Research Journal of Chemical Sciences _____ ISSN 2231-606X
Vol. **2(5),** 85-88, May **(2012)** *Res.J.Chem.Sci.*

Short Communication

A Calorimetric Investigation of Chromium Interaction with Jack bean Urease

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Available online at: www.isca.in
(Received 6th March 2012, revised 26th March 2012, accepted 31st March 2012)

Abstract

Urease activity is often used for characterization of microbial viability in soil. The aim of the investigation was to measure the influence of chromium (III) on urease activity. Urease activity in pure solution was so sensitive for Cr (III), which caused inhibition of urease activity significantly. The complexation between Cr^{3+} and Jack bean urease is examined using isothermal titration calorimetry (ITC). It was found that chromium ions acted as a noncooperative inhibitor of JBU, and there is a set of 12 identical and independent binding sites for Cr^{3+} ions. The association equilibrium constant is 6.79×10^{6} L^{-1}.mol, indicating the strong interaction of Cr^{3+} ion with JBU. The molar enthalpy of binding is $\Delta H = 15.10$ kJmol^{-1}.

Keywords: Isothermal titration calorimetry, jack bean urease, Cr^{3+} ion, binding parameters.

Introduction

Jack Bean Urease is found in plants, fungi and bacteria and has the historical interest of being the first enzyme to be crystallized[1]. Urea is a major nitrogenous waste product of biological actions. In general, urea is short-lived and rapidly metabolized by microbial activities[2].

Urease catalyzes the hydrolysis of urea yielding ammonium carbamate. The ammonium carbamate product is unstable and spontaneously degrades to CO_2 and two molecules of ammonia. This reaction leads to high–volatilization losses of ammonia if urea is surface applied. It can also cause severe germination and seedling damage due to ammonia and nitrite (NO_2^-) when the amount placed near the seed is too large[3-7].

Compounds that inhibit the enzymatic breakdown of nitrogenous compounds present in feces and urine can decrease ammonia production. Urease inhibitors can block the hydrolysis of urinary urea to ammonium and thus decrease ammonia production[8]. Ammonia lost to the atmosphere may be deposited on land or water causing eutrophication and acidification. Urease inhibitors by delaying ammonia formation and subsequent nitrification can reduce the nitrate content in plants and improve the nutritional quality of vegetables and fodder plants. Urease inhibitors have been very important in farming applications where slowing the conversion of urea to ammonium provides further time for the crops to take up the ammonium. The objective of this study was to assess the urease activity and conformational changes of JBU as a result of its binding to Cr^{3+} ion.

Material and Methods

Jack bean urease (JBU; MW=545.34 kDa), Tris salt and Cr^{3+} ions obtained from sigma chemical Co. The isothermal titration microcalorimetric experiments were performed with the four channel commercial microcalorimetric system. Cr^{3+} solution (4 mmol.L^{-1}) was injected by use of a Hamilton syringe into the calorimetric titration vessel, which contained 1.8 mL JBU (37 µmol.L^{-1}). Injection of Cr^{3+} solution into the perfusion vessel was repeated 27 times, with 10 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 Cr^{3+} solution was measured as described above except JBU was excluded. The microcalorimeter was frequently calibrated electrically during the course of the study.

Results and Discussion

We have shown previously that the heats of the ligand + JBU interactions in the aqueous solvent systems, can be calculated via the following equation[9-17]:

$$q = q_{max} x'_B - \delta_A^{\theta}(x'_A L_A + x'_B L_B) - (\delta_B^{\theta} - \delta_A^{\theta})(x'_A L_A + x'_B L_B)x'_B \quad (1)$$

q is the heat of Cr^{3+} + JBU interaction and q_{max} represents the heat value upon saturation of all JBU. The parameters δ_A^{θ} and δ_B^{θ} are the indexes of JBU stability in the low and high Cr^{3+} concentrations, respectively. If the ligand binds at each site

independently, the binding is non-cooperative. p >1 or p <1 indicate positive or negative cooperativity of a macromolecule for binding with a ligand, respectively; p = 1 indicates that the binding is non-cooperative. x'_B can be expressed as follows:

$$x'_B = \frac{px_B}{x_A + px_B} \qquad (2)$$

We can express x_B fractions, as the total Cr^{3+} concentrations divided by the maximum concentration of the Cr^{3+} upon saturation of all JBU as follows:

$$x_B = \frac{[Cr^{3+}]}{[Cr^{3+}]_{\max}}, \quad x_A = 1 - x_B \qquad (3)$$

$[Cr^{3+}]$ is the concentration of Cr^{3+} and $[Cr^{3+}]_{max}$ is the maximum concentration of the Cr^{3+} upon saturation of all JBU. In general, there will be "g" sites for binding of Cr^{3+} per JBU molecule and ν is defined as the average moles of bound Cr^{3+} per mole of total JBU. L_A and L_B are the relative contributions due to the fractions of unbound and bound metal ions in the heats of dilution in the absence of JBU and can be calculated from the heats of dilution of Cr^{3+} in the buffer solution, q_{dilut}, as follows:

$$L_A = q_{dilut} + x_B\left(\frac{\partial q_{dilut}}{\partial x_B}\right), \; L_B = q_{dilut} + x_A\left(\frac{\partial q_{dilut}}{\partial x_B}\right) \qquad (4)$$

The heats of Cr^{3+}+JBU interactions, q, were fitted to equation 1 across the whole Cr^{3+} compositions. In the fitting procedure, p was changed until the best agreement between the experimental and calculated data was approached (figure 1). The binding parameters for Cr^{3+}+JBU interactions recovered from Eq. 1 were listed in table-1. The agreement between the calculated and experimental results (figure 1) is striking, and gives considerable support to the use of equation 1 δ_A^o and δ_B^o values for Cr^{3+}+JBU interactions is negative, indicating that in the low and high concentrations of the metal ions the JBU structure is destabilized, resulting in an decrease in its activity. p=1 indicates that the binding is non-cooperative.

According to the recently data analysis method, a plot of $(\frac{\Delta q}{q_{\max}})M_0$ versus $(\frac{\Delta q}{q})L_0$ should be a linear plot by a slope of 1/g and the vertical-intercept of $\frac{K_d}{g}$, which g and K_d can be obtained.

$$\frac{\Delta q}{q_{\max}} M_0 = (\frac{\Delta q}{q})L_0 \frac{1}{g} - \frac{K_d}{g} \qquad (5)$$

Where g is the number of binding sites, K_d is the dissociation equilibrium constant, M_0 and L_0 are concentrations of JBU and Cr^{3+}, respectively, $\Delta q = q_{\max} - q$, q represents the heat value at a certain Cr^{3+} ion concentration and q_{max} represents the heat value upon saturation of all JBU. If q and q_{max} are calculated per mole of JBU then the molar enthalpy of binding for each binding site (ΔH) will be ΔH= q_{max}/g. Dividing the q_{max} amount of 12100 µJ (equal to 181.68 $kJmol^{-1}$) by g=12, therefore, gives ΔH= 15.10 $kJmol^{-1}$.

To compare all thermodynamic parameters in metal binding process for JBU, the change in standard Gibbs free energy (ΔG°) should be calculated according to the equation (6), which its value can use in equation (7) for calculating the change in standard entropy (ΔS°) of binding process.

$$\Delta G^\circ = -RT \ln K_a \qquad (6)$$

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ \qquad (7)$$

Where K_a is the association binding constant (the inverse of the dissociation binding constant, K_d). The K_a value are obtained 6.79×10^6 $L.mol^{-1}$ Hence:

$\Delta G^\circ = -39.23$ kJ mol^{-1} $\Delta S^\circ = 0.18$ kJ mol^{-1} K^{-1}

It means that the binding process is spontaneous resulted by entropic driven. All thermodynamic parameters for the interaction between JBU and Cr^{3+} ion have been summarized in Table-1. All thermodynamic parameters of the complex formation including ΔG°, ΔH°, ΔS°, indicate that the process is endothermic and entropy driven. This issue shows the predominant role of hydrophobic forces in interaction between Cr^{3+} and JBU. The large association equilibrium constant of the Cr^{3+}+JBU complex, indicates that chromium is strongly associated with JBU. The results show, that Cr^{3+} ions caused inhibition of urease activity significantly.

Conclusion

All thermodynamic parameters of the complex formation including ΔG°, ΔH°, ΔS°, indicate that the process is endothermic and entropy-driven. This issue shows the predominant role of hydrophobic forces in interaction between Cr^{3+} and JBU. The large association equilibrium constant of the Cr^{3+}+JBU complex, indicates that chromium is strongly associated with JBU. The results show, that Cr^{3+} ions caused inhibition of urease activity significantly.

Acknowledgements

The financial support of Islamic azad university, Takestan branch, Takestan IRAN is gratefully acknowledged.

Table-1

Binding parameters for JBU+ Cr^{3+} interactions, *p*=1 indicates that the binding is non-cooperative. The negative δ_A^θ and δ_B^θ values prove that the JBU+Cr^{3+} complexes are not stable, indicating that Cr^{3+} inhibit the JBU activity significantly. The large association equilibrium constant indicates a strong interaction of chromium with JBU

Parameters	T=300K
$K_a / Lmol^{-1}$ K_a / M^{-1}	$6.79\times10^6\pm220$
p	1±0.01
δ_A^θ	-4.43±0.13
δ_B^θ	-9.01±0.15
ΔH / kJmol^{-1}	15.10±0.09
ΔG / kJmol^{-1}	-39.23±0.07
ΔS / kJmol^{-1}K^{-1}	0.18±0.02

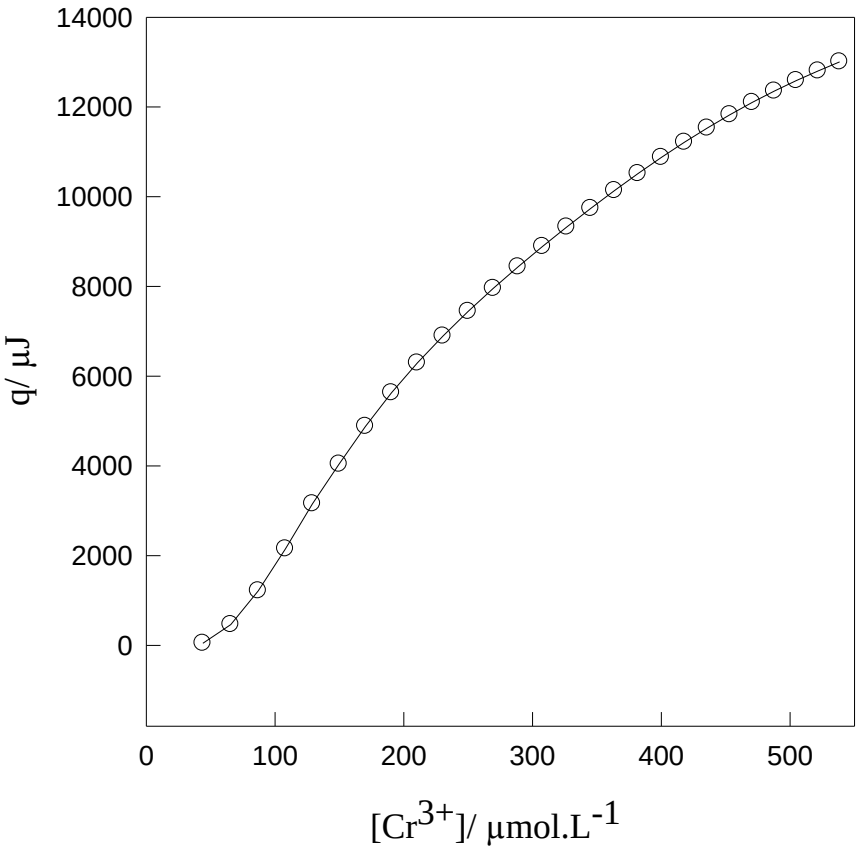

Figure-1
Comparison between the experimental heats (≺) at 300 K, for Cr^{3+} + JBU interactions and the calculated data (lines) via Eq. 1. The [Cr^{3+}] are the concentrations of [Cr $(NO_3)_3$] solution in μmol.L^{-1}

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