J Therm Anal Calorim (2010) 100:1079–1083
DOI 10.1007/s10973-009-0384-x

Thermodynamic study on the interaction of cyanide ion and jack bean urease at different temperatures

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Received: 12 May 2009 / Accepted: 22 July 2009 / Published online: 28 August 2009

Abstract A method based on Isothermal Titration Calorimety (ITC) is described for the thermodynamic assay of jack bean urease. Inhibitory activity of cyanide ion was examined against jack bean urease (JBU), at 27 and 37 °C in 30 mM Tris buffer of pH = 7. The binding parameters of the CN^- + JBU complexation have been calculated. It was found that in the low and high concentrations of the cyanide ions, the JBU structure was destabilized, resulting in a decrease in its biological activity.

Keywords Jack bean urease · Cyanide ion · Isothermal titration calorimetry · Binding parameters · Solvation model

Introduction

Ureases have since been isolated from a wide variety of organisms including high plants, fungi and bacteria [1–3]. Jack bean urease (JBU) was crystallized by Sumner and these were the first crystals of a characterized enzyme [4–6]. Urease is a nickel dependent enzyme and catalyzes the hydrolysis of urea yielding ammonia and carbamate as shown in the following equation:

$$\begin{array}{l} NH_2CONH_2 + H_2O \leftrightarrow NH_2COOH + NH_3 \\ \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad \downarrow \\ \qquad\qquad\qquad\qquad\qquad\quad NH_2COOH + H_2O \leftrightarrow NH_3 + H_2CO_3 \end{array} \quad (1)$$

The carbamate product is unstable and spontaneously degrades to ammonia and carbonic acid [7–9]. Under ideal conditions, the ammonia is converted to ammonium, ready for plant uptake. As Urease makes urea N available to plants, it plays an important role in the utilization of nitrogenous fertilizer and is crucial to the nitrogen cycle. However, under less than ideal conditions (soil pH < 6–6.5) the ammonia can be lost to the atmosphere. Hydrolysis, the fundamental property of urea that greatly affects the management of urea as fertilizer, causes an abrupt overall pH increase, and this is the major cause of the negative side effects. The release of large amounts of ammonia into the atmosphere, as well as the plant damage induced by ammonia toxicity and the increase in soil pH, leads to significant environmental and economic problems [10]. Soil organic matter can undergo nitrification to NO_2^- and NO_3^- under aerobic conditions. NO_2^- and NO_3^- in turn may undergo denitrification to *NO* and N_2O under anaerobic conditions, which cause loss of nitrogenous fertilizer and atmosphere pollution [11]. The rapidly increasing importance of urea fertilizer in world agriculture (urea contains high nitrogen percentage, 46%) has stimulated research to find methods of reducing the problems associated with the use of this fertilizer [10]. The regulating of the rate of the enzymatic urea hydrolysis using urease inhibitors is an important goal to pursue. Thus, the study of urease inhibitors may have medical or agronomic significance, as well as providing insight into the urease catalytic

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mechanism [12]. With recent advances in the sensitivity and versatility of calorimeters, ITC has become an indispensable tool for the direct measurement of thermodynamic parameters such as Gibbs free energy (ΔG), enthalpy (ΔH), and entropy (ΔS) changes along with the dissociation constant (K_d) from a single experiment. In this work, we have attempted to find thermodynamic parameters and conformational changes of JBU due to the binding of cyanide ion.

Materials and methods

Jack bean urease (JBU; $MW = 545.34$ kDa), Tris salt and sodium cyanide obtained from Sigma Chemical Co. Tris-HCl solution (30 mM), pH = 7 was used as a buffer for JBU. The isothermal titration microcalorimetric experiments were performed with the four channel commercial microcalorimetric system, Thermal Activity Monitor 2277, Thermometric, Sweden. The titration vessel was made from stainless steel. The cyanide solution (8 mM) was injected by use of a Hamilton syringe into the calorimetric titration vessel, which contained 1.8 ml JBU (5 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 cyanide solution into the perfusion vessel was repeated 30 times, with 20 μL per injection. The calorimetric signal was measured by a digital voltmeter which was part of a computerized recording system. The heat of each injection was calculated by "Thermometric Digitam 3" software program. The heat of dilution of the cyanide solution was measured as described above except that JBU was excluded. The heats of dilution of the cyanide solutions were

Table 1 The heats of CN^- +JBU interaction at 300 K(O), 310 K (□) in 30 mM Tris buffer solution of pH = 7

[JBU]/μM	[CN^-]/μM	q/μJ (300 K)	q_{dilut}/μJ (300 K)	q/μJ (310 K)	q_{dilut}/μJ (310 K)
4.945	87.912	−144.9	−42.4	−121.2	−39.8
4.891	173.913	−262.8	−78.0	−222.3	−74.1
4.839	258.065	−360.4	−107.9	−307.7	−102.0
4.788	340.425	−442.3	−133.3	−380.8	−125.8
4.737	421.052	−512.0	−154.7	−443.9	−146.2
4.688	500.000	−571.9	−172.3	−499.0	−162.8
4.640	577.320	−623.8	−187.5	−547.4	−177.3
4.592	653.061	−669.3	−201.1	−590.3	−189.6
4.546	727.272	−709.5	−212.1	−628.5	−200.2
4.500	800.000	−745.2	−221.3	−662.8	−208.7
4.455	871.287	−777.2	−229.8	−693.7	−216.5
4.411	941.176	−806.0	−237.2	−721.7	−223.4
4.369	1009.709	−832.0	−243.7	−747.2	−229.6
4.327	1076.923	−855.6	−249.1	−770.5	−234.6
4.285	1142.857	−877.2	−254.1	−791.9	−239.4
4.245	1207.547	−896.9	−258.3	−811.6	−243.4
4.205	1271.028	−915.0	−262.1	−829.8	−247.1
4.166	1333.333	−931.7	−265.8	−846.6	−250.4
4.128	1394.495	−947.2	−269.3	−862.2	−253.5
4.090	1454.545	−961.6	−272.1	−876.8	−256.2
4.054	1513.514	−975.0	−274.6	−890.4	−258.6
4.017	1571.429	−987.5	−277.0	−903.1	−260.8
3.982	1628.319	−999.2	−279.2	−915.0	−262.8
3.947	1684.211	−1010.1	−281.1	−926.2	−264.6
3.913	1739.130	−1020.3	−282.9	−936.7	−266.3
3.880	1793.103	−1029.9	−284.6	−946.6	−267.8
3.846	1846.154	−1039.0	−286.1	−956.0	−269.1
3.813	1898.305	−1047.6	−287.5	−964.9	−270.2
3.781	1949.580	−1055.7	−288.7	−973.3	−271.2
3.750	2000.000	−1063.4	−289.7	−981.3	−272.0

Precision is ± 0.1 μJ or better

subtracted from the heat of CN^- + JBU interaction. The heats of dilution of JBU are negligible. The microcalorimeter was frequently calibrated electrically during the course of the study.

Results and discussion

We have shown previously that the heats of the macromolecules + ligands interactions, q, can be reproduced by Eq. 2 in the aqueous solvent systems [13–24].

$$q = q_{max}x'_B - \delta^{\theta}_A(x'_A L_A + x'_B L_B) - (\delta^{\theta}_B - \delta^{\theta}_A)(x'_A L_A + x'_B L_B)x'_B \quad (2)$$

The parameters δ^{θ}_A and δ^{θ}_B reflect to the net effect of CN^- on the JBU stability in the low and high CN^- concentrations respectively (Table 1). The positive values for δ^{θ}_A or δ^{θ}_B indicate that CN^- stabilized the JBU structure and vice versa. 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 enzyme 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 follows:

$$x'_B = \frac{px_B}{x_A + px_B} \quad (3)$$

x_B is the fraction of the bounded CN^-, and $x_A = 1 - x_B$ is the fraction of unbounded CN^-. We can express x_B fractions, as the total CN^- concentrations divided by the maximum concentration of the CN^- upon saturation of all JBU as follow:

$$x_B = \frac{[CN^-]_T}{[CN^-]_{max}} \quad x_A = 1 - x_B \quad (4)$$

$[CN^-]_T$ is the total concentration of CN^- and $[CN^-]_{max}$ is the maximum concentration of the CN^- upon saturation of all JBU. In general, there will be "g" sites for binding of CN^- per JBU molecule. L_A and L_B are the relative contributions of unbounded and bounded CN^- to the heats of dilution with the exclusion of JBU and can be calculated from the heats of dilution of CN^- in buffer, q_{dilut}, as follows:

$$L_A = q_{dilut} + x_B\left(\frac{\partial q_{dilut}}{\partial x_B}\right), \quad L_B = q_{dilut} - x_A\left(\frac{\partial q_{dilut}}{\partial x_B}\right) \quad (5)$$

The heats of JBU + CN^- interactions were fitted to Eq. 2 over the whole range of CN^- compositions. In the fitting procedure, p parameter varied in the course of an iterative process until the best fit between experimental and calculated data was approached (Fig. 1). δ^{θ}_A and δ^{θ}_B parameters have been also optimized to fit the data. The optimized δ^{θ}_A and δ^{θ}_B values are recovered from the coefficients of the second and third terms of Eq. 2. The binding parameters for JBU + CN^- interactions recovered from Eq. 2 were listed in Table 2. The agreement between the calculated and experimental results (Fig. 1) is excellent, and gives considerable support to the use of Eq. 2. δ^{θ}_A and δ^{θ}_B values for JBU + CN^- interaction are negative, indicating that in the low and high concentrations of the cyanide ions, the JBU structure was destabilized, resulting in a decrease in its biological activity. Destabilization by a ligand indicates that the ligand binds preferentially to the partially unfolded intermediate forms of the enzyme. Such effects are characteristic of nonspecific interactions, in that the nonspecific ligand binds weakly to many different groups at the enzyme/water interface, so that binding becomes a function of ligand concentration and available solvent-exposed enzyme surface area, which is increased through unfolding events.

For a set of identical and independent binding sites, we have before shown [25–30]:

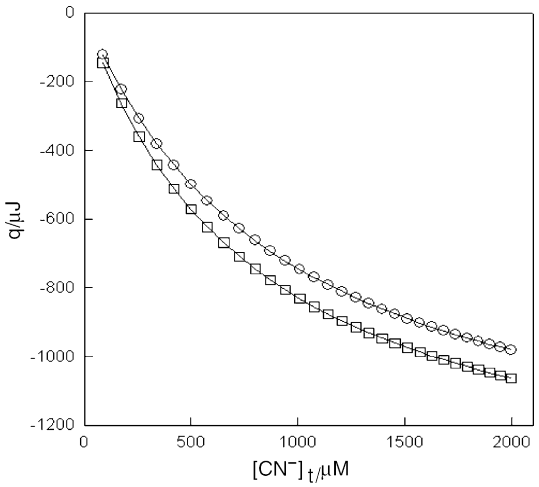

Fig. 1 Comparison between the experimental heats (*open square*) at $T = 300$ K (*open circle*) at $T = 310$ K for CN^- + JBU interactions and the calculated data (*lines*) via Eq. 2. The $[CN^-]_t$ are the total concentrations of NaCN solution in μM

Table 2 Binding parameters for CN^- + JBU interactions recovered from Eqs. 2 and 6. $p = 1$ indicates that the binding is non-cooperative

	p	δ^{θ}_A	δ^{θ}_B	ΔH/kJ mol^{-1}	K_d/μM
$T = 300$ K	1	−3.03	−3.33	−13.6	750
$T = 310$ K	1	−3.07	−4.77	−13.2	892.1

δ^{θ}_A and δ^{θ}_B values indicate that in the low and high concentrations of the cyanide ions, the JBU structure was destabilized, resulting in a decrease in its biological activity

$$\Delta H = \frac{1}{A_i}\left\{(B + K_d) - \left[(B + K_d)^2 - C\right]^{\frac{1}{2}}\right\} \quad (6)$$

where:

$$A_i = \frac{V_i}{2q_i} \quad B = g[M]_T + [L]_T \quad C = 4g[M]_T[L]_T \quad (7)$$

where V_i is the volume of the reaction solution in the calorimetric sample cell in each injection step. M is the total JBU concentration, and L is the total CN^- concentration in the calorimetric sample cell in each injection step. Equation 6 contains two unknown parameters, K_d and ΔH. A series of reasonable value for K_d is inserted into Eq. 6 and corresponding values for ΔH are calculated and the graph ΔH versus K_d is constructed. Curves of all titration steps will intersect in one point, which represents the precise value for ΔH and K_d. The plots of ΔH versus K_d, according to Eq. 6, for 30 injections are shown in Fig. 2.

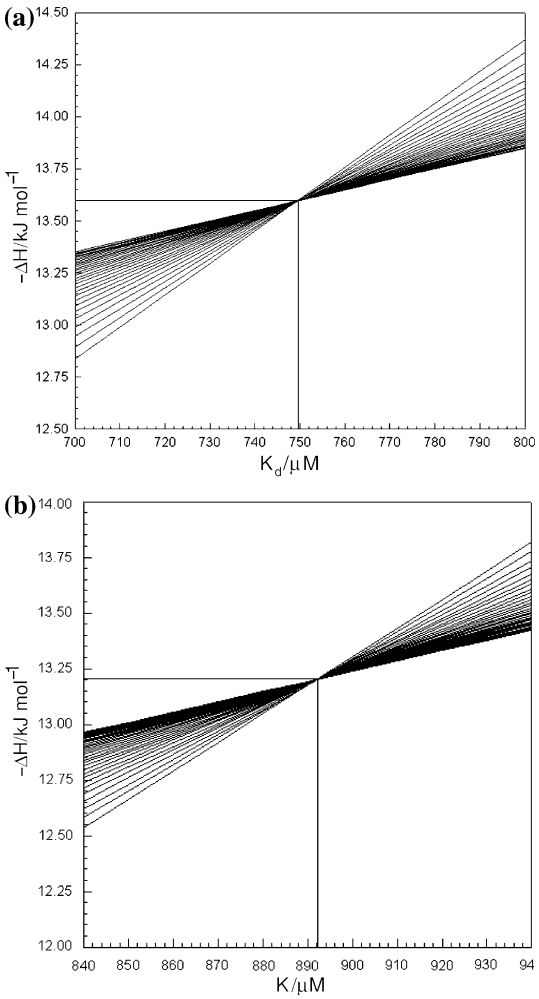

Fig. 2 **a** ΔH versus K_d for all 30 injections in the reasonable values of K_d, according to Eq. 6 at $T = 300$ K. **b** ΔH versus K_d for all 30 injections in the reasonable values of K_d, according to Eq. 6 at $T = 310$ K

The Gibbs free energies as a function of CN^- concentrations can be obtained as follow:

$$\Delta G = -RT\ln K_a \quad (8)$$

Gibbs energies, ΔG, calculated from Eq. 8 have shown graphically in Fig. 3. ΔS values were calculated using ΔG values and have shown in Fig. 4. Calorimetric titrations were carried out at 27 °C and 37 °C, and the change in heat capacity (ΔC_p) associated with the binding reaction was determined by the relationship:

$$\Delta C_p = d\Delta H/dT \quad (9)$$

ΔC_p derived from the temperature dependence of enthalpic changes for enzyme-ligand interaction is one of the most

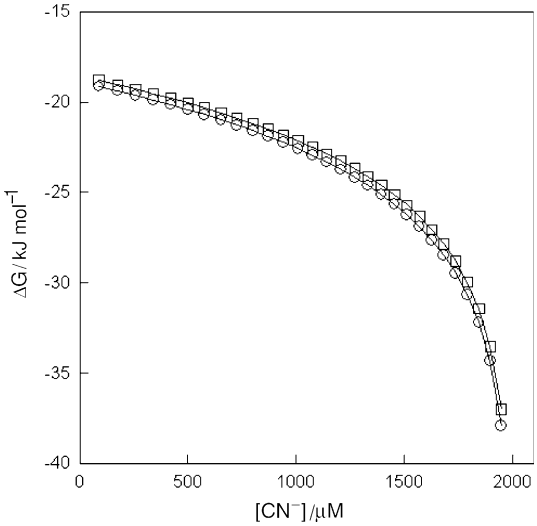

Fig. 3 Comparison between the experimental Gibbs energy values (*open square*) at $T = 300$ K (*open circle*) at $T = 310$ K for CN^- + JBU and calculated values (*lines*) from Eq. 8

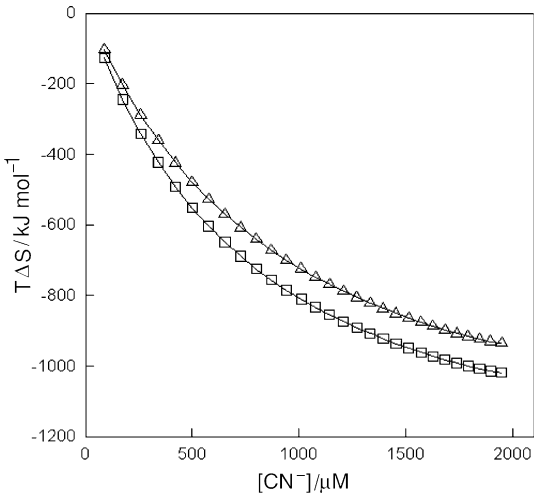

Fig. 4 Comparison between the experimental values (*open square*) at $T = 300$ K (*open circle*) at $T = 310$ K for CN^-–JBU interaction and the calculated values (*lines*)

valuable thermodynamic parameters for inferring the structural changes in the enzyme. The value of ΔC_p obtained from Eq. 9 for $CN^- + JBU$ interaction is ($+0.04$ kJ K^{-1} mol^{-1}).

Conclusions

The extended coordination model, via Eq. 2 will satisfactorily reproduce the enthalpies of JBU + CN^- interactions. Prediction of activity of JBU, structural changes of the enzyme, binding enthalpies using only one set of anion-enzyme enthalpies, makes this theory the most powerful one. JBU + CN^- interaction decreases the stability and the biological activity of JBU.

Acknowledgements Financial support from the Universities of Imam Khomeini International university, Tehran and payam noor (Abhar) are gratefully acknowledged.

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