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Thermochimica Acta 452 (2007) 76–79
thermochimica acta
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Using a new solvation model for thermodynamic study on the interaction of nickel with human growth hormone

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Received 2 July 2006; received in revised form 26 October 2006; accepted 28 October 2006

Abstract

Thermodynamics of the interaction between Ni^{2+} and human growth hormone (hGH) were determined at 27 °C in Nail solution by isothermal titration calorimetry. A new method to predict protein penetration and the effect of metal ions on the stability of proteins is introduced. The new solvation model was used to reproduce the enthalpies of Ni^{2+}–hGH interaction over the whole range of Ni^{2+} concentrations. The solvation parameters recovered from the new equation, attributed to the structural change of hGH and its biological activity.

Keywords: Titration calorimetry; Solvation model; Human growth hormone

1. Introduction

Protein aggregation is the most common manifestation of protein instability. Although a variety of methods have been used to detect protein aggregation, the end results are often unsatisfactory for many proteins. The limited success is due to lack of a clear understanding of the protein aggregation process [1–5]. Human growth hormone (hGH) is a polypeptide hormone, which plays an important role in somatic growth through its effects on the metabolism of proteins, carbohydrates, and lipids [6]. There are some reports on the binding properties and structural changes of hGH due to its interaction with metal ions [7–9]. In this paper, the interaction between Ni^{2+} and hGH has been investigated in neutral aqueous solution to clarify thermodynamics of metal binding properties. This article discusses protein aggregation and its relation with the solvation parameters recovered from the new introduced equation. The new method is able to correlate the solvation parameters to the effect of metals on the stability of a protein in a very simple way.

2. Experimental

2.1. 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.

2.2. Methods

The isothermal titration calorimetric experiments were performed with the four channel commercial calorimetric system, Thermal Activity Monitor 2277, Thermometric, Sweden. The titration vessel was made from stainless steel. A solution of nickel chloride (2 mM) was injected by use of a Hamilton syringe into the calorimetric titration vessel, which contained 1.8 mL hGH (6 μM and 60 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 chloride solution into the perfusion vessel was repeated 30 times, with 20 μL per injection. The heat of each injection was calculated by the "Thermometric Digitam 3" software. The heat of

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0040-6031/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.tca.2006.10.024

dilution of the Ni^{2+} solution was measured as described above except hGH was excluded. The calorimeter was frequently calibrated electrically during the course of the study. The molecular weight of HGH was taken to be 22 kDa [7,8]. Enthalpies of Ni^{2+}–hGH interaction are shown in Fig. 2.

3. Results and discussion

It has been suggested that solvation of a solute in binary solvent mixtures is analogous to complexation, with the better solvent taking the role of the ligand. The model used to analyze the enthalpies of transfer of a solute from a pure solvent into a mixed solvent system has been presented in detail previously [13–18]. Briefly this takes account of preferential solvation by the components of a mixed solvent, the extent to which the solute disrupts or enhances solvent–solvent bonding and the interaction of the solute with the surrounding solvent molecules. 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:

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

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 by curve fitting. ΔH_t^θ is the enthalpy of transfer of the solutes from solvent A to the mixture 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 of A and B in the mixed solvent. 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). The constants 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 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 [13–22]. As the parameters α, β, n, N and $(\alpha n + \beta N)$ are not constant during the solvent compositions; thereby the net effect of the solute on solvent–solvent bonds in mixture $(\alpha n + \beta N)^{mix} = \delta^{mix}$, is changed over the solvent compositions and we can 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. (3) into Eq. (4), leads to:

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

With simple modification of Eq. (6), it is possible to use this equation to reproduce the enthalpies of metal ion–protein interaction 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 Ni^{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 Ni^{2+}–hGH interaction as the total ligand concentrations divided by the maximum concentration of Ni^{2+} as follow:

$$x_B = \frac{[Ni^{2+}]_t}{[Ni^{2+}]_{max}}, \quad x_A = 1 - x_B \quad (8)$$

$[Ni^{2+}]_t$ is the total concentration of Ni^{2+} and $[Ni^{2+}]_{max}$ is the maximum concentration of Ni^{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 Ni^{2+} in water, q_{dilut}, as follow:

$$L_A = q_{bin} + x_B \left(\frac{\partial q_{dilut}}{\partial x_B}\right), \qquad L_B = q_{bin} - x_A \left(\frac{\partial q_{dilut}}{\partial x_B}\right) \quad (9)$$

q values were fitted to Eq. (7) 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 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 Ni^{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 Ni^{2+}, respectively; $p = 1$ indicates random solvation.

The model used to calculate ν values have been presented in detail previously [7–12]. Briefly at any constant heat value due to the binding of ligand molecules per mole of protein (q), the free

concentration of ligand (L^{free}) and $\nu = (L_2 - L_1)/(M_2 - M_1)$ are constant on both curve of (q) against total concentration of either ligand (L_1 and L_2) or protein (M_1 and M_2) in two titration experiments at two different concentration of a protein (Fig. 2). Comparing ν/g values using the previous method [7–9] and x'_B values from Eq. (7), we have realized that $x'_B = \nu/g$.

In general, there will be "g" sites for binding of ligand molecules (Ni^{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. (3) to find "g" value as follow:

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

Therefore, if x'_B values recovered from Eq. (7) are multiplied by "g", which obtains from Eq. (10) ($g = 3$ with the correlation coefficient of $R^2 = 0.99994$), experimental ν values can be calculated with only one set of experimental enthalpies. ν values reproduced from Eq. (10) and experimental values obtained from Eq. (7) have been compared in Fig. 1. By using ν values it is possible to calculate the free concentration of ligand as follow:

$$L^{free} = L_t - \nu M_t \tag{11}$$

where L^{free} and L_t are free concentration and total concentration of ligand, respectively. M_t is the total concentration of hGH. Finally by using the Scatchard equation association binding constant, K, will be obtained as follow:

$$\frac{\nu}{g - \nu} = kL^{free} \tag{12}$$

Therefore, the plot of $\nu/(g - \nu)$ against L^{free} should be a linear plot with a slope of K.

Table 1
Solvation parameters for Ni^{2+}–hGH interaction in 50 mM NaCl solution with water recovered by Eq. (7)

[M] (μM)	p	δ_A^θ	δ_B^θ	q_{max}	ΔH_{bin}	K (μM)
60 μM	3.5	0.33	−2.07	−44.45	−14.81	23.37
6 μM	11	0.33	−0.39	−46.92	−15.64	24.25

Solvation parameters for Ni^{2+}–hGH interaction recovered by Eq. (7) are listed in Table 1 and q values calculated via this equation using these parameters are compared with the experimental data in Fig. 2. The agreement between the calculated and experimental results is striking, and gives considerable support to the use of Eq. (7). In two cases the δ_A^θ values are positive (Table 1), 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 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 part of the highly ordered water in the solvation shell is released to bulk solvent. Therefore, nonpolar moieties come together in aqueous solvent, resulting in formation of multimers and, in extreme cases, aggregation and precipitation. The most common mechanism of protein aggregation is believed to involve protein denaturation, via hydrophobic interfaces and often results in loss of biological activity [23,24]. Hydrophobicity of proteins leads to the loss of entropy, which results in the apparent increase in stability of the native state [25–27]. Therefore, it is possible to introduce a correlation between change

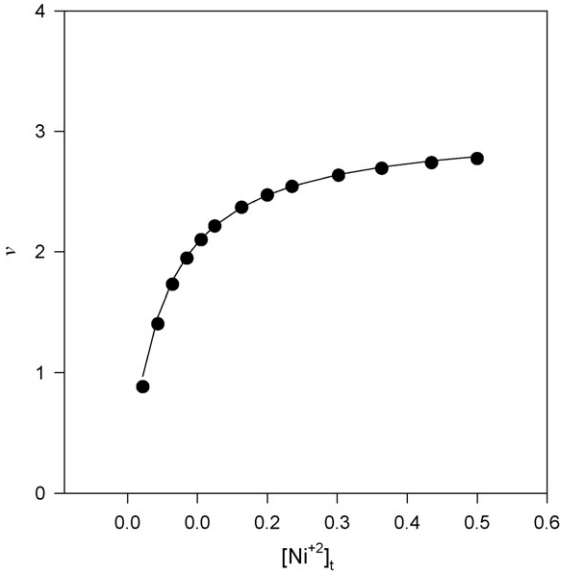

Fig. 1. Comparison between experimental (symbols) ν values (x'_B values recovered from Eq. (7) multiplied by "g") and calculated ν values (line) from Eq. (10).

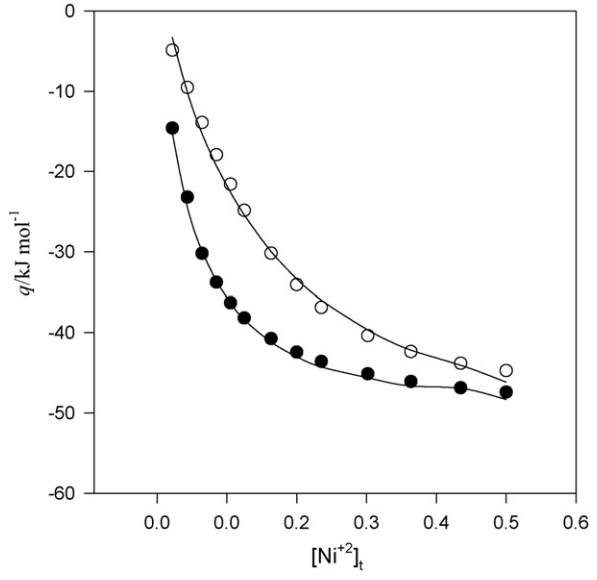

Fig. 2. Comparison between the experimental heat values ((◯) for 6 μM of hGH and (●) for 60 μM of hGH) for Ni^{2+}–hGH interaction in 50 mM NaCl solution and calculated data (lines) via Eq. (7).

in δ_A^θ and increase in the stability of proteins, suggesting that effects on the denatured state are very important in the stability.

The δ_A^θ values reflect the hydrophobic hydration of hGH, leading to the enhancement of water structure and loss of entropy. The greater the extent of this enhancement, the greater the stabilization of the hGH structure and the greater the value of δ_A^θ. The extent to which hGH enhances the aqueous structure is decreased by adding Ni^{2+} ions because the interaction of the Ni^{2+} ions with hydrophilic sites modifies the folding of the protein, affecting the hydrophobic areas indirectly, resulting in loss of hGh hydrophobicity. δ_A^θ values (Table 1) for Ni^{2+}–hGH interaction are small (0.33) indicating that small amounts of Ni^{2+} destabilizes the hGH structure, leading to loss of its native or original characteristic; thereby aggregation. *p*-values for 6 μM hGH and 60 μM hGH are 11 and 3.5, respectively (Table 1) indicating that 6 μM hGH lose its hydrophobicity much faster. In the Ni^{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 Ni^{2+}. Since the hydrophobic property of hGH eventually will disappear by addition of Ni^{2+}, we can attribute decreasing δ_B^θ values (−0.33 and −2.07 for 6 μM and 60 μM of hGH, respectively) to the loss of this property in Ni^{2+}-rich region and destabilizing of the native conformation of hGH. hGH is preferentially bonded by Ni^{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 δ_A^θ and δ_B^θ values, the greater will be the activity of the proteins.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tca.2006.10.024.

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