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A New Approach for Titration Calorimetric Data Analysis on the Binding of Magnesium Ion with Myelin Basic Protein

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Abstract The interaction of the myelin basic protein (MBP) from the bovine central nervous system with divalent magnesium ion was studied by isothermal titration calorimetry at 27 °C in aqueous solution. A simple rapid method for determination of the dissociation binding constants for Mg^{2+}-MBP interaction was introduced using the isothermal titration calometric data. The binding isotherm for Mg^{2+}-MBP interaction is easily obtained by carrying out a titration calorimetric experiment using only one set of concentrations of MBP. There are two identical independent intrinsic association constants equal to 0.021 $\mu mol \cdot L^{-1}$ in the first- and second-binding sites, respectively.

Keywords Myelin basic protein · Magnesium · Isothermal titration calorimetry · Binding parameters

1 Introduction

The energetics of biochemical reactions or molecular interactions at constant temperature are measured by Isothermal Titration Calorimetry (ITC) [1–3]. ITC gives invaluable information about biomacromolecule-ligand interactions. During the last two years we attempted to study the metal ion binding chemistry of different proteins [4–12]. They will change the conformational stability and form aggregates. The importance of metal ions such as Ca^{2+} and Mg^{2+} in determining the stability of proteins is widely reported [13–18]. There are some approximations in previous theories [18] which result in rough average values of binding parameters including a lot of errors. We have introduced a novel method which enables us to determine the binding parameters with minimal errors. This method is successfully applied to the analysis of Mg^{2+} binding MBP.

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2 Materials and Method

2.1 Materials

MBP from bovine central systems (CNS) was obtained from Sigma Chemical Co. Magnesium nitrate was purchased from Merck Co. Protein concentrations were determined from absorbance measurements at 277 nm in 1 cm quartz cuvettes. All other materials and reagents were of analytical grade, and solutions were made from double-distilled water.

2.2 Method

The isothermal titration micro-calorimetric experiments were performed with the four-channel commercial micro calorimetric system, Thermal Activity Monitor 2277, Thermometric, Sweden. The titration vessel was made from stainless steel. The magnesium nitrate solution (500 $\mu mol \cdot L^{-1}$) was injected by use of a Hamilton syringe into the calorimetric titration vessel, which contained 1.8 mL MBP (13.5 $\mu mol \cdot L^{-1}$). Thin (0.15 mm inner diameter) stainless steel hypodermic needles, permanently fixed to the syringe, reached directly into the calorimetric vessel. Injection of magnesium nitrate solution into the perfusion vessel was repeated 30 times, with 30 µL per injection. The calorimetric signal was measured by a digital voltmeter which was part of a computerized recording system. The heat of each injection was calculated by the "Thermometric Digitam 3" software program. The enthalpy 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 micro calorimeter was frequently calibrated electrically during the course of the study. The molecular weight of MBP was taken to be 18500 Da. The enthalpies of Mg^{2+}-MBP interactions were calculated in $kJ \cdot mol^{-1}$ and are shown in Fig. 1.

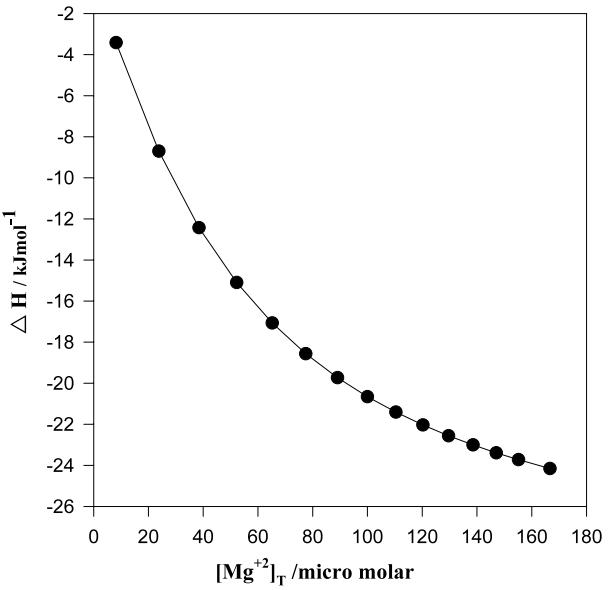

Fig. 1 Comparison between the experimental enthalpies, ΔH, for Mg^{2+}-MBP interactions (•) and calculated curve via Eq. 1. $[Mg^{2+}]_T$ is the total concentration of $Mg(NO_3)_2$ solutions in $\mu mol \cdot L^{-1}$

3 Results and Discussion

We have shown previously that the enthalpies of the solute-solvent (Mg^{2+}-MBP-water in this case) interactions in the aqueous solvent (Mg^{2+}-water in the present case) system, can be accounted for quantitatively in terms of three factors: preferential solvation by the components of a mixed solvent, weakening or strengthening of solvent-solvent bonds by the solute and the change in the enthalpy of the solute-solvent interactions [19–28]. This treatment leads to:

$$\Delta H = \Delta H_{\max} x'_{\mathrm{B}} - \delta^{\theta}_{\mathrm{A}}(x'_{\mathrm{A}} L_{\mathrm{A}} + x'_{\mathrm{B}} L_{\mathrm{B}}) - (\delta^{\theta}_{\mathrm{B}} - \delta^{\theta}_{\mathrm{A}})(x'_{\mathrm{A}} L_{\mathrm{A}} + x'_{\mathrm{B}} L_{\mathrm{B}}) x'_{\mathrm{B}} \tag{1}$$

The parameters $\delta^{\theta}_{\mathrm{A}} = (\alpha n + \beta N)^{\theta}_{\mathrm{A}}$ and $\delta^{\theta}_{\mathrm{B}} = (\alpha n + \beta N)^{\theta}_{\mathrm{B}}$ are the net effects of the solute (MBP in this case) on the water-Mg^{2+} bonds in the water-rich domain and Mg^{2+}-rich region, respectively, with αn resulting from the formation of a cavity wherein n solvent molecules become the nearest neighbors of the solute and βN reflects 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 the solvent–solvent bonds). The constants α and β represent the fraction of the enthalpy of water–Mg^{2+} interaction associated with cavity formation or restructuring, respectively. The superscript θ in all cases refers to the quantities at infinite dilution of the solute. $p < 1$ or $p > 1$ indicate a preference for water or Mg^{2+}, respectively; $p = 1$ indicates random solvation; and the x'_{B} are the local mole fractions of the water and surfactant in the vicinity of the lysozyme or solvation sphere, where the solvent molecules are the nearest neighbors of the solute, which can be expressed as follows:

$$x'_{\mathrm{B}} = \frac{p x_{\mathrm{B}}}{x_{\mathrm{A}} + p x_{\mathrm{B}}} = \frac{\nu}{g} \tag{2}$$

where x_{A} and x_{B} are bulk mole fractions and we can express them as the total Mg^{2+} concentration divided by the maximum concentration of Mg^{2+} upon saturation of all MBP as follows:

$$x_{\mathrm{B}} = \frac{[\mathrm{Mg}^{2+}]_{\mathrm{T}}}{[\mathrm{Mg}^{2+}]_{\max}}, \quad x_{\mathrm{A}} = 1 - x_{\mathrm{B}} \tag{3}$$

$[Mg^{2+}]_{\mathrm{T}}$ is the total concentration of surfactant and $[Mg^{2+}]_{\max}$ is the maximum concentration of the Mg^{2+} upon saturation with respect to MBP. In general, there will be "g" sites for binding of Mg^{2+} per MBP molecule and ν is defined as the average moles of bound Mg^{2+} per mole of total MBP. L_{A} and L_{B} are the relative partial molar enthalpies and can be calculated from heats of dilution of Mg^{2+} in water, $\Delta H_{\mathrm{dilut}}$, as follows:

$$L_{\mathrm{A}} = \Delta H_{\mathrm{dilut}} + x_{\mathrm{B}}\left(\frac{\partial \Delta H_{\mathrm{dilut}}}{\partial x_{\mathrm{B}}}\right), \quad L_{\mathrm{B}} = \Delta H_{\mathrm{dilut}} + x_{\mathrm{A}}\left(\frac{\partial \Delta H_{\mathrm{dilut}}}{\partial x_{\mathrm{B}}}\right) \tag{4}$$

The enthalpies of Mg^{2+}-MBP interactions, ΔH, were fitted by Eq. 1 over the whole range of Mg^{2+} compositions. In this 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 compositions (Fig. 1). Thermodynamic parameters for Mg^{2+}-MBP interactions recovered from Eq. 1 are listed in Table 1. The experimental enthalpies of Mg^{2+}-MBP interactions and calculated data from Eq. 1 are compared in Fig. 1. The agreement between the experimental enthalpies and calculated data via Eq. 1 provides good support for this equation.

Table 1 Thermodynamic parameters for Mg^{2+}-MBP interactions recovered from Eq. 1. $\Delta\Delta H_{12} < 0$ indicates that the interaction of the MBP with Mg^{2+} is stronger than with water. The precision is ±0.005 or better

[MBP] μmol·L^{-1}	p	δ^o_A	δ^o_B	$\Delta\Delta H_{12}$
13.5	5.005	0.434	0.476	−17.774

φ is the fraction of MBP molecule undergoing complexation with Mg^{2+} which can be expressed as follows:

$$\varphi = \frac{(\Delta H_W - \Delta H)}{(\Delta H_W - \Delta H_{max})} \tag{5}$$

where ΔH_W is the enthalpy of Mg^{2+}-MBP interactions in the water-rich domain and $\Delta H_{max} = \Delta\Delta H^o_{12} + \delta^o_B \Delta H^S_{Mg(NO_3)_2} - \delta^o_A \Delta H^{o*}_W$ represents the heat value upon saturation of all MBP. $\Delta\Delta H^o_{12}$ is the difference between the enthalpies of the Mg^{2+}-MBP and water-MBP interactions. $\Delta\Delta H_{12} > 0$ indicates that the interaction of the MBP with Mg^{2+} is weaker than with water. ΔH^{o*}_W is the enthalpy of condensation of pure water (−44.700 kJ·mol^{-1}) and $\Delta H^S_{Mg(NO_3)_2}$ is the enthalpy of solution of magnesium nitrate in water (−3.400 kJ·mol^{-1}). The equilibrium constant values, K_d, as a function of Mg^{2+} concentration can be calculated as follows:

$$K_d = \frac{\varphi}{1-\varphi} \tag{6}$$

The equilibrium constants, K_d, for successive replacement of the Mg^{2+} ions by water molecules are as follows:

$$K_d = x^g_A - \sum_{i=1}^{g} \beta'_i \frac{x^i_B}{x^{i-g}_A} \tag{7}$$

In fact the K_d values are the equilibrium constants for the equilibria:

$$\begin{gathered} MBP(H_2O)_{g-i}(Mg^{2+})_i + iH_2O \rightleftharpoons MBP(H_2O)_g + iMg^{2+}, \\ K_d = \frac{[MBP(H_2O)_g][Mg^{2+}]^i}{[MBP(H_2O)_{g-i}(Mg^{2+})_i]} \end{gathered} \tag{E1}$$

The K_d values obtained from Eq. 6, were fitted by Eq. 7 using a computer program for nonlinear least-squares fitting. Therefore, we can approximate the "g" value simply as $g = 2$ in this work. The β'_i values are the equilibrium products for the equilibria E1. Finally, the ν values can be calculated at any concentration of Mg^{2+} via Eq. 3. The binding parameters obtained from this method are listed in Tables 2 and 3 and shown graphically in Figs. 2 and 3. The Gibbs energies as a function of the Mg^{2+} concentration can be obtained as follows:

$$\Delta G = -RT \ln K_a \tag{8}$$

where K_a is the association equilibrium constant ($1/K_d$) as a function of Mg^{2+} concentration. Gibbs energies, ΔG, calculated from Eq. 8 are shown graphically in Fig. 4. The ΔS values were calculated using the ΔG values and are shown in Fig. 5. Therefore, for the first

Table 2 β_i''s values recovered from the coefficients of Eq. 7, for 2 individual sites of MBP

Number of sites	β_i'/μmol·L^{-1}
1	3.272 ± 0.026
2	3.383 ± 0.013

Table 3 K_d and ν values as a function of Mg^{2+} concentrations for Mg^{2+}-MBP interactions. The precisions are ±0.005 or better

$[Mg^{+2}]_T$/μmol·L^{-1}	K/μmol·L^{-1}	ν
8.197	0	0.411
23.809	0.342	0.909
52.239	0.768	1.200
65.217	1.289	1.391
77.465	1.927	1.526
89.041	2.710	1.626
100.000	3.686	1.703
110.389	4.927	1.765
120.253	6.564	1.815
129.626	8.784	1.857
138.554	12.015	1.892
147.059	17.060	1.922
155.172	26.209	1.948
166.667	47.441	1.970

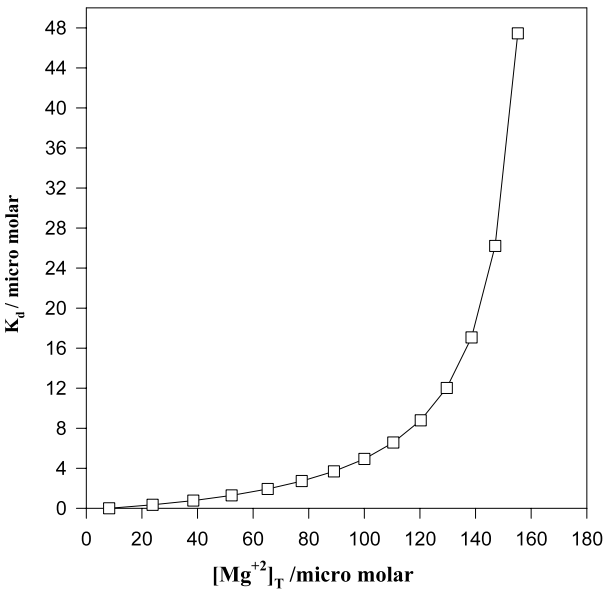

Fig. 2 The experimental dissociation constants (□), K_d, via Eq. 6 and calculated curve for Mg^{2+}-MBP interactions (in μmol·L^{-1}) against the Mg^{2+} concentrations (μmol·L^{-1}) via Eq. 7

time, we managed to calculate ΔG and ΔS values using one set of experimental data at one temperature.

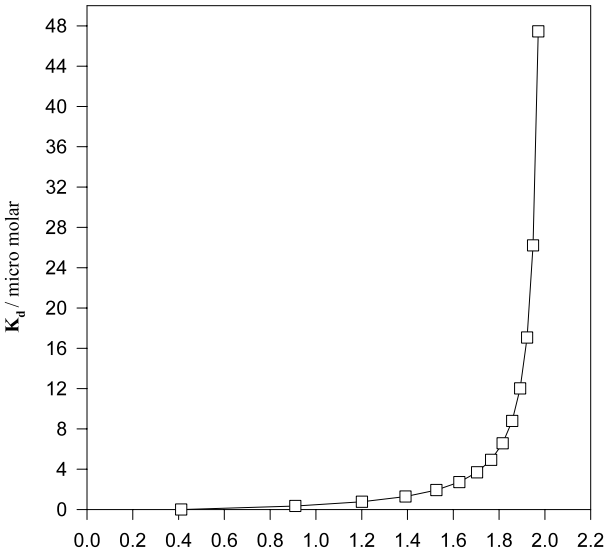

Fig. 3 ν values for Mg^{2+}-MBP interactions ($\mu mol \cdot L^{-1}$) against the K_d ($\mu mol \cdot L^{-1}$)

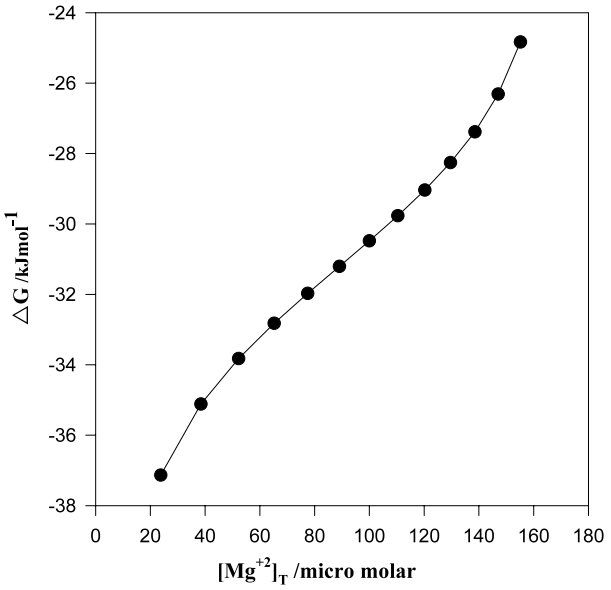

Fig. 4 The experimental ΔG values (•) via Eqs. 6 and 8 ($kJ \cdot mol^{-1}$) and the calculated data from Eqs. 7 and 8 (*curves*) for Mg^{2+}-MBP interactions against the Mg^{2+} concentrations ($\mu mol \cdot L^{-1}$)

If the multiple binding sites on a macromolecule are identical and independent, the ligand binding sites can be reproduced by a model system of monovalent molecules ($(MBP)_g \rightarrow gMBP$) with the same set of dissociation constant, $(K_d)_{max}$, values. Setting $g = i$ in Eq. E1, leads to:

$$MBP(Mg^{2+})_g + gH_2O \rightleftharpoons MBP(H_2O)_g + gMg^{2+}, \tag{E2}$$

$$(K_d)_{max} = \frac{[MBP(H_2O)_g][Mg^{2+}]^g}{[MBP(Mg^{2+})_g][H_2O]^g}$$

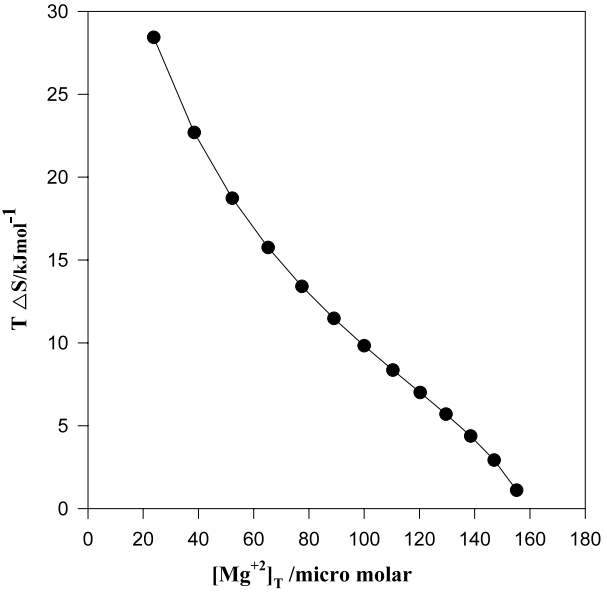

Fig. 5 The experimental ΔS values (•) and calculated curve for Mg^{2+}-MBP interactions ($kJ{\cdot}mol^{-1}$) against the Mg^{2+} concentration ($\mu mol{\cdot}L^{-1}$)

Equilibrium E2 is a special case of the equilibrium E1 in the Mg^{2+}-rich domain where MBP is saturated by Mg^{2+} ions. In other words, the $(K_d)_{max}$ value corresponds to the point where $x'_B = 1$ (or the maximum ν value) (Fig. 3) and can be calculated easily. Binding parameters for the Mg^{2+}-MBP interactions using the new model are as follows:

$$(K_d)_{max} = 47.44\ \mu mol{\cdot}L^{-1} \quad \Delta H = -15.10\ kJ{\cdot}mol^{-1} \quad g = 2$$

As we have reported previously, the free concentration of ligand, $[Mg^{2+}]_F$, can be calculated as follows:

$$[Mg^{2+}]_F = [Mg^{2+}]_T - \nu[MBP]_T \tag{9}$$

The association binding constant, K_a, can be obtained from the Scatchard equation as follows:

$$\frac{\nu}{g - \nu} = K_a[Mg^{2+}]_F \tag{10}$$

The association constant for the Mg^{2+}-MBP interaction obtained from Eq. 10 is $0.021\ \mu mol{\cdot}L^{-1}$ ($K_d = 47.62\ \mu mol{\cdot}L^{-1}$) [27, 28], which is in good agreement with the $(K_d)_{max}$ value calculated from Eqs. 6 and 7. Equation 10 gives us the maximum value for K_d, but Eqs. 6 and 7 allows us to have the K_d values at every concentration of Mg^{2+} ions.

Figure 2 shows the dissociation constant as a function of the total concentration of Mg^{2+}. The low K_d values in the water-rich domain reflect the higher affinity of MBP for Mg^{2+} in this domain which is in agreement with the $\Delta\Delta H_{12}$ value (Table 1). In the Mg^{2+}-rich domain the slope of the curve in Fig. 2 varies sharply, indicating a lower affinity of MBP for Mg^{2+} in this region. The shallow slope of ν against K_d(Fig. 3), corresponds to the higher affinity of MBP in the water-rich domain followed by the steep slope which indicates a lower affinity of MBP in the Mg^{2+}-rich domain. The negative Gibbs energies in the water-rich domain (Fig. 4) also indicate the higher affinity in this region.

We will arrive at the same conclusion using the solvation parameters recovered from Eq. 1 (Table 2). The δ_A^θ values reflect to the hydrophobic hydration of MBP, leading to the enhancement of the water structure. The greater the extent of this enhancement, the greater the stabilization of the MBP structure and the greater the δ_A^θ value and vise versa. In the Mg^{2+}-rich region the δ_B^θ value is more positive (0.476) than that in water-rich domain, indicating that the Mg^{2+}-MBP complex is more stable (lower affinity) in the Mg^{2+}-rich region in comparison to the water-rich domain. The p value (5.005) shows the preferential tendency of Mg^{2+} for occupying the available sites on MBP.

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