A Thermodynamic Study on the Binding of Cobalt Ion with Myelin Basic Protein

G. Rezaei Behbehani,* A. A. Saboury,† and A. Fallah Baghery

*Chemistry Department, Imam Khomeini International University, Qazvin, Iran. *E-mail: grb402003@yahoo.com*
†Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran
Received July 19, 2007

The interaction of myelin basic protein (MBP) from bovine central nervous system with divalent calcium ion was studied by isothermal titration calorimetry at 27 ºC in aqueous solution. The extended solvation model was used to reproduce the enthalpies of Co^{2+}-MBP interaction over the whole Co^{2+} concentrations. The solvation parameters recovered from the solvation model were attributed to the structural change of MBP due to the metal ion interaction. It was found that there is a set of three identical and noninteracting binding sites for Co^{2+} ions. The association equilibrium constant is 0.015 μM^{-1}. The molar enthalpy of binding is $\Delta H = -14.60$ kJ mol^{-1}.

Key Words : Myelin basic protein, Cobalt, Isothermal titration calorimtry, Solvation parameters

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,[5-22] protein denaturation.[23-27] During the last six years we attempt to study the metal ion binding study on different proteins.[28-36] 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.[37-42] 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. Myelin Basic Protein, MBP, is one of the most important proteins of the myelin sheath,[38] and its predominant extrinsic protein in both central and peripheral of the central nervous system myelins. 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. The interactions of MBP with Co^{2+}- MBP have been previously investigated by equilibrium analysis.[39] Binding of Cd, Co, Cu, Hg, Mn, Pb, Zn, Ca, and Mg ions by isolated MBP of bovine central nervous system [CNS] have been recently assessed by centrifugal equilibrium dialysis.[40] MBP is an "intrinsically unstructured" or "natively unfolded" protein; therefore its three-dimensional structure might only be determined in its interaction with another protein.[41,42] 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 Co^{2+} ions with MBP and analyses those using the extended solvation theory.

Experimental

MBP from bovine central systems (CNS) obtained from Sigma chemical Co. Calcium 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 from stainless steel. Cobalt 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 Cobalt 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 cobalt solution was measured as described above except MBP was excluded. The enthalpies of dilution of the cobalt solutions were subtracted from the enthalpy of Co^{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 weight of MBP was taken to be 18500 Da. The heats of Co^{2+}-MBP interactions have been calculated in $kJmol^{-1}$ and reported in Table 1.

Table 1. The enthalpies of Co^{2+}-MBP interactions (Q) with Co^{2+} ion at 300 K in kJmol^{-1} is the enthalpies of dilution of Co^{2+} with water

$[MBP]_T/\mu M$	$[Co^{+2}]_T/\mu M$	Q	Q_{dilut}
13.279	8.197	–3.325	–5.233
12.857	23.809	–8.868	–4.648
12.656	38.460	–13.205	–4.154
12.089	52.239	–16.609	–3.728
11.739	65.217	–19.312	–3.374
11.408	77.465	–21.489	–3.072
11.096	89.041	–23.263	–2.815
10.8	100.000	–24.732	–2.593
10.519	110.389	–25.962	–2.402
10.253	120.253	–27.004	–2.235
10.000	129.626	–27.897	–2.088
9.759	138.554	–28.67	–1.96
9.529	147.059	–29.345	–1.846
9.310	155.172	–29.938	–1.734
9.000	166.667	–30.707	–1.609

Results and Discussion

The solute (MBP in this case) is supposed to occupy a cavity surrounded by n solvent molecules of its nearest neighbours. In forming this cavity each of these n molecules must break some of its solvent-solvent bonds, resulting to an increase in the enthalpy equal to $-\alpha n\Delta H^{o*}$, where α is the fraction of the enthalpy of solvent-solvent bonding, ΔH^{o*}, associated with the broken bonds.

The solute may cause a weakening or strengthening of solvent-solvent bonds over a number of molecular diameters. On average N molecules will be affected ($N \geq n$) and we postulate that the associated enthalpy change may be represented as $-\beta N\Delta H^{o*}$, where β is the proportionality constant and is negative if bonds are strengthened.

Finally, the solute is supposed to interact with the modified solvent, resulting to an enthalpy change $\Delta\Delta H^{o}_{12}$. This treatment leads to:

$$\Delta_t H^o = {}^{A\to B}\Delta_t\ H^o x_B' - (\alpha n + \beta N)(x_A' L_A + x_B' L_B) \quad (1)$$

for the enthalpy of transfer.[42-53] ${}^{A\to B}\Delta_t\ H^o$ 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:

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

$\Delta_t H^o$ is the enthalpy of transfer of the solutes from solvent A to the mixtures 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 for the binary mixtures of A and B components. The parameter $(\alpha n + \beta N)$ reflects the net effect of the solute on the solvent-solvent bonding. The value $(\alpha n + \beta N)$ of is positive if there is a net breaking or weakening of solvent-solvent bonds and is negative if the net effect of the solute is to cause a strengthening of these bonds. The superscript o in all cases refers to the quantities in infinite dilution of the solute. p is a measure of the degree of preferential solvation and p will be < 1 if the solute is preferentially solvated by water and p will be > 1 if the preference is for calcium ion; p = 1 represents random solvation. The derivation of Eq. (1) involves the approximation of constant values for α, β, n, N and $(\alpha n + \beta N)$ over the whole range of solvent compositions.[42-53] As the parameters α, β, n, N and $(\alpha n + \beta N)$ are not constant over the whole range of solvent compositions and the net effect of the solute on solvent-solvent bonds in mixture, $(\alpha n + \beta N)^{mix} = \delta^{mix}$, is changed during the solvent compositions, we suggested to express this parameter as follow:

$$\delta^{mix} = \delta^o_A x_A' + \delta^o_B x_B' = \delta^o_A + (\delta^o_B - \delta^o_A) x_B' \quad (3)$$

δ^o_A and δ^o_B are the net effects of the solute on solvent-solvent bonds in water-rich domain and cosolvent-rich region respectively. Therefore equation 1 changes to:

$$\Delta_t H^o = {}^{A\to B}\Delta_t\ H^o x_B' - \delta^{mix}(x_A' L_A + x_B' L_B) \quad (4)$$

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

$$\Delta_t H^o = {}^{A\to B}\Delta_t\ H^o x_B' - \delta^o_A(x_A' L_A + x_B' L_B) - (\delta^o_B - \delta^o_A)(x_A' L_A + x_B' L_B) x_B' \quad (5)$$

With simple modification of Eq. (5), it is possible to use this equation to reproduce the enthalpies of metal-macromolecules interactions (in kJ mol^{-1}) as follow:

$$Q = Q_{\max} x_B' - \delta^o_A(x_A' L_A + x_B' L_B) - (\delta^o_B - \delta^o_A)(x_A' L_A + x_B' L_B) x_B' \quad (6)$$

Where Q is the heat of Co^{2+}-MBP interactions at certain ligand concentrations and $Q_{\max} = \Delta\Delta H^o_{12} + \delta^o_B \Delta H^S_{Co(NO_3)_2} - \delta^o_A \Delta H^{o*}_W$ represents the heat value upon saturation of all MBP. $\Delta\Delta H^o_{12}$ is the difference between the enthalpies of water-MBP and Co^{2+}-MBP interactions. <0 indicates that the interaction of the MBP with Co^{2+} is stronger than with water. ΔH^{o*}_W is the enthalpy of condensation of pure water (–44.7 kJmol^{-1}) and $\Delta H^S_{Co(NO_3)_2}$ is the enthalpy of solution of Cobalt Nitrate in water (–5.233 kJmol^{-1}). x_A and x_B are bulk mole fractions of the components A and B in solvation shell and we can express them in Co^{2+}-MBP interaction as the total ligand concentrations divided by the maximum concentration of Co^{2+} as follow:

$$x_B = \frac{[Co^{2+}]_T}{[Co^{2+}]_{\max}} \quad (7)$$

$[Co^{2+}]_T$ is the total concentration of Co^{2+} and $[Co^{2+}]_{\max}$ is the maximum consternation of Co^{2+} ion. L_A and L_B are the relative partial molar enthalpies and can be calculated from heats of dilution of Co^{2+} in water, Q_{dilut}, as follow:

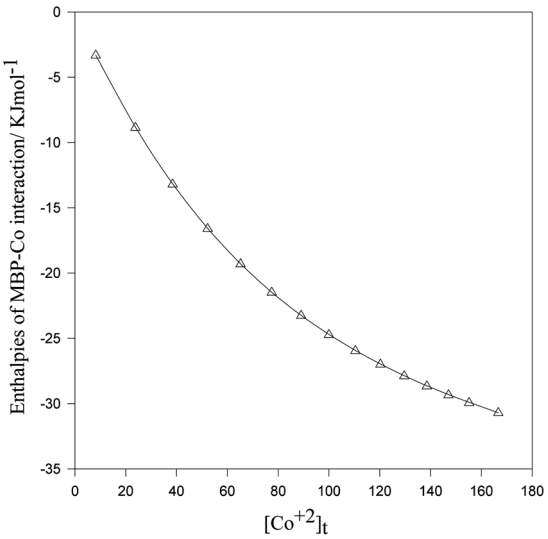

Figure 1. Comparison between the experimental enthalpies (△) for Co^{2+}-MBP interactions and calculated data (lines) *via* Eq. (6). $[Co^{2+}]_t$ are total concentrations of $Co(NO_3)_2$ solutions in μM.

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

The enthalpies of Co^{2+}-MBP interactions (Q) were fitted to Eq. (6) over the whole Co^{2+} compositions. In the calculation 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 (Figure 1). δ_A^o and δ_B^o are the net effects of MBP on solvent-solvent bonds in water-rich region and Co^{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 water or Co^{2+}respectively; $p = 1$ indicates random solvation. The solvation parameters recovered from Eq. (6) have been shown in Table 2.

In general, there will be "g" sites for binding of ligand molecules (Co^{2+} in this case) per one mole of protein and ν is defined as the average moles of bound ligand per mole of total protein.

As $x_B' = n_B/n$ in the solvation sphere, it is possible to change it to $x_B' = \nu/g$ for metal-protein interaction. Therefore if $x_B' = \nu/g$ values recovered from Eq. (6) are multiplied by "g", experimental ν values can be calculated easily with using only one concentration of MBP. By using ν values it is possible to calculate the free concentration of ligand ($[Co^{2+}]_F$) as follow:

$$[Co^{2+}]_F = [Co^{2+}]_T - \nu[MBP]_T \quad (9)$$

Finally by using the Scatchard equation association binding constant, K_a, will be obtained as follow:

$$\frac{\nu}{g-\nu} = K_a[Co^{2+}]_F \quad (10)$$

The association equilibrium constant for Co^{2+}-MBP interaction obtained from Eq. (10) is 0.015 μM^{-1}. Consider a solution containing a ligand (Co^{2+}) and a macromolecule (MBP_g) 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 of dissociation equilibrium constant, K_d, values. Thus, the reaction under consideration can be written:

$$MBP + CO^{2+} \Leftrightarrow MBPCo^{2+} \quad Kd = \frac{[MBP][Co^{2+}]}{[MBPCo^{2+}]} \quad (11)$$

And also

$$[Co^{2+}]_T = [Co^{2+}] + [MBPCo^{2+}] \quad (12)$$

$$[MBP]_{total} = [MBP] + [MBPCo^{2+}] = \frac{K_d[MBPCo^{2+}]}{[Co^{2+}]} + [MBPCo^{2+}] \quad (13)$$

Eq. (12) can be solved for $[Co^{2+}]$ and this then substituted into the Eq. (13), which can be rearranged to:

$$[MBPCo^{2+}] = \frac{1}{2}(B + K_d) - [(B + K_d)^2 - C]^{1/2} \quad (14)$$

Where

$$B = [MBP]_T + [Co^{2+}]_T \quad C = 4[MBP]_T[Co^{2+}]_T \quad (15)$$

The sum of heat evolutions following the i-th titration step, Q_i, can be expressed as

$$Q_i = \Delta H V_i[MBPCo^{2+}]_i \quad (16)$$

Where V_i is the volume of the reaction solution and ΔH is the enthalpy of binding. Combination of Eq. (14) and (16) will leads to

$$\Delta H = \frac{1}{A_i}\{(B_i + K_d) - [(B_i + K_d)^2 - C_i]^{1/2}\} \quad (17)$$

Where

$$A_i = \frac{V_i}{2Q_i} \quad (18)$$

Eq. (17)[61-62] contains two unknowns, K_d and ΔH. A_i, B_i and C_i can be calculated in each injection during the calorimetric titration. A series of reasonable values for K_d is inserted into Eq. (17) and corresponding values for ΔH are calculated and the graph ΔH versus K_d constructed. Curves of all titration steps will intersect in one points (Figure 2), which represents the true value for ΔH and K_d. The intersection of the curves gives:

$$K_d = 63\ \mu M \quad \Delta H = -14.60\ kJmol^{-1}$$

It is possible to use Eq. (19) for calculation of K_d and "g" in

Table 2. Solvation parameters for Co^{2+}-MBP interactions recovered from Eq. (6). $\Delta\Delta H_{12} > 0$ indicates that the interaction of the MBP with water is stronger than with Co^{2+}

13.5 μM	1.59	−0.0038	−3.310	54.952

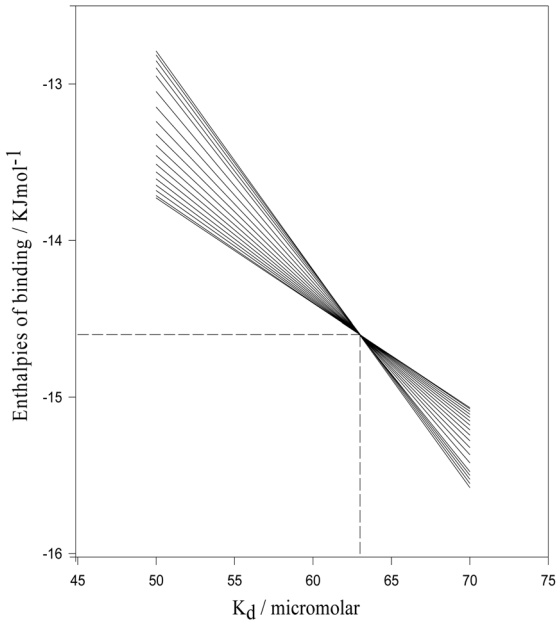

Figure 2. ΔH versus K_d for 17 injections in the reasonable values of K_d, according to Eq. (17). The coordinates of intersection of the curves gives true value for $\Delta H = -14.6$ kJmol^{-1} and $K_d = 63\ \mu$M.

a very simple way as follow[54-55]:

$$\frac{\Delta Q}{Q_{\max}}[MBP]_T = \left[\frac{\Delta Q}{Q}\right][Co^{2+}]_T\frac{1}{g} - \frac{K_d}{g} \qquad (19)$$

Where $\Delta Q = Q_{\max} - Q$. Therefore, the plot of $\Delta Q/Q_{\max}$ $[MBP]_T$ versus $\Delta Q/Q[Co^{2+}]_T$ should be a linear plot by a slope of $1/g$ and the vertical-intercept of K_d/g. K_d and "g" values obtained from Eq. (19) for Co^{2+}-MBP interaction are as follow:

$$K_d = 63\ \mu\text{M} \text{ and } g = 3$$

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.[56-60] 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. Nonpolar moieties come together in aqueous solvent, resulting in the formation of multimers, and in extreme cases, aggregation and precipitation. The δ_A^o and δ_B^o 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 more the extent of this enhancement, the more will be the stabilization of the MBP structure and the greater the values of δ_A^o and δ_B^o. In the Co^{2+}-rich region there was decrease in the δ_B^o value, indicating that MBP was aggregating. One important posttranslational of MBP 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. Co^{2+}-MBP interaction gives rise to a decrease in the hydrophobic property of the MBP as evidenced by the decreased δ_B^o value (−3.3002 in Table 2) in Co^{2+}-rich domain. It is possible to describe the activity of MBP by δ_A^o and δ_B^o values. The greater the δ_A^o and δ_B^o values, the greater the biological activity of MBP. $\Delta\Delta H_{12} > 0$ indicates that the interaction of the MBP with water is stronger than with Co^{2+}. These results ($\delta_B^o < \delta_A^o$ and $\Delta\Delta H_{12} > 0$) were indication of Co^{2+} ability to destabilize the MBP. p value (1.59) 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 Co^{2+} ions. These interpretations are in agreement with the previous reports.[61]

Conclusion

The extended coordination model, via Eq. (6) will satisfactorily reproduce the enthalpies of Co^{2+}-MBP interactions (Figure 1). Prediction of activity of MBP, structural changes of the protein, binding enthalpies and associated equilibrium binding constants using only one set of metal-protein enthalpies, makes this theory the most powerful one. Co^{2+}-MBP interaction destabilizes the MBP molecule.

Acknowledgements. The financial support of the universities of Tehran and Imam Khomeini (Qazvin) are gratefully acknowledged.

References

1. Saboury, A. A. *J. Therm. Anal. Cal.* **2003**, *72*, 93-103.
2. Saboury, A. A. *J. Therm. Anal. Cal.* **2004**, *77*, 997-1004.
3. Ataie, G.; Moosavi-Movahedi, A. A.; Saboury, A. A.; Hakimelahi, G. H.; Ru. Hwu, J.; Tsay, S. C. *International Journal of Biological Macromolecule* **2000**, *27*, 29-33.
4. Saboury, A. A. *J. Iran Chem. Soc.* **2006**, *3*, 1-21.
5. Saboury, A. A.; Dahut, M. U.; Ghobadi, S.; Chamani, J.; Moosavi-Movahedi, A. A. *J. Chin. Chem. Soc.* **1998**, *45*, 667.
6. Bathaie, S. Z.; Moosavi-Movahedi, A. A.; Saboury, A. A. *Nuc. Acid Res.* **1999**, *27*, 1001.
7. Housaindokht, M. R.; Chamani, J.; Saboury, A. A.; Moosavi-Movahedi, A. A. *Bull. Kor. Chem. Soc.* **2001**, *22*, 145.
8. Arabzadeh, A.; Bathaie, S. Z.; Farsam, H.; Amanlou, M.; Saboury, A. A.; Moosavi-Movahedi, A. A. *Inter. J. Pharmac.* **2002**, *237*, 47.
9. Ataie, G.; Moosavi-Movahedi, A. A.; Saboury, A. A.; Hakimelahi, G. H.; Ru. Hwu, J.; Tsay, S. C. *Int. J. Biol. Macromol.* **2000**, *27*, 29.
10. Saboury, A. A.; Karbassi, F. *Thermochimica Acta* **2000**, *362*, 121.
11. Mahmoudi, A.; Nazari, K.; Moosavi-Movahedi, A. A.; Saboury, A. A. *Thermochim. Acta* **2002**, *385*, 33.
12. Ajloo, D.; Moosavi-Movahedi, A. A.; Hakimelahi, G. H.; Saboury, A. A.; Gharibi, H. *Coll. Surf. B: Biointerfaces* **2002**, *26*, 185.
13. Saboury, A. A. *Biologia* **2002**, *57*, 221.
14. Bathaie, S. Z.; Moosavi-Movahedi, A. A.; Ranjbar, B.; Saboury, A. A. *Coll. Surf. B: Biointerfaces* **2003**, *28*, 17.
15. Chamani, J.; Moosavi-Movahedi, A. A.; Saboury, A. A.; Gharanfoli, M.; Hakimelahi, G. H. *J. Chem. Thermodyn.* **2003**, *35*, 199.

16. Bordbar, A. K.; Ghaderi, A. R.; Safaei, E.; Tangestaninejad, S.; Saboury, A. A.; Moosavi-Movahedi, A. A. *Bull. Kor. Chem. Soc.* **2003**, *24*, 547.
17. Mansoori Torshizi, H.; Eslami Moghaddam, M.; Saboury, A. A. *Acta Biochim. Biophys. Sin.* **2003**, *35*, 886.
18. Gharanfoli, A.; Moosavi-Movahedi, A. A.; Safarian, S.; Chamani, J.; Saboury, A. A. *Thermochim. Acta* **2004**, *411*, 37.
19. Moosavi-Movahedi, A. A.; Golchin, A. R.; Nazari, K.; Chamani, J.; Saboury, A. A.; Bathaie, S. Z.; Tangestaninejad, S. *Thermochim. Acta* **2004**, *414*, 233.
20. Nazari, K.; Golchin, A. R.; Moosavi-Movahedi, A. A.; Saboury, A. A.; Hakimelahi, G. H.; Shokravi, A.; Tangestani-Nejad, S. *Thermochim. Acta* **2005**, *428*, 157.
21. Sarraf, N. S.; Mamaghani-Rad, S.; Karbassi, F.; Saboury, A. A. *Bull. Kor. Chem. Soc.* **2005**, *26*, 1051.
22. Saboury, A. A.; Bordbar, A. K.; Moosavi-Movahedi, A. A. *Bull. Chem. Soc. Jpn.* **1996**, *69*, 3031.
23. Saboury, A. A.; Bordbar, A. K.; Moosavi-Movahedi, A. A. *J. Chem. Thermodyn.* **1996**, *28*, 1077.
24. Nazari, K.; Moosavi-Movahedi, A. A.; Saboury, A. A. *Thermochim. Acta* **1997**, *302*, 131.
25. Moosavi-Movahedi, A. A.; Saboury, A. A. *Jour. Chem. Soc. Pak.* **1999**, *21*, 248.
26. Saboury, A. A.; Shamsaei, A. A.; Moosavi-Movahedi, A. A.; Mansori-Torshizi, H. *Chin. Chem. Soc.* **1999**, *46*, 917.
27. Saboury, A. A.; Atri, M. S.; Sanati, M. H.; Sadeghi, M. *J. Therm. Ana. Cal*. **2006**, *83*, 175-179.
28. Saboury, A. A.; Atri, M. S.; Sanati, M. H.; Moosavi-Movahedi, A. A.; Hakimelahi, G. H.; Sadeghi, M. *Biopolymers* **2006**, *81*, 120-126.
29. Saboury, A. A.; Atri, M. S.; Sanati, M. H.; Moosavi-Movahedi, A. A.; Haghbeen, K. *Int. J. Biol. Macromol*. **2005**, *36*, 305-309.
30. Saboury, A. A.; Kordbacheh, M.; Sanati, M. H.; Mizani, F.; Shamsipur, M.; Yakhchali, M. B.; Moosavi-Movahedi, A. A. *Asian J. Chem.* **2005**, *17*, 2773-2782.
31. Atri, M. S.; Saboury, A. A.; Rezaei-Tavirani, M.; Sanati, M. H.; Moosavi-Movahedi, A. A.; Sadeghi, M.; Mansuri-Torshizi, H.; Khodabandeh, N. *Thermochim. Acta* **2005**, *438*, 178-184.
32. Saboury, A. A.; Ghourchaei, H.; Sanati, M. H.; Atri, M. S.; Rezaei-Tawirani, M.; Hakimelahi, G. H. *J. Therm. Ana. Cal.* **2006**, *83*, 175-179.
33. Hindmarsh, P. C.; Brook, C. G. Br. Med. *J. (Clin. Res. Ed.)* **1987**, *295*, 573-583.
34. de Voc, A. M.; Ultsch, M.; Kossiakoff, A. A. *Science* **1992**, *255*, 306-402.
35. Filikov, A. V.; Hayes, R. J.; Luo, P.; Stark, D. M.; Chan, C.; Kundu, A.; Dahiyat, B. I. *Protein Sci*. **2002**, *11*, 1452-1461.
36. Saboury, A. A.; Sarri-Sarraf, N.; Saeidian, S. *J. Termochim. Acta* **2002**, *381*, 147-151.
37. Saboury, A. A. *J. Therm. Analysil. Calorimetry* **2004**, *77*, 1004-1010.
38. Umar Dahot, M.; Saboury, A. A.; Moosavi-Movahedi, A. A. *J. Inhibition. Medicinal. Chemistry* **2004**, *19*, 157-160.
39. Divsalar, A.; Saboury, A. A.; Yousefi, R.; Moosavi-Movahedi, A. A.; Mansoori-Torshizi, A. *International Journal of Biological Macromolecules* **2007**, *40*, 381-386.
40. Saboury, A. A. *J. Chem. Termodynamics* **2003**, *35*, 1975-1981.
41. Sarraf, N. S.; Mamaghani-Rad, S.; Karbassi, F.; Saboury, A. A. *Bull. Korean Chem. Soc*. **2005**, *26*, 1051.
42. Rezaei Behbehani, G. *J. Bull. Korean Chem. Soc*. **2005**, *2*, 238-240.
43. Rezaei Behbehani, G. *Acta Chimica Slov.* **2005**, *52*, 282-285.
44. Rezaei Behbehani, G.; Tazikeh, E.; Saboury, A. A. *J. Bull. Korean Chem. Soc*. **2006**, *2*, 208-210.
45. Rezaei Behbehani, G.; Ghamamy, S. **2006**, *444*, 71-76.
46. Rezaei Behbehani, G.; Ghamamy, S.; Waghorne, W. E. *J. Thermochimica Acta* **2006**, *448*, 37-42.
47. Rezaei Behbehani, G.; Tazikeh, E.; Saboury, A. A. *Acta Chimica Slov.* **2006**, *53*, 363-369.
48. Rezaei Behbehani, G.; Saboury, A. A. *J. Thermochimica Acta* **2007**, *452*, 76-79.
49. Carnegie, P. R.; Moore, W. J.; *Myelin Basic Protein* in *Proteins of the Nervous System*, 2nd Ed.; Bradshaw, R. A.; Schneider, D. M., Eds.; Raven, New York, 1980.
50. Lintner, R. N.; Dyer, C. A. *J. Neurosci. Res*. **2000**, *60*, 437-449.
51. Rezaei Behbehani, G.; Dillon, M.; Smyth, J.; Waghorne, W. E. *J. Solution Chem.* **2002**, *31*, 811-822.
52. Reza Bebahani, G.; Dunnion, D.; Falvey, P.; Hickey, K.; Meade, M.; McCarthy, Y.; Symons, M. C. R.; Waghorne, W. E. *J. Solution Chem.* **2000**, *29*, 521-539.
53. Costigan, A.; Feakins, D.; McStravick, Ita.; O'Duinn, C.; Ryan, J.; Waghorne, W. E. *J. Chem. Soc. Faraday Trans.* **1991**, *87*, 2443-2446.
54. Feakins, D.; O'Neill, R. D.; Waghorne, W. E. *J. Chem. Soc. Faraday Trans.* **1983**, *79*, 2289-2298.
55. Saboury, A. A. *Journal of Thermal Analysis and Calorimetry* **2003**, *72*, 93-103.
56. Saboury, A. A. *Journal of the Iranian Chemical Society* **2006**, *3*, 1-21.
57. Whitaker, J. N.; Mitchell, G. W. *Ann. Neurol.* **1996**, *40*, 3-4.
58. Smith, R. *J. Neurochem.* **1992**, *59*, 1589-1608.
59. Pritzker, L. B.; Joshi, S.; Gowan, J. J.; Harauz, G.; Moscarello, M. A. *Biochemistry* **2000**, *39*, 5374-5381.
60. Nowak, M. W.; Berman, H. A. *Biochemistry* **1991**, *30*, 7642-7651.
61. Moscarello, M. A.; Wood, D. D.; Ackerley, C.; Boulias, C. *J. Clin. Invest.* **1994**, *94*, 146-154.
62. Palma, A. E.; Owh, P.; Fredric, C.; Readhead, C.; Moscarello, M. A. *J. Neurochem.* **1997**, *69*, 1753-1762.