

A Microcalorimetry Study of the Binding of Nickel Ion by Human Growth Hormone

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ABSTRACT: A binding study of nickel ions by a new recombinant human Growth Hormone (hGH), produced as an injected drug, has been done at 27°C in NaCl solution (50 mM) using an isothermal titration calorimetry. There is a set of three identical and non-interacting binding sites for nickel ions. The intrinsic dissociation equilibrium constant and the molar enthalpy of binding are 40 μM and -16.5 kJ/mol, respectively. Thermodynamic parameters of nickel ion binding are compared to the other metal ions. The molar entropy of binding is 29.3 $\text{J K}^{-1} \text{mol}^{-1}$ for Ni^{2+} , less than Cu^{2+} and more than other metal ions, means that the disorder of the protein structure due to the binding of nickel ions is more than to the other ion metals, except Cu^{2+} . It is expected that nickel ions can prevent from the aggregation of the protein.

KEY WORDS: Human growth hormone, Nickel ion, Metal binding, Titration calorimetry.

INTRODUCTION

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 [1]. hGH is a single domain globular protein containing 191 amino acids. The molecular mass is approximately 22 kDa, with pI 5.3 [2-6]. There are two disulfide bridges present in the protein: one connecting distant parts of the molecule involving residues 53 and 165 (large loop) and another near the C terminal between residues 182 and 189 (small loop) [7]. Approximately 55% of the polypeptide backbone exists in a right-handed α -helical conformation [6, 8].

There are some reports on the binding properties and structural changes of hGH due to its interaction with

metal ions [9-19]. The metal-binding site in hGH is located in the hydrophobic core. A well-resolved crystal structure of hGH has been obtained, showing that the metal-binding site is likely composed of His18 and His21 on helix I and Glu 174 on helix IV. Some metal ions such as Zn^{2+} , Cd^{2+} , Hg^{2+} , and Co^{2+} are known to promote hGH reversible dimerization [9-12]. But, in the presence of some other ions such as Ca^{2+} , Ba^{2+} , Mg^{2+} , Pb^{2+} , Al^{3+} , Fe^{2+} , and Fe^{3+} , there is no significant dimerization of hGH in solutions [10]. Some thermodynamic studies have been carried out on the interactions between Ca^{2+} [13-14], Mg^{2+} [15], Fe^{3+} [16], Cu^{2+} [17], and Co^{2+} [18]. There is a set of three identical and non-interacting binding sites for binding of all these metal ions, except Fe^{3+} . There is a set

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of four identical and independent binding sites for Fe^{3+} . The binding process for iron ions is more exothermic ($\Delta H^\circ = -18.7$ kJ/mol) than other four metal ions [16].

The biological importance of nickel ion has been recognized well [20-22]. Nickel ion is now known to be an essential micronutrient for many microorganisms, and it is found in enzymes involved in ureolysis, hydrogen metabolism, methane biogenesis, and acetogenesis. Evidence that nickel plays an important function in animal and bacterial systems also suggests that nickel plays a larger role in plant productivity than is currently recognized. The human abundance of nickel ion is 100 ppb by weight. Binding properties and transportation of nickel ion by human serum albumin are well known [23]. Since there are no exact reports on the metal binding for hGH in the literature, we attempted to find binding properties of hGH due to the binding of nickel ion in natural aqueous solution to clarify thermodynamics of metal binding.

EXPERIMENTAL SECTION

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 277nm in 1cm quartz cuvettes; an absorbance value of 9.3 at 277 nm for 1% hGH solution was used as reported by *Bewley et al.* [24]. Nickel nitrate was purchased from Merck Co. (Darmstadt, Germany). All other materials and reagents were of analytical grade, and solutions were made in 50 mM NaCl using double-distilled water.

The isothermal titration microcalorimetric experiments were performed with the four channels commercial microcalorimetric system, Thermal Activity Monitor 2277, Thermometric (Sweden). The titration vessel was made from stainless steel. Nickel solution (2 mM) was injected by use of a Hamilton syringe into the calorimetric stirred titration vessel, which contained 1.8 mL hGH (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 solution into each perfusion vessel was repeated 30 times, and each injection included 20 μL reagent. 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 nickel 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 aqueous solution, without nickel ion, was injected into the protein solution in the sample cell. The enthalpies of the nickel and protein solutions dilution were subtracted from the enthalpy of hGH-nickel interaction. The microcalorimeter was frequently calibrated electrically during the course of the study. The molecular weight of hGH was taken to be 22 kDa [4, 6].

RESULTS AND DISCUSSION

The raw data obtained from isothermal titration microcalorimetry of hGH interaction with nickel ion have been shown in Fig. 1. Fig. 1 shows the binding heat of nickel ions per mole of hGH versus total concentration of Ni^{2+} (Fig. 1a) or versus total concentration of the protein (Fig. 1b).

Consider a solution containing ligand L, and a biomacromolecule (M_n) that contains n sites capable of binding the ligand. If the multiple binding sites on a biomacromolecule are identical and independent, the ligand binding sites can be reproduced by a model system of monovalent molecules ($M_n \rightarrow nM$) with the same set of dissociation equilibrium constant (K) values. Thus, the reaction under consideration can be written:



For a biomacromolecule that contains g sites capable of binding the ligand with the same dissociation equilibrium constant (K) value, it has been shown [13]:

$$\frac{\Delta q}{q_{\max}} M_0 = \left(\frac{\Delta q}{q} \right) L_0 \frac{1}{n} - \frac{K}{n} \quad (2)$$

where M_0 is the total biomacromolecule concentration, L_0 is the total ligand concentration, q represents the heat value at a certain L_0 , q_{\max} represents the heat value upon saturation of all biomacromolecule and $\Delta q = q_{\max} - q$. Therefore, the plot of $(\Delta q/q_{\max})M_0$ versus $(\Delta q/q)L_0$ should be a linear plot by a slope of $1/n$ and the vertical-intercept of K/n , which n and K can be obtained. The related plot for the binding of nickel ions by hGH is showing in Fig. 2. The linearity of the plot has been examined by different estimated values for q_{\max} to find

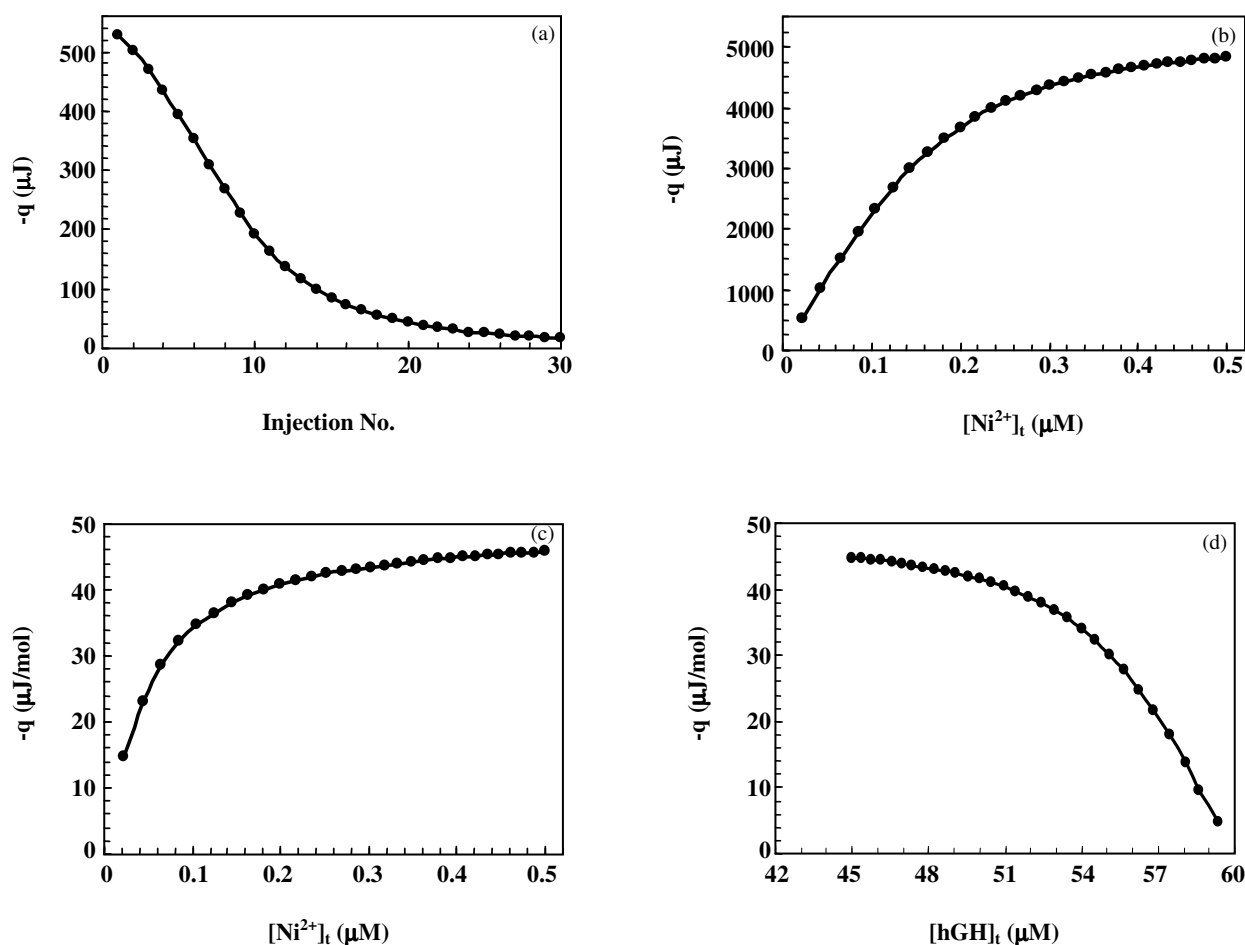


Fig. 1: (a) The heat of binding nickel ions on hGH obtained for 30 automatic cumulative injections, each of 20 μL , 2 mM, into the sample cell containing 1.8 mL hGH solution at an initial concentration of 60 μM at 27°C. (b) The cumulative heat of binding nickel ions on hGH versus total concentration of copper ion, calculated from Fig. 1a. (c) The heat of binding nickel ions per mole of hGH versus total concentration of nickel ions, calculated from Fig. 1b. (d) The heat of binding nickel ions per mole of hGH versus total concentration of hGH, calculated from Fig. 1a.

the best value for the correlation coefficient (near to one). The best linear plot with the correlation coefficient value of 0.99 was obtained using a value of -5346 μJ (equal to -49.5 kJ/mol) for q_{max} . The amounts of n and K , obtained from the slope and vertical-intercept plot, are 3 and 40 μM , respectively. Dividing the q_{max} value of -49.5 kJ/mol by $n=3$, therefore, gives $\Delta H=-16.5$ kJ/mol.

For a set of identical and independent binding sites, we have before shown [25-28]:

$$\Delta H=1/A_i\{(B_i+K)-(B_i+K)^2-C_i\}^{1/2} \quad (3)$$

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

$$A_i=V_i/2q_i \quad B_i=nM_0+L_0 \quad C_i=4nM_0L \quad (4)$$

where V_i and q_i are the volume of the reaction solution and total cumulative heat (by kJ/mol, which can be obtained from Fig. 1) in the calorimetric sample cell in each injection step, respectively. According to data shown in Fig. 1, the total cumulative heats respect to kJ/mol are known in any different values of M_0 and L_0 ; therefore, A_i , B_i and C_i are known in all titration steps. Equation (3) contains two unknowns, K and ΔH . A series of reasonable value for K is inserted into Eq. (3) and corresponding values for ΔH are calculated and the graph ΔH versus K is constructed. Curves of all titration steps will intersect in one point, which represents the true value

Table 1: Thermodynamic parameters of binding for metal ions to hGH obtained by ITC.

Metal ion	n	K (μM)	K_a (M^{-1})	ΔH° (kJ/mol)	ΔG° (kJ/mol)	ΔS° (J/mol K)	Ref
Ca^{2+}	3	52.0	1.92×10^4	-17.4	-24.60	24.0	13-14
Mg^{2+}	3	46.0	2.17×10^4	-17.7	-24.9	24.0	15
Fe^{3+}	4	40.0	2.50×10^4	-18.7	-25.3	22.0	16
Cu^{2+}	3	8.6	11.63×10^4	-16.7	-29.1	41.3	17
Co^{2+}	3	1250	800	-16.70	-16.67	-0.10	18
Ni^{2+}	3	40.0	2.5×10^4	-16.5	-25.3	29.3	-

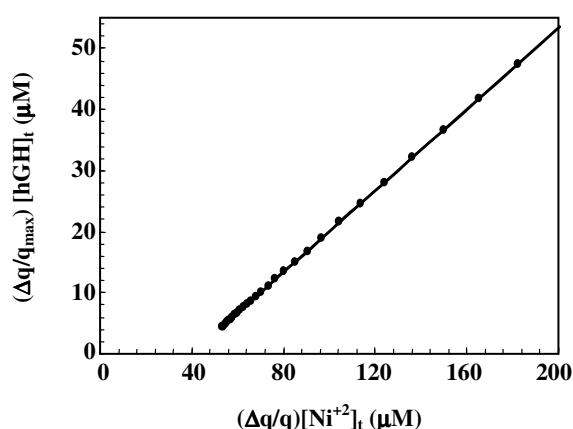


Fig. 2: The best linear plot of $(\Delta q/q_{\max})M_0$ versus $(\Delta q/q)L_0$, according to Eq. (2), using a value of $5346 \mu\text{J}$ (equal to 49.5 kJ/mol) for q_{\max} to obtain the best correlation coefficient value ($R^2 = 0.999$). The initial concentration of hGH was $60 \mu\text{M}$. Values of n and K can be obtained from the slope and the vertical-intercepts, respectively.

for ΔH and K . Actually, this method represents a simple graphical non-linear fitting method. The plots of ΔH versus K , according to equation (3), for all injections are shown in Fig. 3. The intersection of curves gives:

$$K = 40 \mu\text{M} \quad \Delta H = -16.5 \text{ kJ/mol}$$

The conformity of K and ΔH values obtained from two methods are observed.

To compare all thermodynamic parameters in metal binding process for hGH, the change in standard Gibbs free energy (ΔG°) should be calculated according to the Eq. (5), which its value can use in Eq. (6) for calculating the change in standard entropy (ΔS°) of binding process.

$$\Delta G^\circ = -RT \ln K_a \quad (5)$$

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ \quad (6)$$

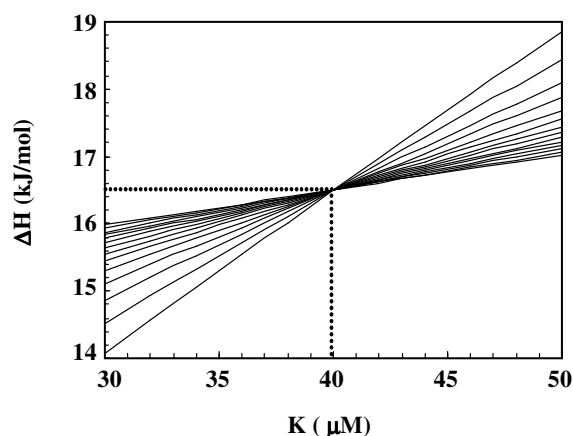


Fig. 3: ΔH versus K for the first 15 injections in the reasonable values of K , according to Eq. (3), using data in Fig. 1. The hGH first concentration was $60 \mu\text{M}$. (The coordinates of the intersection point of the curves give the true values for ΔH and K .)

Where K_a is the association binding constant (the inverse of the dissociation binding constant, K). The K_a value is obtained 25000 M^{-1} . Hence:

$$\Delta G^\circ = -25.3 \text{ kJ/mol} \quad \Delta S^\circ = +29.3 \text{ J/K mol}$$

It means that the binding process is spontaneous resulted by not only enthalpic but also entropic driven.

CONCLUSIONS

All thermodynamic parameters for the interaction between hGH and metal ions studied are summarized in Table 1. There is a set of three identical and non-interacting binding sites for binding of all these metal ions, except Fe^{3+} . The binding process for iron ions is more exothermic ($\Delta H^\circ = -18.7 \text{ kJ/mol}$) than other metal ions. The affinity of binding for copper ion is the highest ($K_a = 11.67 \times 10^4$). The affinity of binding for nickel ion is identical to the iron ion ($K_a = 2.50 \times 10^4$), and both these

ions have a higher affinity respect to the other ions after copper ion. Nickel ion has the lowest value of the enthalpy of binding. The molar entropy of binding means that the difference between the entropy of metal-hGH complex (S_{M-hGH}) and the entropy of native hGH (S_{hGH}): $\Delta S = S_{M-hGH} - S_{hGH}$. The value of S_{hGH} is identical for all cases. The ΔS value is $29.3 \text{ J K}^{-1} \text{ mol}^{-1}$ for Ni^{2+} binding to the protein, less than Cu^{2+} and more than other metal ions. Hence, the disorder of the protein structure due to the binding of nickel ions is more than to the other ion metals, except Cu^{2+} .

Some metal ions like Zn^{2+} , Cd^{2+} , Hg^{2+} , Co^{2+} and Fe^{3+} are known to promote hGH reversible dimerization [9-12, 16]. According to the binding of Ni^{2+} to hGH, similar to other metal ions, it may be also expected that nickel ions can prevent from the aggregation of the protein.

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