Turk J Chem
34 (2010) , 631 – 638.

doi:10.3906/kim-0911-27

A calorimetric study of the interaction of silver ions with jack bean urease

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Received 10.11.2009

A thermodynamic study of silver ions by jack bean urease (JBU) was carried out at 2 temperatures, 27 and 37 °C, in Tris buffer (30 mM; pH 7.0) using isothermal titration calorimetry (ITC). There was a set of 12 identical and noninteracting binding sites for the silver ions. The intrinsic dissociation equilibrium constant and the molar enthalpy of binding were 185 μM and -16.7 kJ mol^{-1} at 27 °C, and 229 μM and -16.3 kJ mol^{-1} at 37 °C, respectively. The molar entropy of binding was $+15.7$ J K^{-1} mol^{-1} at 27 °C and $+17.1$ J K^{-1} mol^{-1} at 37 °C. Hence, the binding process of silver ions to JBU is not only enthalpy driven but is also entropy driven, and the role of entropy should be made more effective by increasing the temperature.

Key Words: Urease; silver ion; isothermal titration calorimetry; binding constant; enthalpy of binding; entropy of binding

Introduction

Jack bean urease (urea amidohydrolase; EC 3.5.1.5) is the first crystallized enzyme[1] and is also the first enzyme shown to contain nickel.[2,3] The crystal structure of urease has not been determined yet. The best resolution obtained at 3.5 Å only allowed for the assigning of the octahedral crystals of this urease to a cubic space group.[4] Jack bean urease has 6 identical subunits. Each subunit consists of a single kind of polypeptide chain containing 840 amino acid residues with a relative molecular mass of 90,770, excluding the 2 nickel ions per subunit.[5] Hence, the mass of the hexamer urease, including 12 nickel ions, is 545.340 kDa[5] (590 kDa by a

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sedimentation method[6]). The subunit of urease from microorganisms appears to be smaller than jack bean urease in size and number.[7,8]

Urease catalyzes the hydrolysis of urea to carbonic acid and 2 molecules of ammonia.[9] The specificity of urease was thought to be absolute[10] until Fishbein et al. reported that N-hydroxyurea was a substrate.[11,12] N-hydroxyurea,[11–13] (N,N')dihydroxyurea,[14,15] semicarbazide,[16] N-methylurea,[17] formamide,[18] and acetamide[17] are other examples of substrates for urease. The mechanisms of hydrolysis of urea by urease presently contemplated are those by Benini et al.[19] and Karplus et al.,[20] which take their origins from the mechanism proposed by Zerner's group.[17] Zerner et al. have proposed a model for jack bean urease catalysis in which one nickel coordinates the oxygen atom of urea, polarizing the carbonyl group, and a second nickel coordinates hydroxide ion, the catalytic nucleophile.[17]

Ureases are inhibited by a number of compounds. The study of urease inhibitors may have medical or agronomic significance, as well as providing insight into the urease catalytic mechanism.[21,22] Substrate urea, product ammonium ions, and substrate analogues are weak inhibitors of urease.[21,23,24] Thiols inhibit urease competitively in their thiolate anion form, R-S^-.[22] Amides and esters of phosphoric acid are also slow-binding inhibitors of urease, classified as the strongest inhibitors. Boric and boronic acids are rapidly binding urease inhibitors, comparatively weak. Fluoride ion was found to be a competitive inhibitor for jack bean urease.[25] There are some reports on the inhibition of urease by bismuth compounds, which are of medical importance since they are widely used as bactericidal agents.[26–29] The inhibition of ureases by quinones has been mainly tested for its potential application with urea fertilizers.[29,30]

Inhibition of urease by heavy metal ions is important not only in view of heavy metal ion pollution, as appropriate levels of urease activity in agricultural soils may be endangered, but this inhibition may also be exploited in constructing urease inhibition-based sensing systems[31–33] for in situ and real time determination of trace levels of ions, e.g. in environmental monitoring, food control, and biomedical analysis. Heavy metal ions inhibit both plant[31,34–38] and bacterial ureases[39,40] in the following order of effectiveness:[36,38]

$$Hg^{2+} \approx Ag^{+} > Cu^{2+} >> Ni^{2+} > Cd^{2+} > Zn^{2+} > Co^{2+} > Fe^{3+} > Pb^{2+} > Mn^{2+}$$

Hg^{2+}, Ag^{+}, and Cu^{2+} ions are nearly always listed as the strongest inhibitors.[31–36,38,41] The inhibition has been habitually ascribed to the reaction of the metal ions with the thiol groups of the enzyme.[33,41,42] However, both copper and silver ions coordinate to nitrogen (histidine)- and possibly oxygen (aspartic and glutamic acids)-containing functional groups in urease.[43,44] Notwithstanding the fact that heavy metal ion binding to urease is important, there is not a comprehensive binding study in this case. Here we applied isothermal titration calorimetry (ITC) as a powerful tool for studying silver ion binding to jack bean urease, for which all thermodynamic parameters for the binding process can be found.

Experimental

Materials

Jack bean urease (JBU) was obtained from Sigma Chemical Co, USA. Silver nitrate was obtained from Merck, Germany. The buffer solution used in the experiments was 30 mM Tris using double-distilled water, pH 7.0, which was obtained from Merck, Germany. Experiments were carried out at 2 temperatures, 27 and 37 °C.

Method

The experiments were performed with a 4-channel commercial microcalorimetric system, Thermal Activity Monitor 2277 (Thermometric, Sweden). Each channel is a twin heat conduction calorimeter (multijunction thermocouple plates) positioned between the vessel holders and the surrounding heat sink. Both the sample and reference vessels were made from stainless steel. The limited sensitivity for the calorimeter is 0.1 μJ. Silver ion solution (10 mM) was injected into the calorimetric titration vessel, which contained 1.8 mL of JBU, 4 μM (2.2 mg mL^{-1}), in Tris buffer (30 mM, pH 7.0), using a Hamilton syringe. Thin (0.15 mm inner diameter) stainless steel hypodermic needles, permanently fixed to the syringe, reached directly into the calorimetric vessel. Injection of silver ion solution into the perfusion vessel was repeated 30 times and each injection included 20 μL of silver ion solution. The calorimetric signal was measured by a digital voltmeter that was part of a computerized recording system. The heat of the injection was calculated by Thermometric Digitam 3 software. The heat of the dilution of the silver ion solution was measured as described above, except that JBU was excluded. The heat of the 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.[45] The enthalpies of the silver ion and protein solution dilution were subtracted from the enthalpies of silver ion solutions in JBU solutions. The microcalorimeter was frequently calibrated electrically during the course of the study.

Results and discussion

The raw data obtained from ITC at 2 temperatures, 27 and 37 °C, are shown in Figure 1. Figure 1a shows the heat of each injection and Figure 1b shows the cumulative heat at each total concentration of silver ion, $[Ag^+]_t$. For a set of identical and independent binding sites, we have previously shown different methods of ITC data analysis.[45] For a set of identical and independent binding sites, we have previously introduced the following equation:[45,46]

$$\frac{\Delta q}{q_{\max}} M_0 = (\frac{\Delta q}{q}) L_0 \frac{1}{g} - \frac{K_d}{g}, \tag{1}$$

where g is the number of binding sites; K_d is the dissociation equilibrium constant; M_0 and L_0 are the total concentrations of biomacromolecules and metal ions, respectively; $\Delta q = q_{max}\text{-}q$; q represents the heat value at a certain L_0; and q_{max} represents the heat value upon saturation of all biomacromolecules. If q and q_{max} are calculated per mole of biomacromolecule, then the standard molar enthalpy of binding for each binding site (ΔH°) will be $\Delta H^\circ = \frac{q_{max}}{g}$. According to Eq. (1), a plot of $(\Delta q/q_{max})M_0$ versus $(\Delta q/q)L_0$ should be a linear plot with a slope of 1 g^{-1} and the vertical-intercept of K_d g^{-1}, for which g and K_d can be obtained. The related plot for the binding of Ag^+ ions by JBU is shown in Figure 2. The linearity of the plot has been examined by different estimated values for q_{max} to find the best value for the correlation coefficient (close to 1). The best linear plot with the correlation coefficient ($R^2 = 0.99$) value (close to 1) was obtained using -1443 and -1408 μJ (equal to -200.4 and -195.6 kJ mol^{-1}) at 27 and 37 °C, respectively, for q_{max} (Figure 2). The lack of a suitable value for q_{max} to obtain a linear plot of $(\frac{\Delta q}{q_{max}})M_0$ versus $(\frac{\Delta q}{q})L_0$ may be related to the existence of nonidentical binding sites or the interaction between them. The value of g is 12, obtained from the slope. The value of K_d can be obtained from the vertical-intercept plot, which is 185 mM at 27 °C and 229 μM at 37 °C.

Dividing the q_{max} amounts of -200.4 and -195.6 kJ mol^{-1} by g = 12, therefore, gives ΔH° = -16.7 and -16.3 kJ mol^{-1} at 27 and 37 °C, respectively.

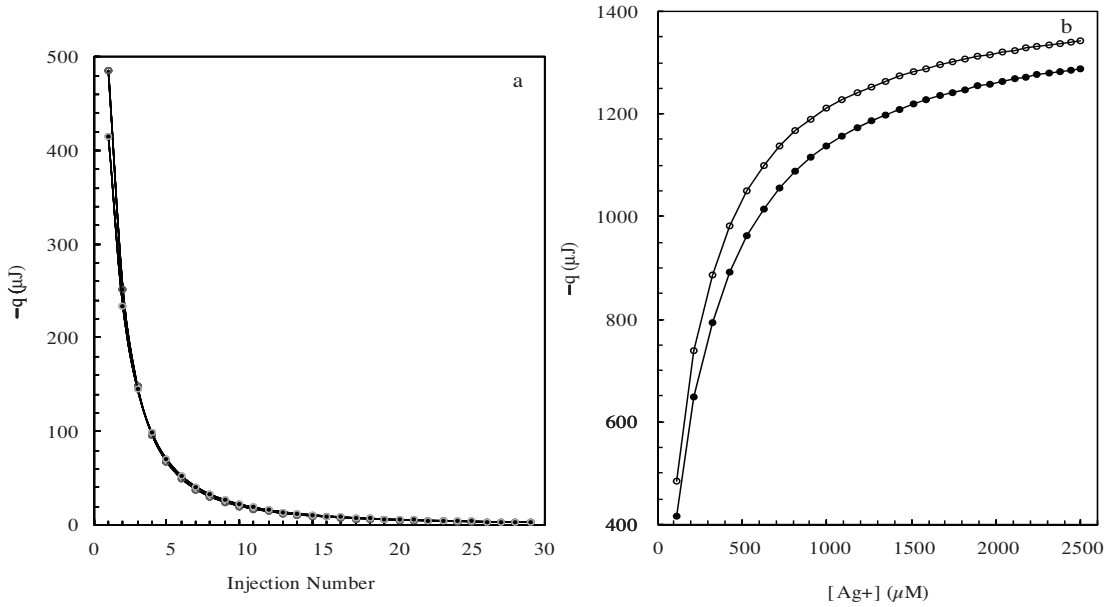

Figure 1. (a) The heat of silver ion binding to JBU for 30 automatic cumulative injections, each of 20 μL of 10 mM silver nitrate solution, into sample cells containing 1.8 mL of 4 μM protein solution at 300 K (○) and 310 K (●). (b) The total cumulative heat of binding versus the total concentration of silver ions calculated from Figure 1a.

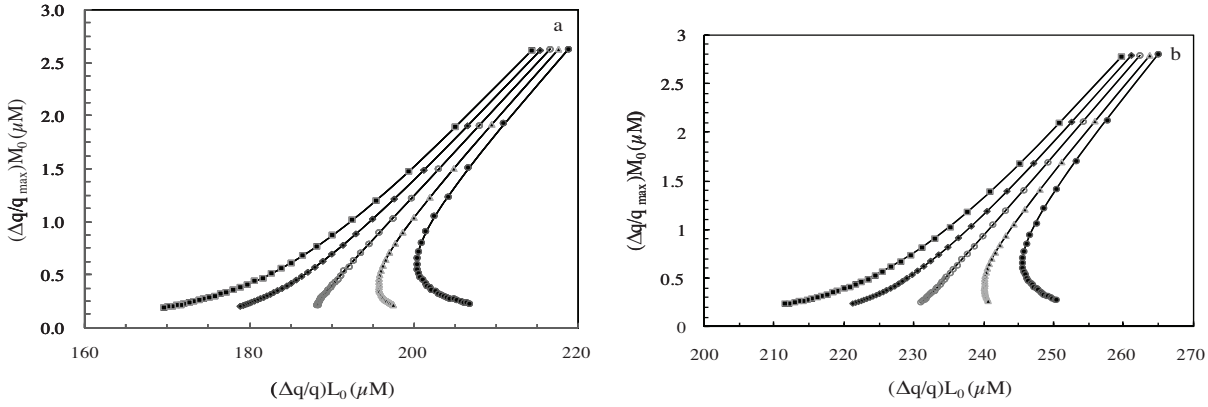

Figure 2. (a) The best linear plot of $(\frac{\Delta q}{q_{max}})M_0$ versus $(\frac{\Delta q}{q})L_0$ according to Eq. (1), using values of -1433 μJ (■), -1438 (♦, -1443 μJ (○, -1448 μJ (Δ), and -1453 μJ (●) at 300 K for q_{max} to obtain the best correlation coefficient value (R^2 = 0.999) for a linear plot. (b) The best linear plot of $(\frac{\Delta q}{q_{max}})M_0$ versus $(\frac{\Delta q}{q})L_0$ according to Eq. (1), using values of -1398 μJ (■), -1403 (♦), -1408 μJ (○), -1413 μJ (Δ), and -1418 μJ (●) at 310 K for q_{max} to obtain the best correlation coefficient value (R^2 = 0.999) for a linear plot.

The molar enthalpy of each binding site (ΔH°) and its dissociation equilibrium constant (K_d) in a set of biomacromolecule binding sites can also be obtained via a simple graphical nonlinear fitting method using

the following equation:[45,47,48]

$$\Delta H = 1/A\,((B + K_d) - [(B + K_d)^2 - C]^{1/2}) \tag{2}$$

A, B, and C are constants in each injection, which have been defined as follows:

$$A = V/2q \quad B = gM_0 + L_0 \quad C = 4gM_0L_0, \tag{3}$$

where V is the volume of the reaction solution in the calorimetric sample cell in each injection step. Eq. (2) contains 2 unknown parameters, K_d and ΔH°. A series of reasonable values for K_d were inserted into Eq. (2), corresponding amounts for ΔH° were calculated, and the graph of ΔH° versus K_d was constructed. Curves of all titration steps will intersect at one point, which represents the true amounts for ΔH° and K_d. The plots of ΔH° versus K_d according to Eq. (2) for all injections are shown in Figure 3. The intrinsic dissociation equilibrium constant and the molar enthalpy of binding were obtained as 185 μM and -16.7 kJ mol^{-1} at 27 °C and 229 μM and -16.3 kJ mol^{-1} at 37 °C, respectively (see Figure 3). These results are identical with results obtained by the previous method described above.

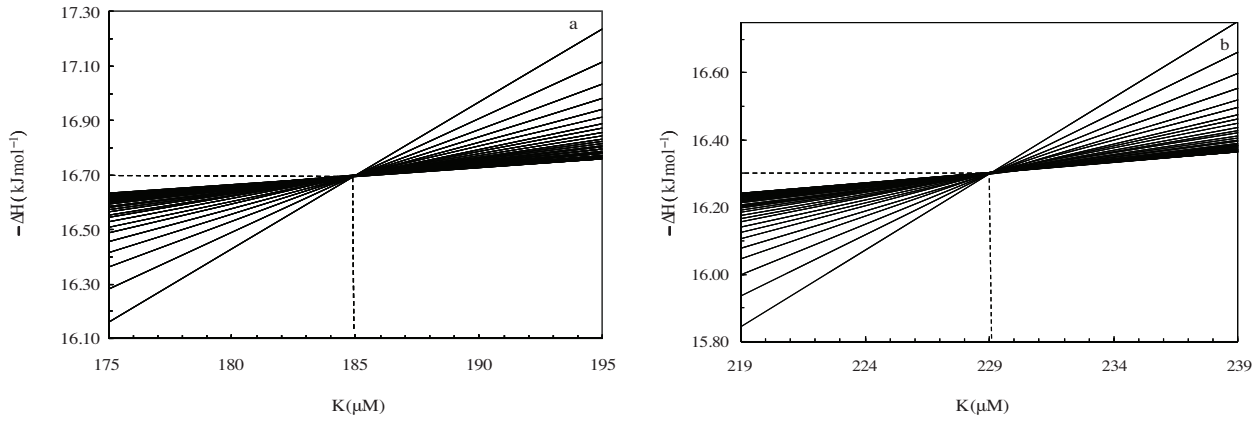

Figure 3. ΔH versus K_d for all 30 injections at 300 K (a) and 310 K (b) using data from Figure 1b. The coordinates of the intersection point of the curves give the true values for ΔH and K_d.

To compare all thermodynamic parameters in the metal binding process for JBU, the change in standard Gibbs free energy (ΔG^o) should be calculated according to Eq. (4), whose value can be used in Eq. (5) for calculating the change in standard entropy (ΔS^o) of binding process.

$$\Delta G^o = -RTlnK_a \tag{4}$$

$$\Delta G^o = \Delta H^o - T\Delta S^o, \tag{5}$$

where K_a is the association binding constant (the inverse of the dissociation binding constant, K_d). The K_a values obtained are 5405 and 4367 M^{-1} at 27 and 37 °C, respectively. Hence:

$$\Delta G^o = \text{-21.4 kJ mol}^{-1} \quad \Delta S^o = +15.7 \text{ J K}^{-1} \text{ mol}^{-1} \qquad (\text{at } 27\ ^\circ\text{C})$$

$$\Delta G^o = \text{-}21.6 \text{ kJ mol}^{-1} \quad \Delta S^o = +17.1 \text{ J K}^{-1} \text{ mol}^{-1} \qquad (\text{at } 37\ ^\circ\text{C})$$

This means that the binding process is the spontaneous result of not only enthalpic but also entropic driven forces.

All thermodynamic parameters for the interaction between JBU and silver ion at 2 temperatures, 27 and 37 °C, have been summarized in the Table. There is a set of 12 identical and noninteracting binding sites for silver ions to JBU. The binding process is exothermic at both temperatures. The binding process is not only enthalpy driven but also entropy driven. The role of entropy in the binding process should be made more effective by increasing the temperature. The molar entropy of binding means that the difference between the entropy of the Ag^+-JBU complex (S_{Ag}-JBU) and the entropy of native JBU (S_{JBU}) is: $\Delta S = S_{Ag-JBU}$ - S_{JBU}. Hence, the disorder of the protein structure has been increased due to the binding of copper ions.

Table. Thermodynamic parameters of binding for silver ions to JBU obtained by ITC.

T (K)	g g	K (μM)	K_a (M^{-1})	ΔH° (kJ mol^{-1})	ΔG° (kJ mol^{-1})	ΔS° (J mol^{-1} K^{-1})
300	12	185	5405	-16.7	-21.4	15.7
310	12	229	4367	-16.3	-21.6	17.1

Copper and silver ions are listed as the strongest inhibitors for jack bean urease.[31−36,49,50] The number of binding sites for copper ion binding to JBU is 12, but the dissociation equilibrium constant (K_d) is 285 and 346 μM at 27 and 37 °C, respectively.[51] Silver ions should be more effective for inhibiting the enzyme for the K_d value, for the silver ion is smaller than the copper ion.

Acknowledgement

Financial support from the University of Tehran is gratefully acknowledged.

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