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A thermodynamic study on the interaction of nickel ion with myelin basic protein by isothermal titration calorimetry

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Abstract The interaction of myelin basic protein (MBP) from the bovine central nervous system with divalent nickel ion was studied by isothermal titration calorimetry at 37 and 47 °C in Tris buffer solution at pH = 7. The new solvation model was used to reproduce the heats of MBP + Ni^{2+} interaction over the whole Ni^{2+} concentrations. It was found that MBP has three identical and independent binding sites for Ni^{2+} ions. The intrinsic dissociation equilibrium constant and the molar enthalpy of binding are 89.953 μM, −14.403 kJ mol^{-1} and 106.978 μM, −14.026 kJ mol^{-1} at 37 and 47 °C, respectively. The binding parameters recovered from the new solvation model were correlated to the structural changes of MBP due to its interaction with nickel ion interaction. It was found that in the low and high concentrations of the nickel ions, the MBP structure was destabilized.

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

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Introduction

Myelin is the multilamellar membranous sheath surrounding nerve axons and myelin basic protein (MBP) is an integral component of the myelin sheath in the central nervous system (CNS). It is the second most abundant protein in the CNS, comprising roughly 30% of the proteins found in CNS myelin. MBP is primarily located in the cytoplasmatic spacing of the myelin sheath and is believed to play an active role in stabilizing the periodic myelin structure via non-specific interactions with the apposing lipid bilayers. MBP has been extensively studied as the autoantigen responsible for experimental autoimmune encephalomyelitis (EAE), a model for multiple sclerosis, that Multiple sclerosis (MS), for example, is an acquired CNS disorder of young adults and characterized by inflammatory demyelination as well as axonal degeneration [1–3]. The structure of the human MBP transcription until has been determined to consist of seven exons [4]. The 18.5 kDa isoform of MBP is one of the most abundant proteins of the myelin sheath of the adult mammalian CNS, and has been the most thoroughly studied because of its importance in maintenance of the stability of the sheath [5]. The functions both in membrane adhesion, and as a linker connecting the oligodendrocyte membrane to the underlying cytoskeleton. Based on various spectroscopic measurements, all known forms of MBP (both classic and Golli) belong to the class of intrinsically disordered proteins (IDPs), sometimes also called intrinsically unstructured protein [6]. The conformation of MBP is highly dependent on its environment, it is highly extended and flexible in aqueous solution. Aqueous solution of MBP from bovine brain alone exhibited a circular dichroism (CD) spectrum characteristic of random coil protein molecules without any apparent secondary structures.

However, attains ordered secondary structure in the presence of detergents and lipids. The detailed high-resolution tertiary structure of MBP is not known [7–9]. This protein is extremely cationic (PI $\sim$ 10.6), and exists as a number of charge isomers or components (named C_1–C_8) that are the result of myriad post-translational modifications, where C_1 is the least modified and most highly positively charged [10, 11]. The energy of biochemical binding processes or molecular interactions at constant temperature are measured by Isothermal Titration Calorimetry (ITC) [12, 13]. ITC gives invaluable thermodynamics information about biomacromolecule–ligand interactions. During the last 2 years, we attempted to study the metal ion binding chemistry of different protein [14–19]. They will change the conformational stability and form aggregates. The importance of metal ions such as Ca^{2+}, Mg^{2+}, Cu^{2+}, and Co^{2+} in determining the stability of MBP by ITC is widely reported [20–25]. The interactions of Cd, Co, Cu, Hg, Mn, Pb, Zn, Ca, and Mg ions by isolated MBP of bovine central nervous system [CN] have been recently assessed by centrifugal equilibrium dialysis. Some metal ions (Zn^{2+}, Co^{2+}, and Cu^{2+}) inhibits dissociation of MBP from the membrane [26–28]. There are some approximations in previous theories which result in rough average values of binding parameters including a lot of errors. We have introduced new solvation model which enables us to determine the binding parameters with minimal errors. This model is able to correlate the binding parameters to the effect of metals on the stability of a protein in a very simple way that successfully applied to the analysis of Ni^{2+} binding MBP. 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 new solvation theory.

Materials and methods

Myelin basic protein (MBP; MW = 18.5 kDa) from bovine central systems (CNS) obtained from Sigma chemical Co. Nickel nitrate was purchased from Merk Co. All other materials and reagents were of analytical grade, and solutions were made in double-distilled water. Tris solutions with 30 mM concentration, pH = 7.0, was used as a buffer.

Isothermal Titration Calorimetry, ITC, measurements were carried out on a VP-ITC ultrasensitive microcalorimeter (MicroCal, Northampton, MA). 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. Nickel nitrate solution (1 mM) was injected by use of a Hamilton syringe into the calorimetric titration vessel, which contained 1.8 mL MBP (27 μM). Thin (0.15 mm inner diameter) stainless steel hypodermic needles, permanently fixed to the syringe, reached directly into the calorimetric vessel. Injection of nickel nitrate 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 heats of dilution of the metal ion solutions were measured as described above, except that MBP was excluded. The microcalorimeter was frequently calibrated electrically during the course of the study. The heats of MBP + Ni^{2+} interactions reported in Table 1. The molecular weight of MBP was taken to be 18.5 kDa.

Table 1 Heats of Ni^{2+} + MBP interactions, q, at 310 K (▲) and 320 K (■)

$[Ni^{2+}]$/μM	[MBP]/μM	q/μJ(▲)	q_{dilut}/μJ(▲)	q/μJ(■)	q_{dilut}/μJ(■)
10.989	26.703	−1310	−33.0	−116.1	−24.6
21.739	26.413	−251.5	−63.3	−223.0	−47.3
32.258	26.129	−362.0	−91.1	−321.3	−68.1
42.553	25.851	−463.0	−116.7	−411.5	−87.2
52.631	25.579	−555.2	−140.0	−494.2	−104.8
62.500	25.312	−639.3	−161.3	−570.0	−120.9
72.165	25.051	−715.9	−179.9	−639.5	−135.7
81.633	24.796	−785.7	−196.8	−703.2	−149.3
90.909	24.545	−849.4	−212.4	−761.7	−161.8
100.000	24.300	−907.6	−226.6	−815.4	−173.3
108.911	24.059	−960.8	−239.6	−864.8	−183.9
117.647	23.823	−1009.5	−251.5	−910.4	−193.7
126.214	23.592	−1054.1	−262.4	−952.4	−202.7
134.615	23.365	−1095.1	−272.5	−991.3	−211.1
142.857	23.143	−1132.9	−281.8	−1027.3	−218.9
150.943	22.924	−1167.7	−290.4	−1060.7	−226.1
158.878	22.710	−1199.9	−298.3	−1091.7	−232.8
166.666	22.500	−1229.7	−305.7	−1120.6	−239.1
174.312	22.294	−1257.4	−312.6	−1147.5	−245.0
181.818	22.091	−1283.1	−319.0	−1172.7	−250.5
189.189	21.892	−1307.0	−324.9	−1196.2	−255.6
196.428	21.696	−1329.4	−330.6	−1218.3	−260.4
203.539	21.504	−1350.3	−335.8	−1239.0	−265.0
210.526	21.316	−1369.9	−340.7	−1258.5	−269.3
217.391	21.130	−1388.3	−345.3	−1276.8	−273.3
224.138	20.948	−1405.5	−349.7	−1294.1	−277.1
230.769	20.769	−1421.7	−353.8	−1310.4	−280.7
237.288	20.593	−1437.0	−357.7	−1325.8	−284.1
243.697	20.420	−1451.4	−361.4	−1340.4	−287.4
250.000	20.250	−1465.0	−364.8	−1354.2	−290.5

q_{dilut} are the heats of dilution of Ni $(NO_3)_2$ with water. The precision is ±0.1 μJ or better

Results and discussion

We have shown previously [29–38] that the heats of the macromolecules + ligand interactions in the aqueous solvent systems can be calculated via the following equation:

$$q = q_{\max} x'_{\mathrm{B}} - \delta^{\circ}_{\mathrm{A}}(x'_{\mathrm{A}}L_{\mathrm{A}} + x'_{\mathrm{B}}L_{\mathrm{B}}) - (\delta^{\circ}_{\mathrm{B}} - \delta^{\circ}_{\mathrm{A}})(x'_{\mathrm{A}}L_{\mathrm{A}} + x'_{\mathrm{B}}L_{\mathrm{B}})x'_{\mathrm{B}} \quad (1)$$

where q is the heat of MBP + Ni^{2+} interaction at certain ligand concentrations and $q_{\max}$ represents the heat value upon saturation of all MBP. The parameters $\delta^{\circ}_{\mathrm{A}}$ and $\delta^{\circ}_{\mathrm{B}}$ reflect to the net effect of Ni^{2+} on the MBP stability in the low and high Ni^{2+} concentrations, respectively. $p > 1$ or $p < 1$ indicate positive or negative cooperativity of macromolecule for binding with ligand, respectively; $p = 1$ indicate that the binding is non-cooperative. x'_{B} can be expressed as follows:

$$x'_{\mathrm{B}} = \frac{px_{\mathrm{B}}}{x_{\mathrm{A}} + px_{\mathrm{B}}} \quad (2)$$

x_{B} is the fraction of the Ni^{2+} ions needed for saturation of the binding sites, and $x_{\mathrm{A}} = 1 - x_{\mathrm{B}}$ is the fraction of unbounded Ni^{2+} ions. x_{B} fractions can be expressed as follows:

$$x_{\mathrm{B}} = \frac{[Ni^{2+}]_{\mathrm{T}}}{[Ni^{2+}]_{\max}} \quad x_{\mathrm{A}} = 1 - x_{\mathrm{B}} \quad (3)$$

$[Ni^{2+}]_{\mathrm{T}}$ total concentration of Ni^{2+} and $[Ni^{2+}]_{\max}$is the maximum concentration of Ni^{2+} ion upon saturation of all MBP. In general, there will be "g" sites for binding of Ni^{2+} per MBP molecule. L_{A} and L_{B} are the relative contributions due to the fractions of unbounded and bounded Ni^{2+} in the heats of dilution in the absence of MBP and can be calculated from the heats of dilution of nickel nitrate solution in buffer, q_{dilut}, as follows:

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

The heats of MBP + Ni^{2+} interactions, q, were fitted to Eq. 1 over the whole Ni^{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 (Fig. 1). The small relative standard coefficient errors and the high r^2 values (1) support the method. The binding parameters for MBP + Ni^{2+} interactions recovered from Eq. 1 are listed in Table 2.

Consider a solution containing ligand, (Ni^{2+}), and a macromolecule (MBP) that contains "g" sites capable of binding the ligand. 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

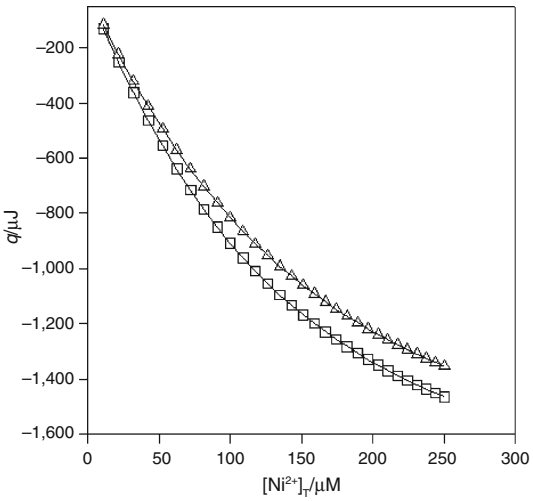

Fig. 1 Comparison between the experimental heats for MBP + Ni^{2+}interactions at 310 K (*square*), 320 K (*triangle*), and calculated data (*lines*) via Eq. 1. The $[Ni^{2+}]_{\mathrm{T}}$ are the total concentrations of $Ni(NO_3)_2$ solutions in 1,000 μM

Table 2 Binding parameters for MBP + Ni^{2+} interactions via Eq. 1

Parameters	$T = 310$ K	$T = 320$ K
K_{d}/μM	89.953 ± 0.0038	106.978 ± 0.0053
p	1.20	1
$\delta^{\theta}_{\mathrm{A}}$	−4.964 ± 0.037	−4.757 ± 0.010
$\delta^{\theta}_{\mathrm{B}}$	−6.471 ± 0.030	−4.708 ± 0.013
$\Delta H_{\max}$ = kJ mol^{-1}	−43.209	−42.078

p Values (1 and 1 at 37 and 47 °C, respectively) suggest that Ni^{2+} ions bind preferentially to the native state of MBP. $\delta^{\theta}_{\mathrm{A}}$ and $\delta^{\theta}_{\mathrm{B}}$ values for MBP + Ni^{2+} interactions are negative at 37 and 47 °C (Table 2), indicating that in the low and maximum concentration of the nickel ions the MBP structure was destabilized as a result of binding to Ni^{2+} ions

of dissociation equilibrium constant, K_{d}, values. Thus, the reaction under consideration can be written as:

$$M + L \Leftrightarrow ML \quad K_{\mathrm{d}} = \frac{[M][L]}{[ML]} \quad (5)$$

For a set of identical and independent binding sites, we have shown before [12, 14, 16, 17, 39]:

$$\Delta H = \frac{1}{A_i}\left\{(B + K_{\mathrm{d}}) - \left[(B + K_{\mathrm{d}})^2 - C\right]^{\frac{1}{2}}\right\} \quad (6)$$

A_i, B_i, and C_i are constants in each injection i, which have been defined as follows:

$$A_i = \frac{V_i}{2q_i} \quad B = g[M]_{\mathrm{T}} + [L]_{\mathrm{T}} \quad C = 4g[M]_{\mathrm{T}}[L]_{\mathrm{T}} \quad (7)$$

where V_i is the volume of the reaction solution in the calorimetric sample cell in each injection step. M_{T} is the total MBP concentration and L_{T} is the total Ni^{2+}

concentration in the calorimetric sample cell in each injection step. Equation 6 contains two unknown parameters, K_d and ΔH. A series of reasonable value for K_d is inserted into Eq. 6 and corresponding values for ΔH are calculated and the graph ΔH versus K_d is constructed. Curves of all titration steps will intersect in one point, which represents the precise value for ΔH and K_d. The plots of ΔH versus K_d, according to Eq. 6, for 30 injections are shown in Fig. 2.

It is possible to use Eq. 8 for calculation of K_d, and g in a very simple way as follows [13, 14]:

$$\frac{\Delta q}{q_{max}} M_T = \left(\frac{\Delta q}{q}\right) L_T \frac{1}{g} - \frac{K_d}{g} \quad (8)$$

where $\Delta q = q_{max} - q$. Therefore, the plot of $(\Delta q/q_{max})M_T$ versus $(\Delta q/q)L_T$ should be a linear plot with a slope of $(1/g)$ and a vertical intercept of (K_d/g). The linear plot has been examined by different appraised values for q_{max} to find the best value for the correlation coefficient (near to one). The best linear plots (Figs. 5 and 6) were obtained using a value of −2,100 and −2,045 μJ (equal to −43.209 and −42.078 kJ/mol at 37 and 47 °C, respectively) for q_{max}. The amounts of g and K_d, obtained from the slope and vertical intercept plot, are 3 and 89.953 and 106.978 μM at 37 and 47 °C, respectively. If q and q_{max} are calculated per mole of biomacromolecule than the molar enthalpy of binding for each binding site (ΔH) will be $\Delta H = q_{max}/g$. Dividing the q_{max} value of −43.209 and −42.078 kJ mol^{-1} by $g = 3$, therefore, gives −14.403 and −14.026 kJ mol^{-1} at 37 and 47 °C, respectively.

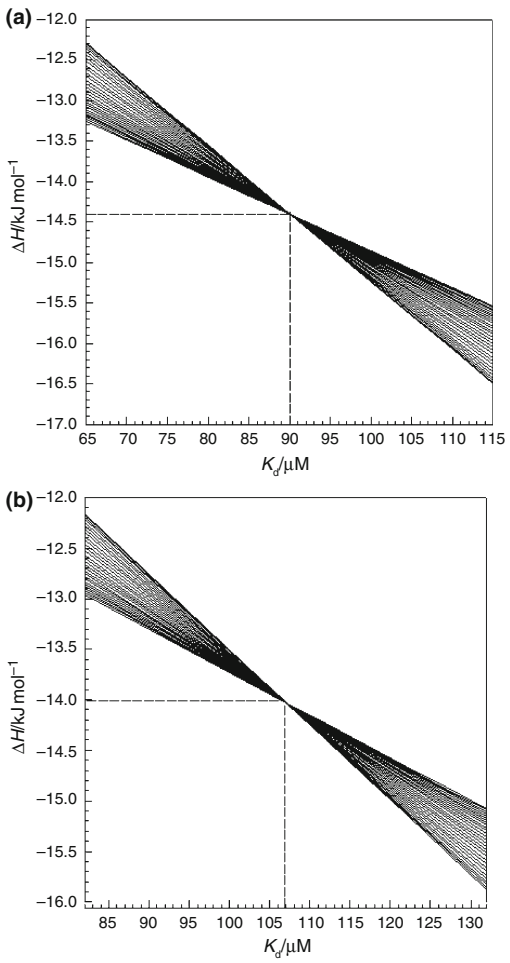

Fig. 2 **a** ΔH versus K_d for all 30 injections in the reasonable values of K_d, according to Eq. 6 at $T = 310$ K. **b** ΔH versus K_d for all 30 injections in the reasonable values of K_d, according to Eq. 6 at $T = 320$ K

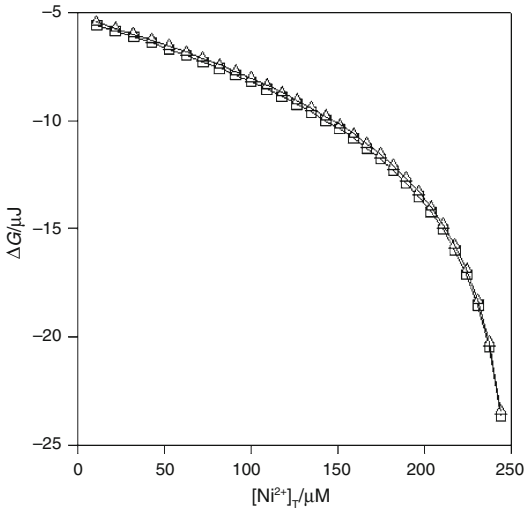

Fig. 3 Comparison between the experimental Gibbs free energies at 310 K (*square*) and 320 K (*triangle*) for Ni^{2+} interactions and calculated data (*lines*) via Eq. 9

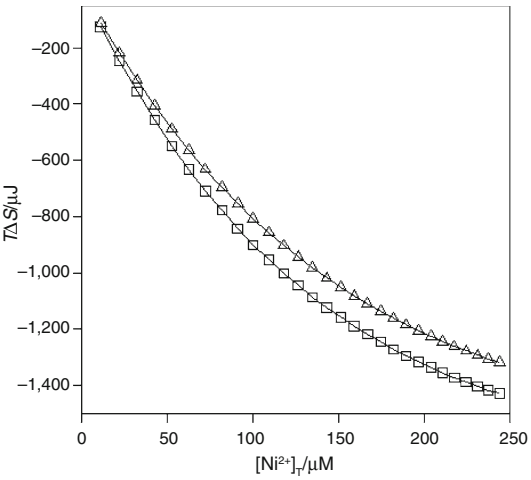

Fig. 4 Comparison between the experimental entropies at 310 K (*square*) and 320 K (*triangle*) for MBP + Ni^{2+} interactions and calculated data (*lines*) via Eq. 9

The standard Gibbs free energies as a function of Ni^{2+} concentrations can be obtained as follows:

$$\Delta G^{\circ} = -RT\mathrm{Ln}\, K_{\mathrm{a}} \quad (9)$$

The standard Gibbs energies, ΔG°, calculated from Eq. 9 have shown graphically in Fig. 3. $T\Delta S$ values were calculated using ΔG° values at different temperatures and have shown in Fig. 4. $\delta_{\mathrm{A}}^{\theta}$ and $\delta_{\mathrm{B}}^{\theta}$ values for MBP + Ni^{2+} interactions are negative at 37 and 47 °C (Table 2), indicating that in the low and high concentration of the nickel ions, the MBP structure was destabilized as a result of binding to Ni^{2+} ions resulting in a decrease in its biological activity (Figs. 5, 6).

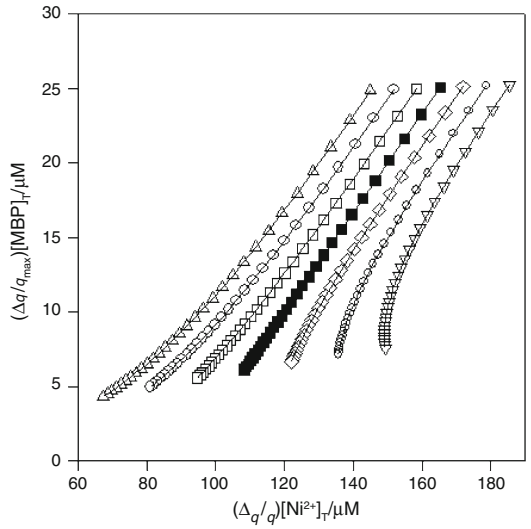

Fig. 5 The best linear plot of $(\Delta q/q_{\max})[\mathrm{MBP}]_{\mathrm{T}}$ versus $(\Delta q/q)[\mathrm{Ni}^{2+}]_{\mathrm{T}}$ at 310 K, according to the Eq. 8, using a value of −2,100 μJ for $q_{\max}$ (■), −2,020 μJ (□), −1,940 μJ (O), −1,860 μJ (Δ), −2,180 μJ (◇), −2,260 μJ (○), −2,340 μJ (▽)

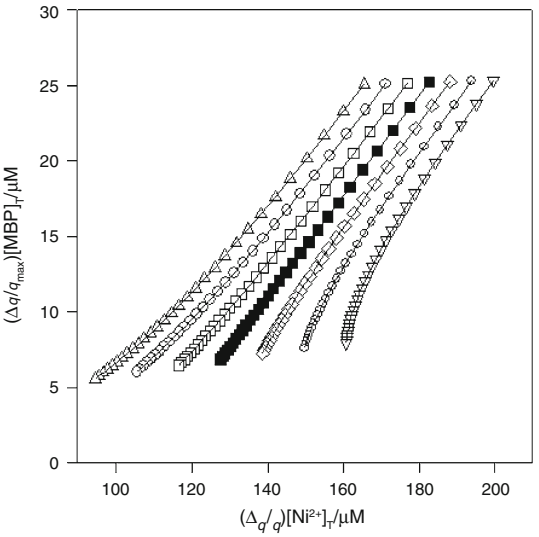

Fig. 6 The best linear plot of $(\Delta q/q_{\max})[\mathrm{MBP}]_{\mathrm{T}}$ versus $(\Delta q/q)[\mathrm{Ni}^{2+}]_{\mathrm{T}}$ at 320 K, according to the Eq. 8, using a value of −2,045 μJ for $q_{\max}$ (■), −1,985 μJ (□), −1,925 μJ (O), −1,865 μJ (Δ), −2,105 μJ (◇), −2,165 μJ (○), −2,225 μJ (▽)

Conclusions

The extended coordination model, via Eq. 1 reproduces satisfactorily the heats of MBP + Ni^{2+} interactions (Fig. 1). The binding parameters derived from the new solvation model were attributed to a structural change of MBP due to its interactions with nickel ion. The ability to predict of activity of protein, binding enthalpies and dissociated binding constant, make this theory the most powerful one. The results of this study are further evidence of a possible role of Ni^{2+} in adding to the stability of MBP and its function as an extrinsic protein of the myelin sheath in vitro. The MBP + Ni^{2+} interaction exhibit decrease the stability and biological activity of MBP in the low and high concentration of the nickel ion at 37 and 47 °C.

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