J Therm Anal Calorim (2010) 100:283–288
DOI 10.1007/s10973-009-0012-9

A thermodynamic study of nickel ion interaction with bovine carbonic anhydrase II molecule

G. Rezaei Behbehani · A. A. Saboury · E. Yahaghi

Received: 17 March 2009 / Accepted: 12 May 2009 / Published online: 10 June 2009

Abstract A thermodynamic study on the interaction of bovine carbonic anhydrase II (CAII) with nickel ions was performed by using isothermal titration calorimetry (ITC) at 27 °C in Tris buffer solution at pH = 7.5. The enthalpies of Ni^{2+} + CAII interaction are reported and analysed in terms of the new solvation theory. It was indicated that there are three identical and non-cooperative sites for Ni^{2+}. The binding of a nickle ion is exothermic with dissociation equilibrium constants of 81.306 and 99.126 μM at 27°C and 37°C, respectively. The binding of nickel ions can cause some changes in the stability of the enzyme at low and high Ni^{2+} concentrations.

Keywords Bovine carbonic anhydrase · Nickel ion · Isothermal titration calorimetry

Introduction

Metal ions play a crucial role in many biological processes such as: catalytic activity of metalloenzymes, regulation of nucleic acids replication, pharmacological activity of many metal complexes [1].

G. R. Behbehani (✉)
Chemistry Department, Imam Khomeini International University, Qazvin, Iran
e-mail: grb402003@yahoo.com

A. A. Saboury
Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran

E. Yahaghi
School of Chemistry, University of Tehran, Tehran, Iran

The carbonic anhydrase (CA, EC 4.2.1.1) is ubiquitous zinc enzymes, presents in Archaea, prokaryotes and eukaryotes, being encoded by three distinct, evolutionarily unrelated gene families: the α-CA, β-CA and the γ-CA [2–4]. CA is one of the fastest enzymes known, with a maximal turnover rate for CO_2 hydration of $\sim 10^6$ s^{-1} at 25°C, which is probably the reason why the activation of CA has not been much studied. In contrast, inhibition of CA has been widely investigated and several crystal structures of CA complexes with inhibitor molecules have been reported [1, 5]. Seven distinct isozymes are presently known in higher vertebrates, though their physiological function is not completely known.

CAII is novel as a metal protein due to its unusually high affinity for zinc, so that the CAII + Zn^{2+} dissociation constant is 1–10 pM [1]. The role of highly conserved aromatic residues surrounding the zinc binding site of human carbonic anhydrase II (CAII) in determining the metal ion binding specificity of this enzyme has been previously examined by mutagenesis [6, 7]. Residues F93, F95, and W97 are located along a β-strand containing two residues that coordinate zinc, H94 and H96, and these aromatic amino acids contribute to the high zinc affinity and slow zinc dissociation rate constant of CAII. Substitutions of these aromatic amino acids with smaller side chains enhance the copper affinity (up to 100-fold) while decreasing the affinity of both cobalt and zinc, thereby altering the metal binding specificity up to 10^4-fold. Furthermore, the free energy of the stability of native CAII, determined by solvent-induced denaturation, correlates positively with increased hydrophobicity of the amino acids at positions 93, 95, and 97 as well as with cobalt and zinc affinity. [8, 9] Conversely, increased copper affinity correlates with decreased protein stability. Although CAII is loaded with zinc in its physiologically relevant format, it

can bind a number of other metal ions in the zinc binding site, such as Co^{2+}, Ni^{2+}, Cu^{2+}, Cd^{2+}, Hg^{2+} and Pb^{2+} with various affinities [4, 7].

Some Zn(II) and Cu(II) metal complexes of sulfonamides incorporating polyaminopolycarboxylated tails have also been reported, which indeed showed very good in vitro CA inhibitory activity against isoforms CA I, II, and IV. Rami et al. reported the preparation and inhibition assay of some Cu(II) complexes of aromatic/heterocyclic sulfonamides incorporating EDTA and DTPA tails. In addition, such copper (II) derivatives with potent CA IX/XII inhibitory activity might also be important for developing positron emission tomography (PET) imaging agents for tumor hypoxia [10, 11]. In this article the effect of the nickel ion on the stability of the CAII, in addition to some investigations on the binding parameters of Ni^{2+} to the enzyme has been considered.

Materials and method

Erythrocyte bovine carbonic anhydrase was obtained from Sigma. Nickel (II) nitrate was obtained from Merck. The buffer solution used in the experiments was 30 mM Tris, pH = 7.5, which was obtained from Merck. All the experiments were carried out in two temperatures of 300 and 310 K. The experiments were performed with the 4-channel commercial microcalorimetric system, Thermal Activity Monitor 2277, Thermometric, Sweden. Each channel is twin heat conduction calorimeter (multijuction thermocouple plates) positioned between the vessel holders and the surrounding heat sink. Both sample and reference vessels were made from stainless steel. The limited sensitivity for the calorimeter is 0.1 μcal. Nickel nitrate solution (5 mM) was injected by use of a Hamilton syringe into the calorimetric titration vessel, which contained 1.8 mL CA, 30 μM, in Tris buffer (30 mM), pH = 7.5. 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 and each injection included 20 μL nickel nitrate solution. The calorimetric signal was measured by a digital voltmeter that was part of a computerized recording system. The heat of injection was calculated by the "Thermometric Digitam 3'' software program. The heat of dilution of the nickel solution was measured as described above except CAII was excluded. Also, the heat of dilution of the protein solution was measured as described above except that the buffer solution was injected to the protein solution in the sample cell. The enthalpies of nickel nitrate dilution were subtracted from the enthalpies of nickel nitrate solutions in CAII solutions. The determined enthalpies for Ni^{2+}+CAII interactions, were listed in Table 1 (in μJ). The microcalorimeter was frequently calibrated electrically during the course of the study.

Table 1 Enthalpies of Ni^{2+}+CAII interactions, Q, at 300 K(O) and 310 K (□)

$[Ni^{2+}]$/mM	[CAII]/μM	Q (O)/μJ	Q_{dilut} (O)/μJ	Q (□)/μJ	Q_{dilut} (□)/μJ
0.055	29.670	−705.6	−594.7	−620.8	−552.3
0.109	29.348	−1181.2	−1106.5	−1048	−1027.6
0.161	29.032	−1490.9	−1525.4	−1338.3	−1416.5
0.213	28.723	−1697.7	−1881.9	−1539.6	−1747.2
0.263	28.421	−1841.4	−2187.5	−1683.9	−2031.1
0.312	28.125	−1945.4	−2436.1	−1790.9	−2261.9
0.361	27.835	−2023.5	−2653.5	−1872.8	−2463.7
0.408	27.551	−2084	−2840.5	−1937.2	−2637.5
0.454	27.273	−2132.1	−2997.2	−1989	−2783.1
0.500	27.000	−2171.1	−3125	−2031.4	−2901.6
0.544	26.733	−2203.4	−3242.5	−2066.8	−3010.9
0.588	26.470	−2230.5	−3345.5	−2096.7	−3106.5
0.631	26.214	−2253.6	−3438.4	−2122.3	−3192.8
0.673	25.962	−2273.5	−3514.7	−2144.5	−3263.5
0.714	25.714	−2290.8	−3585.2	−2163.9	−3329
0.755	25.472	−2306	−3645.8	−2180.9	−3385.1
0.794	25.233	−2319.4	−3702.2	−2196	−3437.3
0.833	25.000	−2331.4	−3753.4	−2209.5	−3485.1
0.872	24.771	−2342.1	−3800.4	−2221.6	−3528.7
0.909	24.545	−2351.8	−3841.7	−2232.6	−3567.3
0.946	24.324	−2360.6	−3878.6	−2242.5	−3601.6
0.982	24.107	−2368.6	−3911.2	−2251.6	−3631.7
1.018	23.894	−2375.9	−3941.4	−2259.9	−3659.7
1.053	23.684	−2382.6	−3969.6	−2267.5	−3686
1.087	23.478	−2388.8	−3995.2	−2274.5	−3709.9
1.121	23.276	−2394.5	−4017.5	−2281	−3730.7

ΔH_{dilut} are the enthalpies of dilution of $Ni(NO_3)_2$ with water. Precision is ±0.400 μJ or better

Results and discussion

It has been shown previously [12–26] that the enthalpies of interactions of biopolymers with ligands (Ni^{2+}+ CAII in this case) in the aqueous solvent (Ni^{2+} + water in the present case) mixtures, can be reproduced via the following equation.

$$Q = Q_{max}x'_B - \delta^{\theta}_A\left(x'_A L_A + x'_B L_B\right) - \left(\delta^{\theta}_B - \delta^{\theta}_A\right)\left(x'_A L_A + x'_B L_B\right)x'_B \quad (1)$$

The parameters δ^{θ}_A and δ^{θ}_B are the indexes of the CAII stability as a result of interaction with Ni^{2+} in the low and high Ni^{2+} concentrations, respectively. Cooperative

binding requires that the macromolecule have more than one binding site, since cooperativity results from the interactions of identical binding sites with some similar ligands. If the binding of ligand at one site increases the affinity for ligand at another site, the macromolecule exhibits positive cooperativity. Conversely, if the binding of ligand at one site lowers the affinity for ligand at another site, the protein exhibits negative cooperativity. If the ligand binds at each site independently, the binding is non-cooperative. $p < 1$ or $p > 1$ indicate positive or negative cooperativity of macromolecule for binding with ligand respectively; $p = 1$ indicates that the binding is non-cooperative. x'_B can be expressed as follow:

$$x'_B = \frac{px_B}{x_A + px_B} \tag{2}$$

x_B is the fraction of the Ni^{2+} needed for saturation of the binding sites, and $x_A = 1 - x_B$ is the fraction of unbounded Ni^{2+}. Now the model is a simple mass action treatment, with metal ions replacing water molecules, at the binding sites in the present case. We can express x_B fractions, as the total Ni^{2+} concentrations divided by the maximum concentration of the Ni^{2+} upon saturation of all CAII as follow:

$$x_B = \frac{[Ni^{2+}]_T}{[Ni^{2+}]_{max}} \quad x_A = 1 - x_B \tag{3}$$

$[Ni^{2+}]_T$ is the total concentration of nickel and $[Ni^{2+}]_{max}$ is the maximum concentration of the nickel upon saturation of all CAII. In general, there will be "g" sites for binding of Ni^{2+} per CAII molecule and v is defined as the average moles of bound Ni^{2+} per mole of CAII. L_A and L_B are the relative contributions of unbounded and bounded Ni^{2+} to the enthalpies of dilution with the exclusion of CAII and can be calculated from the enthalpies of dilution of Ni^{2+} in buffer, Q_{dilut}, as follows:

$$L_A = Q_{dilut} + x_B\left(\frac{\partial Q_{dilut}}{\partial x_B}\right), L_B = Q_{dilut} - x_A\left(\frac{\partial Q_{dilut}}{\partial x_B}\right) \tag{4}$$

The enthalpies of Ni^{2+}+CAII interactions,Q, were fitted to Eq. 1 over the whole Ni^{2+} compositions. In the procedure, the only adjustable parameter (p) was changed until the best agreement between the experimental and calculated data was approached (Fig. 1). δ_A^θ and δ_B^θ parameters have been also optimized to fit the data. The optimized δ_A^θ and δ_B^θ values are recovered from the coefficients of the second and third terms of Eq. 1. The small relative standard coefficient errors and the high r^2 values (0.99999) support the method. The binding parameters for Ni^{2+}+CAII interactions recovered from Eq. 1 were listed in Table 2. The agreement between the calculated and experimental results (Fig. 1) is striking, and gives considerable support to the use of Eq. 1.

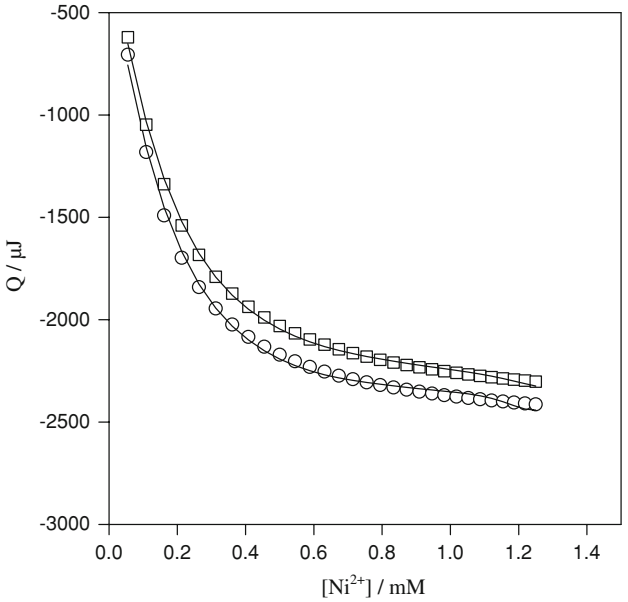

Fig. 1 Comparison between the experimental enthalpies for Ni^{2+} + CAII interactions at 300 K (*open circle*), 310 K (*open square*) and calculated data (*lines*) via Eq. 1

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

	$T = 300$ K	$T = 310$ K
K_1/μM	81.306 ± 0.036	99.126 ± 0.012
K_2/μM	81.306 ± 0.036	99.126 ± 0.012
K_3/μM	81.306 ± 0.036	99.126 ± 0.012
p	0.800 ± 0.002	0.760 ± 0.002
δ_A^θ	−0.249 ± 0.027	−0.374 ± 0.015
δ_B^θ	4.840 ± 0.041	1.932 ± 0.017
ΔH_{max}/kJ mol^{-1}	−23.880 ± 0.34	−23.102 ± 0.012

p value (0.800 and 0.76) shows the overall negative cooperativity for the interaction of Ni^{2+} ions with CAII including both specific and non-specific interactions. $p < 1$ suggests that Ni^{2+} ion binds preferentially to partially denatured CAII species

Φ is the fraction of CAII molecule undergoing complexation with Ni $^{2+}$ which can be expressed as follow:

$$\Phi = \frac{Q}{Q_{max}} \tag{5}$$

Q_{max} represents the heat value upon saturation of all CAII. The appearance equilibrium constant values, K_a, as a function of free concentration of Ni^{2+}, $[Ni^{2+}]_F$, can be calculated as follow:

$$K_a = \frac{\Phi}{(1-\Phi)[Ni^{2+}]_F} = \frac{\Phi}{(1-\Phi)[Ni^{2+}]_T(1-x_B)} \tag{6}$$

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

$$\Delta G = -RTLnK_a \tag{7}$$

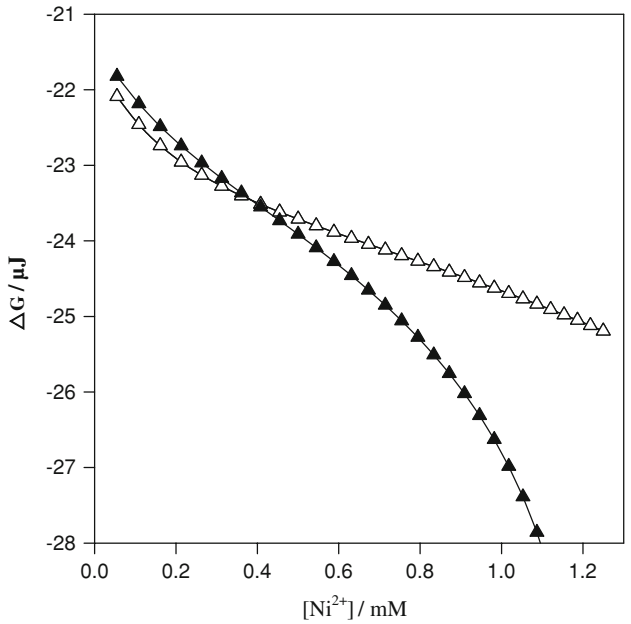

Fig. 2 Comparison between the experimental Gibbs free energies at 300 K (*open triangle*) and 310 K (*filled triangle*) for Ni^{2+}+CAII interactions and calculated data (*lines*) via Eqs. 7 and 8. The linearity of ΔG against Ni^{2+} concentrations indicates that the structural effects compensate each other in the free energy which supports the extended solvation model

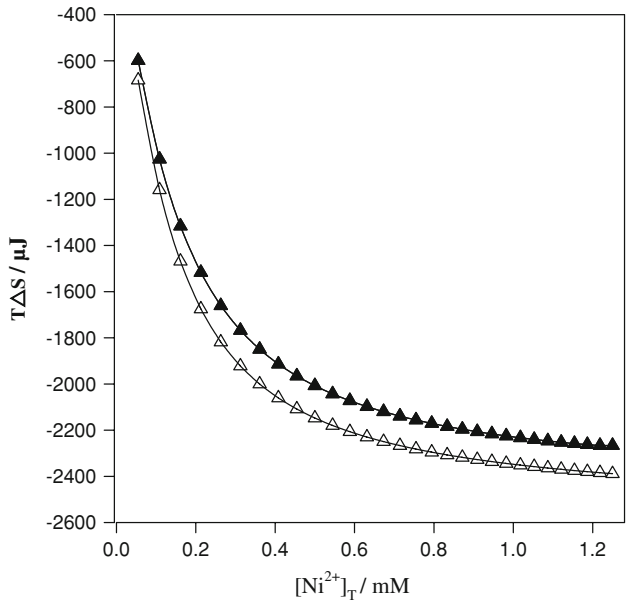

Fig. 3 Comparison between the experimental entropies at 300 K (*filled triangle*) and 310 K (*open triangle*) for Ni^{2+}+CAII interactions in and calculated data (*lines*) via Eqs. 7 and 8

Gibbs energies, ΔG, at different temperatures calculated from Eq. 7 have shown graphically in Fig. 2. ΔS values were calculated using ΔG values at different temperatures and have shown in Fig. 3.

Consider a solution containing a ligand (Ni^{2+}) and a macromolecule 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 with the same set of dissociation equilibrium constant, K_d, values. Thus, the reaction under consideration can be written:

$$M + L \Leftrightarrow ML \qquad K_d = \frac{[M][L]}{[ML]} \tag{8}$$

If α is defined as the fraction of free binding sites on the biomacromolecule, M_0 is the total biomacromolecule concentration, and L_0 is the total ligand concentration, then the free concentrations of monovalent molecule $[M]$ and ligand $[L]$ as well as the concentration of bound ligand $[ML]$ can be deduced as follows:

$$[ML] = g(1-\alpha)M_0 \tag{9}$$

$$[L] = L_0 - [ML] = L_0 - g(1-\alpha)M_0 \tag{10}$$

$$[M] = gM_0 - [ML] = gM_0 - g(1-\alpha)M_0 = \alpha g M_0 \tag{11}$$

Substitution of free concentrations of all these components in Eq. 8 gives [27]:

$$K_d = \left(\frac{\alpha}{1-\alpha}\right)L_0 - \alpha g M_0 \tag{12}$$

or

$$\alpha M_0 = \left(\frac{\alpha}{1-\alpha}\right)\frac{1}{g}L_0 - \frac{K_d}{g} \tag{13}$$

The value of $1 - \alpha$ as the fraction of occupied binding sites on the biomacromolecule:

$$1 - \alpha = \frac{Q}{Q_{\max}} \tag{14}$$

where q represents the heat value at a certain L_0 and $Q_{\max}$ represents the heat value upon saturation of all biomacromolecules. The combination of Eqs. 13 and 14 yields:

$$\frac{\Delta Q}{Q_{\max}}M_0 = \left(\frac{\Delta Q}{Q}\right)L_0\frac{1}{g} - \frac{K_d}{g} \tag{15}$$

Where $\Delta Q = Q_{\max} - Q$. Therefore, the plot of $\frac{\Delta Q}{Q_{\max}}M_0$ versus $\frac{\Delta Q}{Q}L_0$ should be a linear plot with a slope of $\frac{1}{g}$ and a vertical-intercept of $\frac{K_d}{g}$.

The linearity of the plot has been examined by different estimated values for $Q_{\max}$ to reach the best value for the correlation coefficient. The best linear plot with the correlation coefficient value ($r^2 \approx 1$) was obtained using −2574 μJ and −2495 μJ (equal to −23.880 kJ/mol and −23.102 kJ/mol at 27 °C and 37 °C, respectively). The values of g and K_d, obtained from the slope and vertical-intercept plot, are listed in Table 2 and have shown graphically in Fig. 4.

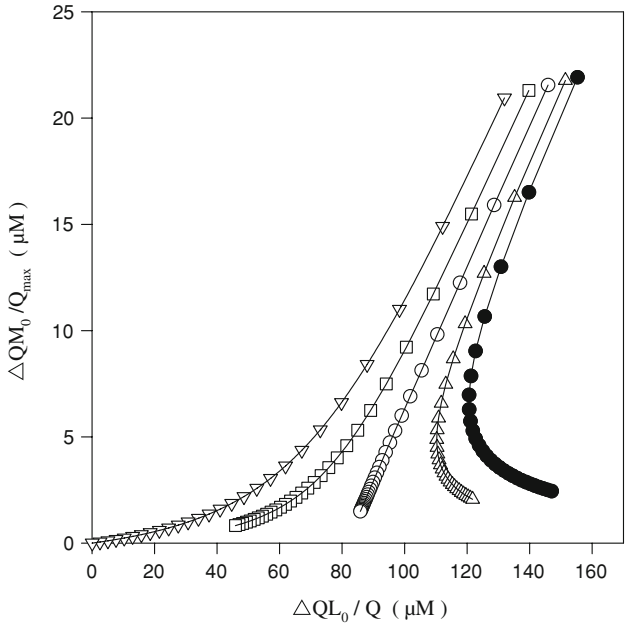

Fig. 4 A graphical representation of how to approach the best linear plot (*open circle*) of $\frac{\Delta Q}{Q_{\max}}M_0$ against $\frac{\Delta Q}{Q}L_0$ with a slope of $\frac{1}{g}$ and a vertical-intercept of $\frac{K_d}{g}$, using −2,576 μJ (*open circle*), −2,700 μJ (*filled circle*), −2,650 μJ (*filled triangle*), −2,500 μJ (*open square*) and −2,400 μJ (*inverted triangle*) as $Q_{\max}$ in Eq. 15

The calorimetric method described recently allows obtaining the number of binding sites (g), the molar enthalpy of binding site $\left(\Delta H_{\text{bin}} = \frac{\Delta H_{\max}}{g}\right)$ and the dissociation equilibrium constant (K_d) for a set of biomacromolecule binding sites. The lack of a suitable value for $Q_{\max}$ to obtain a linear plot of $(\Delta Q/Q_{\max})M_0$ vs. $(\Delta Q/Q)L_0$ may be related to the existence of non-identical binding sites or the interaction between them. Using this method shows that there is a set of three identical and non-interacting binding sites for Nickle ions. Binding parameters for Ni^{2+}+CAII interactions using the new model are listed in Table 2.

The negative value for δ_A^θ (−0.249) in the low concentration of Ni^{2+}, indicates that CAII structure is destabilized as a result of binding to Ni^{2+} ions. Destabilization of CAII by Ni^{2+} ions indicates that the nickel ions bind preferentially to the partially unfolded intermediate form of the protein. The K_d values (Table 2) point toward specific interactions between CAII and Ni^{2+}, but the δ_A^θ values recovered from Eq. 1 suggest that destabilization is the result of both specific and nonspecific interactions. The negative values for δ_A^θ are characteristic of nonspecific interactions, in that 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. These results are inconsistent with the specific interactions in the certain sites on CAII, indicating the existence of some partially unfolded intermediate forms of CAII. Figure 2 shows that there was a relationship, with negative slope, between Ni^{2+} ion concentration and the ΔG values for Ni^{2+}+CAII interaction. The positive value of δ_B^θ (4.840) reflects stabilization of the CAII structure in the high concentration of Ni^{2+}. In other words, the positive values of δ_B^θ suggest that Ni^{2+} ions bind preferentially to the native folded state of CAII. The negative slope of ΔG values against Ni^{2+} ion concentration supports this interpretation. p value (0.800) shows the overall negative cooperativity for the interaction of Ni^{2+} ions with CAII including both specific and non-specific interactions. $p < 1$ suggests that non-specific interactions of Ni^{2+} ion with partially unfolded CAII species are dominant.

References

1. Orioli P. Structural studies on the interactions between metal ions and biological macromolecules. Croat Chem Acta. 1998;71:659–72.
2. Lyer R, Barrese AA, Parker CN, Tripp BC. Inhibition profiling of human carbonic anhydrase II by high-throughput screening of structurally diverse, biologically active compounds. J Biomol Screen. 2006;11:782–91.
3. Sarraf NS, Saboury AA, Ranjbar B, Moosavi-Movahedi AA. Structural and functional changes of bovine carbonic anhydrase as a consequence of temperature. Acta Biochem Polo. 2004;51:665–71.
4. Supuran CT, Scozzafava A, Mastrolorenzo A. Bacterial proteases: current therapeutic use and future prospects for the development of new antibiotics. Exp Opin Ther Patent. 2001;11:221–59.
5. Bertini I, Lanini G, Luchinat C. Equilibrium species in cobalt(II) carbonic anhydrase. J Am Chem Soc. 1983;105:5116–8.
6. Lindskog S, Nyman PO. Metal-binding properties of human erythrocyte carbonic anhydrases. Biochim Biophys Acta. 1964;85:462–72.
7. Sarraf NS, Mamaghani-Rad S, Karbassi F, Saboury AA. Thermodynamic studies on the interaction of copper ions with carbonic anhydrase. Bull Korean Chem Soc. 2005;26:1051–6.
8. Stadie WC, O'Brien H. The catalysis of the hydration of carbon dioxide and dehydration of carbonic acid by an enzyme isolated from red blood cells. J Biol Chem. 1933;103:521–9.
9. Nishino S, Ishikawa Y, Nishida Y. Interaction between a copper(II) compound and protein investigated in terms of the capillary electrophoresis method. Inorg Chem Commun. 1999;2:438–41.
10. Winum JY, Rami M, Montero JL, Scozzafava A, Supuran CT. Carbonic anhydrase IX: a new druggable target for the design of antitumor agents. Med Res Rev. 2007;28:445–63.
11. Alzuet G, Casanova J, Borrfis J, Garcia-Granda S, Gutidrrez-Rodrfguez A, Supuran CT. Copper complexes modelling the interaction between benzolamide and Cu-substituted carbonic anhydrase. Crystal structure of Cu(bz)(NH3)(4) complex. Inorg Chim Acta. 1998;273:334–8.
12. Rezaei Behbehani G, Divsalar A, Saboury AA, Faridbod A, Ganjali R. A high performance theory for thermodynamic study on the binding of human serum albumin with erbium chloride. Chin J Chem. 2009;27:289–94.
13. Rezaei Behbehani G, Divsalar A, Saboury AA, Gheibi N. A new approach for thermodynamic study on binding some metal ions

with human growth hormone. J Solution Chem. 2008;37:1645–55.
14. Rezaei Behbehani G, Divsalar A, Saboury AA, Bagheri MJ. A thermodynamic study on the binding of human serum albumin with new synthesized anti cancer Pd (II) complex. J Solution Chem. 2008;37:1785–94.
15. Saboury AA. A simple method for determination of binding isotherm by isothermal titration calorimetry and its application to the interaction between Cu^{2+} and myelin basic protein. J Therm Anal Cal. 2004;77:997–1004.
16. Rezaei Behbehani G, Saboury AA. A new method for thermodynamic study on the binding of magnesium with human growth hormone. J Therm Anal Cal. 2007;89:852–61.
17. Rezaei Behbehani G, Saboury AA, Bagheri AF. A thermodynamic study on the binding of calcium ion with myelin basic protein. J Solution Chem. 2007;36:1311–20.
18. Rezaei Behbehani G, Tazikeh E, Saboury AA. Using the new developed equation to reproduce the enthalpies of transfer of urea from water to aqueous ethanol, propan-1-ol and acetonitrile at 298 K. Bull Korean Chem Soc. 2006;27:208–11.
19. Rezaei Behbehani G, Ghamamy S, Waghorne WE. Enthalpies of transfer of acetonitrile from water to aqueous methanol, ethanol and dimethylsulphoxide mixtures at 298.15 K. Thermochim Acta. 2006;448:37–42.
20. Rezaei Behbehani G, Saboury AA. Using a new solvation model for thermodynamic study on the interaction of nickel with human growth hormone. Thermochim Acta. 2007;452:76–9.
21. Rezaei Behbehani G, Saboury AA, Taleshi E. A direct calorimetric determination of denaturation enthalpy for lysozyme in sodium dodecyl sulfate. Colloids Surf B: Biointerfaces. 2008;61:224–8.
22. Rezaei Behbehani G. Application of a new method to reproduce the enthalpies of transfer of NaI from water to aqueous methanol, ethanol and iPrOH solvent systems at 289.15 K. Bull Korean Chem Soc. 2005;26:238–40.
23. Rezaei Behbehani G. Application of the new solvation theory to reproduce the enthalpies of transfer of LiBr, tetrabuthylammonium bromide and tetrapenthylamonium bromide from water to aqueous acetonitrile at 298 K. Acta Chim Slov. 2005;52:282–5.
24. Rezaei Behbehani G. Enthalpies of transfer of tetraalkylammonium bromides and CsBr from water to aqueous DMF at 298.15 K. J Solution Chem. 2007;36:939–45.
25. Rezaei Behbehani G, Saboury AA, Taleshi E. A comparative study on direct calorimetric determination of denaturation enthalpy for lysozyme in sodium dodecyl sulfate and dodecyltrimethylammonium bromide. J Solution Chem. 2008;37:619–29.
26. Rezaei Behbehani G, Saboury AA, Divsalar A. Thermodynamic study of the binding of calcium and magnesium ions with myelin basic protein using the extended solvation theory. Acta Biochim Biophys Sin. 2008;40:964–9.
27. Saboury AA. A review on the ligand binding studies by isothermal titration calorimetry. J Iran Chem Soc. 2006;3:1–21.