \delta_A^\theta (x_A' L_A + x_B' L_B) - (\delta_B^\theta - \delta_A^\theta)(x_A' L_A + x_B' L_B) x_B' \quad (5)$$

With simple modification of Eq. (5), it is possible to use this equation to reproduce the enthalpies of surfactant–protein interaction as follow:

$$Q = Q_{max} x_B' - \delta_A^\theta (x_A' L_A + x_B' L_B) - (\delta_B^\theta - \delta_A^\theta)(x_A' L_A + x_B' L_B) x_B' \quad (6)$$

where Q is the heat of Mg^{2+}–MBP interactions at certain ligand concentrations and $Q_{max} = \Delta\Delta H_{12}^0 + \delta_B^0 \Delta H^S_{Mg(NO_3)_2} - \delta_A^0 \Delta H_W^{0*}$ represents the heat value upon saturation of all MBP. $\Delta\Delta H_{12}^0$ is the difference between the enthalpies of water–MBP and Mg^{2+}–MBP interactions. $\Delta\Delta H_{12}^0 < 0$ indicates that the interaction of the MBP with Mg^{2+} is stronger than with water. ΔH_W^{0*} is the enthalpy of condensation of pure water (–44.7 kJ mol^{-1}) and $\Delta H^S_{Mg(NO_3)_2}$ is the enthalpy of solution of magnesium nitrate in water (3.4 kJ mol^{-1}). x_A and x_B are bulk mole fractions in solvation model theory and we can express them in Mg^{2+}–MBP interaction as the total ligand concentrations divided by the maximum concentration of Mg^{2+} as follow:

$$x_B = \frac{\lfloor Mg^{2+} \rfloor_T}{\lfloor Mg^{2+} \rfloor_{max}} \quad (7)$$

where $\lfloor Mg^{2+} \rfloor_T$ is the total concentration of $Mg^{2+}$and $\lfloor Mg^{2+} \rfloor_{max}$ is the maximum consternation of Mg^{2+} ion. L_A and L_B are the relative partial molar enthalpies and can be calculated from heats of dilution of Mg^{2+} in water, Q_{dilut}, as follow:

$$L_A = Q_{dilut} + x_B \left(\frac{\partial Q_{dilut}}{\partial x_B} \right), \quad L_B = Q_{dilut} - x_A \left(\frac{\partial Q_{dilut}}{\partial x_B} \right) \quad (8)$$

The enthalpies of Mg^{2+}–MBP interactions (Q) were fitted to Eq. (6) over the whole Mg^{2+} compositions. In the procedure the only adjustable parameter (p) was changed until the best agreement between the experimental and calculated data was approached over the whole range of solvent composition (Fig. 1). δ_A^0 and δ_B^0 are the net effects of MBP on solvent–solvent bonds in water-rich region and Mg^{2+}-rich region respectively which are recovered from the coefficients of the second and third terms of Eq. (6). $p<1$ or $p>1$ indicate a preferential solvation of MBP by or Mg^{2+} respectively; $p=1$ indicates random solvation. The solvation parameters recovered from Eq. (6) have been shown in Table 2.

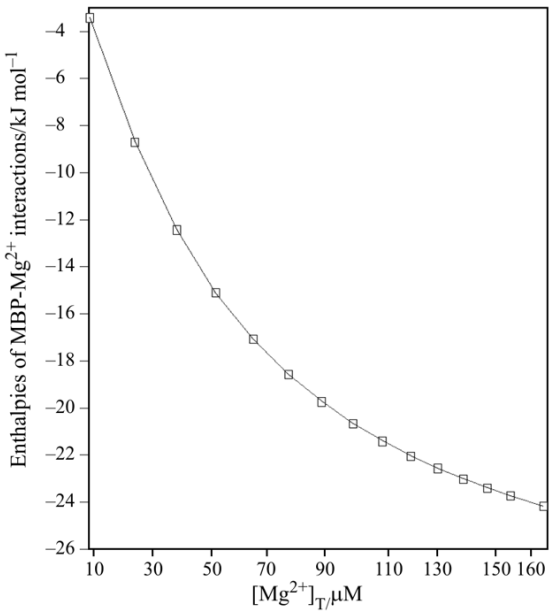

Fig. 1 Comparison between the experimental enthalpies (□) for Mg^{2+}–MBP interactions and calculated data (lines) via Eq. (6). $[Mg^{2+}]_T$ is total concentrations of Mg^{2+} solutions

Table 2 Thermodynamic parameters for Mg^{2+}–MBP interactions via Eq. (6). $\Delta\Delta H_{12}>0$ indicates that the interaction of the MBP with Mg^{2+} is weaker than with water

p	δ_A^θ	δ_B^θ	$\Delta\Delta H_{12}$/kJ mol^{-1}
5.005	0.43	0.48	–21.01

In general, there will be 'g' sites for binding of ligand molecules (Mg^{2+} in this case) per one mole of protein and ν is defined as the average moles of bound ligand per mole of protein. As $x_B' = \frac{n_B}{n}$ in the solvation sphere of the solute, it is possible to change it to $x_B' = \frac{\nu}{g}$ for metal–protein interaction. Therefore if $x_B' = \frac{\nu}{g}$ values recovered from Eq. (6) are multiplied by 'g', experimental values can be calculated easily with using only one concentration of MBP.

It is possible to use Eq. (9) for calculation of K_d and 'g' in a very simple way as follow [11, 12]:

$$\frac{\Delta Q}{Q_{max}}[MBP]_T = \left[\frac{\Delta Q}{Q}\right][Ca^{2+}]_T \frac{1}{g} - \frac{K_d}{g} \quad (9)$$

where $\Delta Q = Q_{max} - Q$. Therefore, the plot of $\frac{\Delta Q}{Q_{max}}[MBP]_T$ *vs.* $\left[\frac{\Delta Q}{Q}\right][Mg^{2+}]_T$ should be a linear plot by a slope of $\frac{1}{g}$ and the vertical-intercept of $\frac{K_d}{g}$. K_d

and 'g' values obtained from Eq. (9) for Mg^{2+}–MBP interaction are as follow:

$$K_d = 45.5\ \mu M \text{ and } g = 2$$

The molar enthalpy of binding of Mg^{2+} with MBP, ΔH, is obtained by:

$$\Delta H = \frac{Q_{max}}{g} = \frac{-30.48}{2} = -15.24\ kJ\ mol^{-1}$$

Protein denaturation occurs when a polypeptide loses its higher level of structure, and leads to aggregation. The most common mechanism of protein aggregation is believed to involve protein denaturation, via hydrophobic interfaces and often results in loss of biological activity [13–41]. When two nonpolar groups come together on the folding of a polypeptide chain, the surface area exposed to the solvent is reduced and part of the highly ordered water in the solvation shell is released to the bulk solvent. Therefore nonpolar moieties to come together in aqueous solvent, resulting in the formation of multimers, and in extreme cases, aggregation and precipitation. The δ_A^0 and δ_B^0 values reflect to the hydrophobic hydration of MBP and give a measure of relative enhancement of water structure result in the loss of entropy. The greater the extent of this enhancement, the greater will be the stabilization of the MBP structure and the greater the values of δ_A^0 and δ_B^0. In the Mg^{2+}-rich region there was little increase in the δ_B^0 value, indicating that Mg^{2+} stabilized MBP. One important post-translational of MBP that correlates with the severity of autoimmune disease multiple sclerosis (MS) is deimination, the enzymatic conversion of arginine to citrulline by peptidylarginine deiminase. Deimination limits MBP ability to maintain a compact myelin sheath by disrupting both its tertiary structure and its interaction with lipids. Mg^{2+}–MBP interaction gives rise to an increase in the hydrophobic property of the MBP as evidenced by the increased value δ_B^0 (0.48 in Table 2) in Mg^{2+}-rich domain. Therefore despite the deimination processes, Mg^{2+}–MBP interaction gives rise to a minor increase in the stability of MBP. It is possible to describe the activity of MBP by δ_A^0 and δ_B^0 values. The greater the δ_A^θ and δ_B^θ values, the greater the biological activity of MBP. $\Delta\Delta H_{12}<0$ indicates that the interaction of the MBP with Mg^{2+} is stronger than with water. These results ($\delta_B^0>\delta_A^0$ and $\Delta\Delta H_{12}<0$) were indicative of Mg^{2+} ability to stabilize the MBP. p value (5.005) shows the tendency of metal ions for occupying the available sites on MBP. In other word p is the mean stability constant for the successive replacement of water molecules in the solvation shell of the MBP by Mg^{2+} ions.

Conclusions

The extended coordination model, via Eq. (6) will satisfactorily reproduce the enthalpies of Mg^{2+}–MBP interactions (Fig. 1). Prediction of activity of MBP, structural changes of the protein, binding enthalpies and dissociated equilibrium binding constants using only one set of metal-protein enthalpies, makes this theory the most powerful one. Mg^{2+}–MBP interaction increases the stability and the biological activity of MBP.

Acknowledgements

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

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Received: August 6, 2007
Accepted: November 20, 2007

DOI: 10.1007/s10973-007-8674-7