Journal of Thermal Analysis and Calorimetry, Vol. 89 (2007) 3, 857–861

A THERMODYNAMIC STUDY ON THE BINDING OF MAGNESIUM WITH HUMAN GROWTH HORMONE
Consideration of the new extended coordination model solvation parameters

G. Rezaei Behbehani[1]* *and A. A. Saboury*[2]

[1]Institute of Biochemistry and Biophysics, University of Tehran Iran
[2]Department of Chemistry, Imam Khomeini International University Qazvin Iran

The thermodynamic parameters underlying the binding of Mg^{2+} to the hydrophobic core of human growth hormone, hGH, are determined using isothermal titration calorimetry. The interaction between Mg^{2+} and hGH (35 μM) was studied at 27°C in NaCl solution. A new solvation model was used to reproduce the enthalpies of Mg^{2+}-hGH interaction over the whole Mg^{2+} concentrations. The solvation parameters recovered from the new solvation model, were correlated to the structural changes of hGH due to the metal ion interaction.

Keywords: *human growth hormone, isothermal titration calorimetry, magnesium, solvation model*

Introduction

The method of isothermal titration calorimetry (ITC) is now widely used to obtain thermodynamics information about biochemical binding processes at constant temperature [1–3]. Experiments are performed by titration of a reactant into a sample solution containing the other reactant(s) necessary for reaction. ITC gives invaluable information about biomacromolecule-ligand interaction. During the last two years we attempt to study the metal ion binding study on the human growth hormone (hGH) [4–9]. hGH is a polypeptide hormone, which plays an important role in somatic growth through its effects on the metabolism of proteins, carbohydrates and lipids [10–12]. Electrolytes have complex effects on protein stability. They will change the conformational stability and formation of aggregates. The importance of metal ions such as Zn^{2+} and Cd^{2+} in determining the stability of proteins is widely reported [13–17].

We have previously developed a theory to account for the solvation of solutes in mixed solvent systems. This new 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 [18–23]. The present paper reports some interesting experimental data for the heats of interaction of Mg^{2+} ions with the human growth hormone and analyses these using the new solvation theory. 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 new solvation model.

Experimental

Materials

Highly purified preparations of hGH were provided by the National Research Center of Genetic Engineering and Biotechnology (NRCGEB), Tehran, Iran. 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 in 50 mM NaCl using double-distilled water.

Microcalorimetric experiments

The isothermal titration microcalorimetric experiments were performed with the four channel commercial microcalorimetric system, Thermal Activity Monitor 2277, Thermometric, Sweden. Each channel is a twin heat-conduction calorimeter where the heat-flow sensor is a semiconducting thermopile (multi-junction thermocouple plates) positioned between the vessel holders and surrounding heat sink. The insertion vessel was made from stainless steel. A magnesium chloride solution (2 mM) was injected by use of a Hamilton syringe into the calorimetric titration vessel, which contained

* Author for correspondence: grb402003@yahoo.com

1388–6150/$20.00

Akadémiai Kiadó, Budapest, Hungary
Springer, Dordrecht, The Netherlands

1.8 mL hGH (35 μM). Thin (0.15 mm inner diameter) stainless steel hypodermic needles, permanently fixed to the syringe, reached directly into the calorimetric vessel. Injection of the magnesium chloride solution into the perfusion vessel was repeated 30 times, with 20 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 Mg^{2+} solution was measured as described above except hGH was excluded. Also, the heat of dilution of the protein solution was measured as described above except the buffer solution was injected into the protein solution in the sample cell. The enthalpies of magnesium and protein solutions dilution were subtracted from the enthalpy of Mg^{2+}-hGH interaction. The microcalorimeter was frequently calibrated electrically during the course of the study. The molecular mass of hGH was taken to be 22 kDa [11–12].

Results and discussion

The observation of preferential solvation in mixed solvents was particularly striking and suggested that solvation in these media was analogous to complexation, with better solvent taking the role of the ligand. It has been shown previously [24–29] that the enthalpies of transfer of a solute from a pure solvent into a mixed solvent system can be accounted for quantitatively in terms of three factors: preferential solvation by the components of the mixed solvent, weakening or strengthening of solvent-solvent bonds by the solute and the change in the enthalpy of the solute-solvent interactions. This treatment leads to:

$$\Delta H_t^\theta = {}^{A\to B}\Delta H_t^\theta x'_B - (\alpha n+\beta N)(x'_A L_A + x'_B L_B) \quad (1)$$

${}^{A\to B}\Delta H_t^\theta$ 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:

$$x'_B = \frac{px_B}{x_A + px_B} = \frac{n_B}{n}, \quad x'_A = 1 - x'_B \quad (2)$$

The composition of the coordination sphere, $x'_B = n_B/n$, of the solute is calculable via:

$$x'_B = \frac{n_B}{n} = \frac{1}{n}\sum_{i=1}^{2n} b_i \left(\frac{x_B}{x_A}\right)^i \quad (3)$$

where b_i coefficients are calculated easily by curve fitting. ΔH_t^θ 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 with αn resulting from the formation of a cavity wherein *n* solvent molecules become the nearest neighbours of the solute and βN reflecting 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 solvent-solvent bonds). α and β represent the fraction of the enthalpy of solvent-solvent bonding associated with the cavity formation or restructuring respectively. The superscript θ in all cases refers to the quantities in infinite dilution of the solute. $p<1$ or $p>1$ indicate a preference for solvent *A* or *B* respectively; $p=1$ indicates random solvation. The ΔH_t^θ values could not be reproduced quantitatively by Eq. (1) across the whole range of solvent compositions [24–29]. The significant reason for the failure of Eq. (1) is the approximation of constant values for α, β, *n*, *N* and ($\alpha n+\beta N$) over the entire range of solvent compositions.

The failure of Eq. (1) in most cases led us to introduce the new extended coordination model of solvation [27–29]. However, it is unreasonable to suppose that the number of the molecules neighboring the solute and the molecules around the cavity is the same in the solvent mixtures with different concentrations of cosolvent, due to the different size of the molecule of cosolvent and the different interactions between the solvent molecules. 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:

$$\delta^{mix} = \delta_A^\theta x'_A + \delta_B^\theta x'_B = \delta_A^\theta + (\delta_B^\theta - \delta_A^\theta)x'_B \quad (4)$$

x'_A and x'_B mole fractions of the components *A* and *B* in the vicinity of the solute or solvation sphere. δ_A^θ and δ_B^θ are the net effects of the solute on solvent-solvent bonds in water-rich domain and cosolvent-rich region respectively. Therefore Eq. (1) changes to:

$$\Delta H_t^\theta = {}^{A\to B}\Delta H_t^\theta x'_B - \delta^{mix}(x'_A L_A + x'_B L_B) \quad (5)$$

Substituting δ^{mix} from Eq. (4) into Eq. (5), leads to:

Table 1 Enthalpies (Q) of Mg^{2+}-hGH interaction in 2 mM Mg^{2+} solution with water at 300 K in kJ mol^{-1}. Q_{dilu} is the enthalpies of dilution of Mg^{2+} with water

$[Mg^{2+}]_t$	Q	Q_{dilu}
0.058	–9.27	–2.92
0.213	–24.54	–2.42
0.395	–45.08	–1.77
0.553	–51.74	–1.36
0.674	–55.17	–1.08
0.781	–57.23	–0.89
0.874	–58.60	–0.77
0.916	–59.11	–0.71
0.987	–59.92	–0.63

$$\Delta H_t^\theta = \overset{A\to B}{\Delta} H_t^\theta x'_B - \delta_A^\theta (x'_A L_A + x'_B L_B) - (\delta_B^\theta - \delta_A^\theta)(x'_A L_A + x'_B L_B) x'_B \quad (6)$$

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, with simple modification of Eq. (6), it is possible to use this equation to reproduce the enthalpies of metal-macromolecules interactions as follow:

$$Q = Q_{max} x'_B - \delta_A^\theta (x'_A L_A + x'_B L_B) - (\delta_B^\theta - \delta_A^\theta)(x'_A L_A + x'_B L_B) x'_B \quad (7)$$

where Q is heat of Mg^{2+}-hGH interactions at certain ligand concentrations and Q_{max} represents the heat value upon saturation of all hGH. x_A and x_B are bulk mole fractions in solvation model theory and we can express them in Mg^{2+}-hGH interaction as the total ligand concentrations divided by the maximum concentration of Mg^{2+} as follow:

$$x_B = \frac{[Mg^{2+}]_t}{[Mg^{2+}]_{max}} \quad (8)$$

$[Mg^{2+}]_t$ is the total concentration of Mg^{2+} and $[Mg^{2+}]_{max}$ is the maximum consternation of Mg^{2+} that is calculable by setting dQ/dx_B to zero. L_A and L_B are the relative partial molar enthalpies and can be calculated from heats of dilution of Mg^{2+} in water, Q_{dilu}, as follow:

$$L_A = Q_{dilu} + x_B \left(\frac{\partial Q_{dilu}}{\partial x_B}\right), \quad L_B = Q_{dilu} - x_A \left(\frac{\partial Q_{dilu}}{\partial x_B}\right) \quad (9)$$

Q values were fitted to Eq. (7) over the whole Mg^{2+} compositions. In the 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. δ_A^θ and δ_B^θ are the net effects of hGH on solvent-solvent bonds in water-rich region and Mg^{2+}-rich region respectively which are recovered from the coefficients of the second and third terms of Eq. (1). $p<1$ or $p>1$ indicate a preferential solvation of hGH by or Mg^{2+} respectively; $p=1$ indicates random solvation.

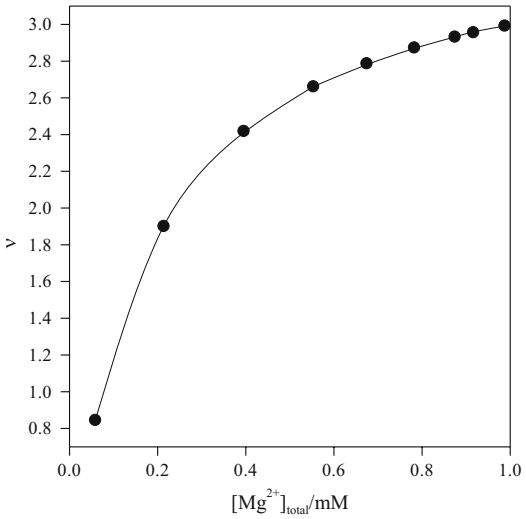

Fig. 1 Comparison between experimental values of ν (●) [5] and calculated ν values using Eqs (7) and (10)

In general, there will be 'g' sites for binding of ligand molecules (Mg^{2+} in this case) per protein macromolecule and ν is defined as the average moles of bound ligand per mole of total protein. It is possible to use the similar Eq. of (4) to find 'g' as follow:

$$\frac{\nu}{g} = \frac{1}{g}\sum_{i=1}^{2g} b_i \left(\frac{x_B}{x_A}\right)^i \quad (10)$$

Therefore if $x'_B = \nu / g$ values recovered from Eq. (6) are multiplied by 'g', experimental ν values can be calculated easily (Fig. 1) with using only one set of concentrations of hGH. Experimental values of ν recovered by Eq. (7) are fitted to Eq. (10) with the R=0.999. By using ν values it is possible to calculate the free concentration of ligand as follow:

$$L^{free} = L_t - \nu M_t \quad (11)$$

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

$$\frac{\nu}{g - \nu} = K L^{free} \quad (12)$$

Table 2 Thermodynamic parameters for Mg^{2+}-hGH interaction in 2 mM Mg^{2+} solution with water via Eq. (7)

[hGH]	p	δ_A^θ	δ_B^θ	Q_{max}	ΔH_{bin}	K/mM
35 μM	6.4	8.79	–0.68	–59.44	–19.81	47.47

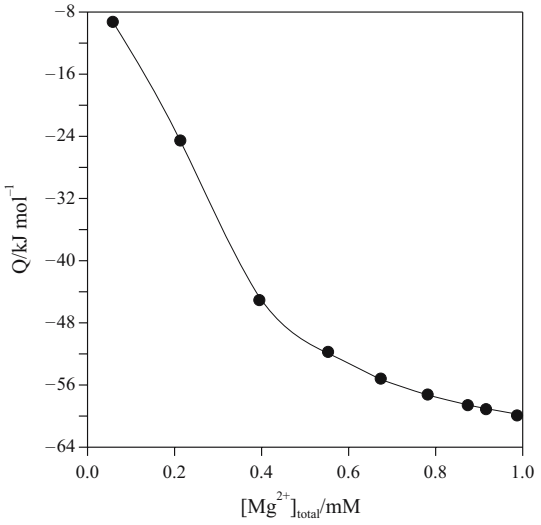

Fig. 2 Comparison between the experimental heats values (● for 35 μM of hGH) for Mg^{2+}-hGH interaction in 2 mM Mg^{2+} solution and calculated data (lines) via Eq. (7)

Association binding constant recovered for Mg^{2+}-hGH interaction is 47.47 mM.

δ_A^θ value is positive, indicating that the net effect of the hGH is breaking of solvent-solvent bonds. Hydrophobic interactions are clearly important in stabilizing protein conformation; it is important to realize that the strength of a hydrophobic interaction is not due to a high intrinsic attraction between nonpolar groups, but rather to properties of the water solvent in which the nonpolar groups are dissolved. A nonpolar residue dissolved in water, induces in the water solvent, a solvation shell, in which water molecules are highly ordered. When two nonpolar groups, come together on the folding of a polypeptide chain, the surface area exposed to the solvent is reduced, and a part of the highly ordered water in solvation shell is released to bulk solvent. Therefore nonpolar moieties to come together in aqueous solvent, resulting in the formation of multimers, and in extreme cases, aggregation and precipitation. A 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. The δ_A^θ values reflect to the hydrophobic hydration of hGH, leading to the enhancement of water structure. The greater the extent of this enhancement, the greater stabilization of the structure of the hGH structure and the greater the δ_A^θ value and vise versa. The extent to which hGH enhances the aqueous structure, decreases as adding Mg^{2+} ions occupy the hydrophobic sites of hGH, resulting in loss of hGh hydrophobicity. δ_A^θ values for Mg^{2+}-hGH interaction is high (8.79) indicating that Mg^{2+} stabilizes the hGH structure in water-rich domain, leading to sustain its native or original characteristic and there is no significant aggregation. In the Mg^{2+}-rich region δ_B^θ values are negative, as would be expected because the hydrophobic sites of hGH have been saturated in this region and hydrophobic hydration by hGH is rapidly decreased by the addition of Mg^{2+}. Since the hydrophobic property of hGH eventually will disappear by addition of Mg^{2+}, we can attribute decreasing of the δ_B^θ values (–0.68) to the loss of this property in Mg^{2+}-rich region and destabilizing of the native conformation of hGH. hGH are preferentially solvated by Mg^{2+} as the p values are more than one (p>1). It is possible to attribute the δ_A^θ and δ_B^θ values to the biological activity of proteins. The greater the δ_A^θ and δ_B^θ values, the greater the biological activity of proteins and vice versa.

Conclusions

Operationally it has been confirmed that the new extended coordination model, via Eq. (7) will satisfactorily reproduce the enthalpies of Mg^{2+}-hGH interaction (Fig. 2). Prediction of biological activity of protein, binding enthalpies and associated equilibrium binding constant using only one set of metal-protein enthalpies, makes this theory the most powerful one.

Acknowledgements

The financial support given by the Imam Khomeini International University, Qazvin, the University of Tehran and the Iran National Science Foundation (INSF) are gratefully acknowledged.

References

1 A. A. Saboury, J. Therm. Anal. Cal., 72 (2003) 93.
2 A. A. Saboury, J. Therm. Anal. Cal., 77 (2004) 997.
3 L. Peng, L. Yi, H. Jia, D. Fengjiao, P. Daiwen and Q. Songsheng, J. Therm. Anal. Cal., 73 (2003) 843.
4 A. A. Saboury, M. S. Atri, M. H. Sanati and M. Sadeghi, J. Therm. Anal. Cal., 83 (2006) 175.
5 A. A. Saboury, M. S. Atri, M. H. Sanati, A. A. Moosavi-Movahedi, G. H. Hakimelahi and M. Sadeghi, Biopolymers, 81 (2006) 120.
6 A. A. Saboury, M. S. Atri, M. H. Sanati, A. A. Moosavi-Movahedi and K. Haghbeen, Int. J. Biol. Macromol., 36 (2005) 305.
7 A. A. Saboury, J. Iran. Chem. Soc., 3 (2006) 1.
8 M. S. Atri, A. A. Saboury, M. Rezaei-Tavirani, M. H. Sanati, A. A. Moosavi-Movahedi, M. Sadeghi, H. Mansuri-Torshizi and N. Khodabandeh, Thermochim. Acta, 438 (2005) 178.
9 A. A. Saboury, H. Ghourchaei, M. H. Sanati, M. S. Atri, M. Rezaei-Tawirani and G. H. Hakimelahi, J. Therm. Anal. Cal., in press (2006).

10 P. C. Hindmarsh and C. G. Brook, Br. Med. J. (Clin. Res. Ed.), 295 (1987) 573.
11 A. M. de Voc, M. Ultsch and A. A. Kossiakoff, Science, 255 (1992) 306.
12 A. V. Filikov, R. J. Hayes, P. Luo, D. M. Stark, C. Chan, A. Kundu and B. I. Dahiyat, Protein Sci., 11 (2002) 1452.
13 T. Arakawa and S. N. Timasheff, Biochemistry, 23 (1984) 5912.
14 D. S. MacLean, O. S. Qian and C. R. Middaugh, J. Pharm. Sci., 91 (2002) 2220.
15 C. Nishimura, V. N. Uversky and A. L. Fink, Biochem., 40 (2001) 2113.
16 R. A. Curtis J. Ulrich, A. Montaser and H. W. Blanch, Biotechnol. Bioeng., 79 (2002) 367.
17 Y. F. Maa and C. C. Hsu, Int. J. Pharm., 140 (1996) 155.
18 G. Rezaei Behbehani, Bull. Kor. Chem. Soc., 2 (2005) 285.
19 G. Rezaei Behbehani, Acta Chimica Slov., 52 (2005) 282.
20 G. Rezaei Behbehani, E. Tazikeh and A. A. Saboury, Bull. Kor. Chem. Soc., 2 (2006) 208.
21 G. Rezaei Behbehani and S. Ghamamy, Thermochim. Acta, 444 (2006) 71.
22 G. Rezaei Behbehani, S. Ghamamy and W. E. Waghorne, Thermochim. Acta, 448 (2006) 37.
23 G. Rezaei Behbehani, E. Tazikeh and A. A. Saboury, Acta Chimica Slov., 53 (2006) 363.
24 G. Rezaei Behbehani, M. Dillon, J. Smyth and W. E. Waghorne, J. Solution Chem., 31 (2002) 827.
25 G. Rezaei Behbehani, D. Dunnion, P. Falvey, K. Hickey, M. Meade, Y. McCarthy, M. C. R. Symons and W. E. Waghorne, J. Solution Chem., 29 (2000) 521.
26 D. Feakins, J. Mullally and W. E. Waghorne, J. Chem. Soc. Faraday. Trans., 87 (1991) 87.
27 E. de Valera, D. Feakins and W. E. Waghorne, J. Chem. Soc. Faraday Trans., 79 (1983) 1061.
28 D. Feakins, C. O' Duinn and W. E. Waghorne, J. Solution Chem., 16 (1987) 907.
29 B. G. Cox and W. E. Waghorne, J. Chem. Soc. Faraday Trans., 80 (1984) 1267.

Received: September 26, 2006
Accepted: January 23, 2007

DOI: 10.1007/s10973-006-8206-x