Thermodynamic Study of Myelin Basic Protein upon Interaction with [Hg^{2+}] Using Extension Solvation Model

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Mercury ion interaction with myelin basic protein (MBP) was studied at 300 K in 30 mmol/L tris buffer, pH = 7 by isothermal titration calorimetry (ITC). An extended solvation model was used for Hg^{2+} + MBP interaction over the whole range of Hg^{2+} concentrations. The binding parameters recovered from the solvation model were attributed to the structural changes of MBP due to its interaction with mercury ion. It was found that mercury ion acted as a noncooperative effector of MBP, and there is a set of two identical and independent binding sites for Hg^{2+} ions. The dissociation equilibrium constant is 97.6 µmol/L. The molar enthalpy change of binding is -11.25 kJ•mol^{-1}.

Keywords mercury ion, myelin basic protein, isothermal titration calorimetry, binding parameter

Introduction

Thermodynamic of biomacromolecule-ligand interaction is very important to understand the structure function relationship in proteins. Isothermal titration calorimetry (ITC) gives invaluable information about biomacromolecule-ligand interaction, protein denaturation, kinetic parameters and enzyme inhibition. The correlation of structural and calorimetric measurements is one of the fundamental areas of advance incorporating ITC data. The number of publications on ITC has grown exponentially over the last decade, reflecting the general utility of the method. We attempted to study the metal ion binding to different proteins during the last years. We have before developed a theory to account for the solvation of solutes in mixed solvent systems. The extended solvation model, ESM, satisfactorily reproduced all the experimental enthalpy transfer of the solutes from pure solvents into mixed solvent systems across the whole range of solvent compositions. 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 ESM. Myelin basic protein, MBP, is one of the most important proteins of the myelin sheath, and the predominant extrinsic protein in both central and peripheral parts of the central nervous system myelin. It is thought to be involved in the stabilizing interactions between myelin membranes, and it may play an important role in demyelinating diseases such as multiple sclerosis. MBP is an “intrinsically unstructured” or “natively unfolded” protein; therefore its three-dimensional structure might only be determined in its interaction with another protein. Binding of Cd, Co, Cu, Hg, Mn, Pb, Zn, Ca, and Mg ions to isolated MBP of bovine central nervous system [CNS] has been recently assessed by centrifugal equilibrium dialysis. 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 Hg^{2+} ions with MBP and analyses using ESM.

Materials and methods

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

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 of stainless steel. The mercury solution (1000 µmol/L) was injected by use of a Hamilton syringe into the calorimetric titration vessel, which contained 1.8 mL of MBP (27 µmol/L). Thin (0.15 mm inner diameter) stainless steel hypodermic needles, permanently fixed to...
the syring, reached directly into the calorimetric vessel. Injection of the mercury solution into the perfusion vessel was repeated 30 times, with 20 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 Thermometric Digitam 3 software program. The heat of dilution of the mercury nitrate solution was measured as described above except that MBP was excluded. The heats of dilution of the mercury nitrate solutions were subtracted from the heat of Hg\({\text{2}^+}\)+MBP interaction. The heats of dilution of MBP are negligible. The microcalorimeter was frequently calibrated electrically during the course of the study.

**Results and discussion**

One of the unique aspects of our approach is to study the stability of proteins by using ESM. We have shown before that the interaction heats of the macromolecules+ligands can be reproduced by Eq. 1 in the aqueous solvent systems.\(^{\text{28-39}}\)

\[
q = q_{\text{max}}x_B - \delta^x_A(x_A'q_{\text{max}} + x_B'q_{\text{max}}) - \\
(\delta^x_A - \delta^x_B)(x_A'q_{\text{max}} + x_B'q_{\text{max}})x_B^* 
\]

(1)

\(q\) is the heat of Hg\(2^+\)+MBP interaction and \(q_{\text{max}}\) represents the heat value upon saturation of all MBP. The parameters \(\delta^x_A\) and \(\delta^x_B\) are the indexes of MBP destability at the low and high Hg\(2^+\) concentrations respectively. Cooperative binding requires that the macromolecule has more than one binding sites, since cooperativity results from the interactions between identical binding sites with the same ligand. If the ligand binds at each site independently, the binding is non-cooperative.

\(p < 1\) or \(p > 1\) indicates negative or positive cooperativity of macromolecule for binding with ligand respectively; \(p = 1\) indicates that the binding is non-cooperative. \(x_B^*\) can be expressed as follows:

\[
x_B^* = \frac{px_B}{x_A + px_B} 
\]

(2)

\(x_B^*\) is the fraction of bound Hg\(2^+\) with MBP molecule, and \(x_A = 1 - x_B\) is the fraction of unbound Hg\(2^+\). Now the model is a simple mass action treatment, with Hg\(2^+\) molecules replacing water molecules, at the binding sites of MBP. We can express \(x_B\) fractions, as the total Hg\(2^+\) concentrations divided by the maximum concentration of the Hg\(2^+\) upon saturation of all MBP as follows:

\[
x_B = \frac{[\text{Hg}^{2+}]_B}{[\text{Hg}^{2+}]_{\text{max}}}, \quad x_A = 1 - x_B 
\]

(3)

[\(\text{Hg}^{2+}\)]\(_B\) is the total concentration of Hg\(2^+\) and [\(\text{Hg}^{2+}\)]\(_{\text{max}}\) is the maximum concentration of the Hg\(2^+\) upon saturation of all MBP. In general, there will be “\(g\)” sites for binding of Hg\(2^+\) per MBP molecule. \(L_A\) and \(L_B\) are the relative contributions of unbound and bound Hg\(2^+\) in the heats of dilution with the exclusion of MBP and can be calculated from the heats of dilution of Hg\(2^+\) in buffer, \(q_{\text{dilut}}\), as follows:

\[
L_A = q_{\text{dilut}} + x_B \left( \frac{\partial q_{\text{dilut}}}{\partial x_B} \right), \quad L_B = q_{\text{dilut}} - x_A \left( \frac{\partial q_{\text{dilut}}}{\partial x_B} \right) 
\]

(4)

The heats of MBP+Hg\(2^+\) interactions were fitted to Eq. (1) over the whole Hg\(2^+\) compositions. In the fitting procedure, the only adjustable parameter (\(p\)) was changed until the best agreement between the experimental and calculated data was approached (Figure 1).

Figure 1 Comparison between the experimental heats (\(\Delta\)) for Hg\(2^+\)+MBP interactions and the calculated data (lines) via Eq. (1). The [\(\text{Hg}^{2+}\)]\(_T\) means the total concentrations of Hg(NO\(_3\))\(_2\) solutions in µmol/L.

Parameters \(\delta^x_A\) and \(\delta^x_B\) have been also optimized to fit the data. The optimized \(\delta^x_A\) and \(\delta^x_B\) values were recovered from the coefficients of the second and third terms of Eq. (1). The small relative standard coefficient errors, the high \(r^2\) values (0.99999) and least miscue (\(\pm 0.03\%\)) support the method. The binding parameters for MBP+Hg\(2^+\) interactions recovered from Eq. (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 Eq. (1). \(\delta^x_A\) and \(\delta^x_B\) values for MBP+Hg\(2^+\) interaction are negative, indicating that in the low and high concentrations of the mercury ions, the MBP structure was destabilized, resulting in a decrease in its biological activity. Destabilization by a ligand indicates that the mercury binds preferentially to the partially unfolded intermediate forms of the protein. Such effects are characteristic of nonspecific interactions, in which the nonspecific ligand binds weakly to many different groups at the protein/water interface, so that binding becomes a function.
of ligand concentration and available solvent-exposed protein surface area, which is increased through unfolding events.

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

\[ \Phi = \frac{q}{q_{\text{max}}} \]  

\(q_{\text{max}}\) represents the heat value upon saturation of all MBP. The appearance equilibrium constant values, \(K_a\), as a function of free concentration of the Hg\(^{2+}\) ions, [Hg\(^{2+}\)]\(_f\), can be calculated as follows:

\[ K_a = \frac{\Phi}{(1-\Phi)[\text{Hg}^{2+}]_f} = \frac{\Phi}{(1-\Phi)[\text{Hg}^{2+}]_l(1-x_n)} \]  

The Gibbs energies as a function of Hg\(^{2+}\) concentrations can be obtained as follows:

\[ \Delta G = -RT \ln K_a \]  \hspace{1cm} (7)

where \(K_a\) is the apparent association equilibrium constant. Gibbs energies, \(\Delta G\), calculated from Eq. (7) have been shown graphically in Figure 2. \(T\Delta S\) values were calculated using \(\Delta G\) and heat values, and have been shown in Figure 3.

The calorimetric data analysis using Eq. (8) is a graphical fitting model, which has been extensively applied to studies on the protein inhibition\(^2,16,18,19\) and metal binding to proteins.\(^30-39\) Consider, a solution containing ligand and a biomacromolecule, that contains \(g\) sites capable of binding the ligand. If the multiple binding sites on a biomacromolecule are identical and independent, the binding parameters can also be reproduced by Eq. (8).

**Table 1** Heats of Hg\(^{2+}\)+MBP interaction at 300 K in 30 mmol/L Tris buffer solution of pH=7

<table>
<thead>
<tr>
<th>[MBP](_f)/(µmol*L(^{-1}))</th>
<th>[Hg(^{2+})](_f)/(µmol*L(^{-1}))</th>
<th>(q/(\text{kJ}*\text{mol}^{-1}))</th>
<th>(q_{\text{dilut}}/(\text{kJ}*\text{mol}^{-1}))</th>
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<tr>
<td>26.7033</td>
<td>10.98901</td>
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<td>-759.1</td>
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Table 2  Binding parameters for Hg$^{2+}$ + MBP interactions recovered from Eq. (1)

<table>
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<tr>
<th>[MBP]/(µmol•L$^{-1}$)</th>
<th>$p$</th>
<th>$\delta^c$</th>
<th>$\delta^v$</th>
<th>$k_d$(µmol•L$^{-1}$)</th>
<th>$\Delta H$(kJ•mol$^{-1}$)</th>
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<td>27</td>
<td>1.0</td>
<td>$-1.675\pm0.025$</td>
<td>$-0.528\pm0.050$</td>
<td>98.0±0.2</td>
<td>$-11.25\pm0.05$</td>
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</tbody>
</table>

$p=1$ indicates that the binding is non-cooperative in two identical and non interacting binding sites which is in agreement with two similar dissociation equilibrium constants recovered from Eqs. (8) and (15).

\[ M + L \leftrightarrow ML \quad K_d = \frac{[M][L]}{[ML]} \]  

(9)

If $q$ and $q_{max}$ are calculated per mole of biomacromolecular, then the molar enthalpy of binding for each binding site ($\Delta H$) will be $\Delta H = \frac{q_{max}}{g}$. Therefore, the plot of $\left(\frac{\Delta q}{q_{max}}\right)K_d$ vs. $\left(\frac{\Delta q}{q}\right)L_0$ should have a linear plot slope of $\frac{1}{g}$ and the vertical-intercept of $\frac{K_d}{g}$, from which $g$ and $K_d$ can be obtained. The linearity of the plot has been examined by different estimated values for $q_{max}$ to find the best value for the correlation coefficient (near to one). The best linear plot was obtained using a value of $-1100 \mu$J (equal to $-22.63$ kJ•mol$^{-1}$) for $q_{max}$. The amounts of $g$ and $K_d$, obtained from the slope and vertical intercept plot, are 2 and 98.2 µmol/L, respectively (Figure 4). The lack of a suitable value for $q_{max}$ to obtain a linear plot of $\frac{\Delta q}{q_{max}}$ vs. $\left(\frac{\Delta q}{q}\right)L_0$ may be related to the existence of non-identical binding sites or the interaction between them.

The method introduced for ligand binding study in mercury interaction with MBP includes an assumption that the concentration of bound ligand is negligible in comparison with the total concentration of ligand. The more generalized method should be developed as follows:

\[ [L]_T = [L] + [ML] \]  

(10)
factorily reproduce the heats of MBP$^+$+Hg$^{2+}$ interactions. Prediction of stability of MBP and its structural changes, binding enthalpies using only one set of heats of interactions, makes this theory the most powerful one.

**Acknowledgements**

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**References**

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