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Short Communication

Inhibitory effect of Cobalt (II) ion on Jack Bean Urease

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Abstract

The interaction of Jack Bean Urease with cobalt (II) ion was studied by isothermal titration calorimetry (ITC) at 300 K and 310 K in 30 mM Tris buffer, pH=7. The stability of the enzyme increases due to its binding with cobalt ions. The extended solvation model was used to reproduce the heats of Co^{2+}+JBU interaction. It was found that there is a set of 12 equivalent and non-interacting binding sites for Co^{2+} ions. The association equilibrium constant and the molar enthalpy of binding are 4260.76M^{-1}, -16.5 kJmol^{-1} at 300 K and 3438M^{-1}, -16 kJmol^{-1} at 310 K, respectively.

Keywords: Isothermal titration calorimetry, jack bean urease, cobalt ion.

Introduction

Urease is found in bacteria, fungi and plants, and catalyzes the hydrolysis of urea yielding ammonia and carbamate as shown in equation 1.

$$NH_2CONH_2 + H_2O \leftrightarrow NH_2COOH + NH_3 \quad (1)$$
$$\downarrow$$
$$NH_2COOH + H_2O \leftrightarrow NH_3 + H_2CO_3$$

The carbamate product is unstable and spontaneously degrades to ammonia and carbonic acid[1, 2]. There are some reports on the binding properties and structural changes of JBU due to its interaction with metal ions. Jack bean urease has many SH groups at its surface and this enzyme can be immobilized directly to the metal surface by adsorption[3, 4]. The interaction of JBU with some of divalent metal ions (Cu^{2+} and Cd^{2+}) in aqueous solution was studied using different techniques. Cd^{2+} addition did not affect jack bean urease growth in plant[5-7]. The heavy metal ions were found to inhibit urease in the following decreasing order: Hg^{2+} > Cu^{2+} > Zn^{2+} > Cd^{2+} > Ni^{2+} > Pb^{2+} > Co^{2+} > Fe^{3+} > As^{3+} [8]. In this paper, the interaction between Co^{2+} and JBU has been investigated in neutral Tris buffer to clarify thermodynamics of metal binding properties. The binding parameters recovered from the extended solvation model were correlated to the effect of metals on the stability of protein [5-9].

Material and Methods

Jack bean urease (JBU; MW=545.34 kDa) and cobalt nitrate were obtained from Merck. The buffer solution used in the experiments was 30 mM tris, pH=7.0, which was obtained from Merck. Experiments were carried out in 300 K and 310K. 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. Cobalt solution (10 mM) was injected by use of a Hamilton syringe into the calorimetric titration vessel, which contained 1.8 mL JBU (4 µM). Thin (0.15 mm inner diameter) stainless steel hypodermic needles, permanently fixed to the syringe, reached directly into the calorimetric vessel. Injection of cobalt solution into the perfusion vessel was repeated 30 times, with 20 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 Co^{2+} solution was measured as described above except JBU was excluded.

Results and Discussion

We have shown previously [4-10] that the enthalpies of the ligand+ JBU interactions in the aqueous solvent systems, can be calculated via the following equation:

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

q is the heat of Co^{2+}+ JBU interactions and $q_{\max}$ represents the heat value upon saturation of all JBU. The parameters δ^{θ}_A and δ^{θ}_B are the indexes of JBU stability in the low and high Co^{2+} concentrations respectively. If the ligand binds at each site independently, the binding is non-cooperative. p<1 or p>1 indicate negative or positive cooperativity of macromolecule for binding with ligand respectively; p = 1 indicates that the binding is non-cooperative. x'_B can be expressed as follows:

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

x'_B is the fraction of bound Co^{2+} to the binding sites on JBU, and $x'_A = 1 - x'_B$ is the fraction of unbound Co^{2+}. The model is a simple mass action treatment, with Co^{2+} molecules replacing

water molecules, at the binding sites. We can express x_B fractions, as the total Co^{2+} concentrations divided by the maximum concentration of the Co^{2+} upon saturation of all JBU as follows:

$$x_B = \frac{[Co^{2+}]}{[Co^{2+}]_{\max}}, \qquad x_A = 1 - x_B \qquad (4)$$

$[Co^{2+}]$ is the concentration of metal ions and $[Co^{2+}]_{max}$ is the maximum concentration of the Co^{2+} upon saturation of all JBU. In general, there will be "g" sites for binding of Co^{2+} per JBU molecule. L_A and L_B are the relative contributions due to the fractions of unbounded and bounded metal ions in the hearts of dilution in the absence of JBU and can be calculated from the heats of dilution of Co^{2+} in buffer, q_{dilut}, as follows:

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

The heats of Co^{2+}+JBU interactions, q, were fitted to equation 2 across the whole Co^{2+}concenterations. In the fitting procedure the only adjustable parameter (p) was changed until the best agreement between the experimental and calculated data was approached. The optimized δ_A^θ and δ_B^θ values are recovered from the coefficients of the second and third terms of equation 2. The agreement between the calculated and experimental results (figure 1) is striking, and gives considerable support to the use of equation 2. δ_A^θ value for Co^{2+}+JBU interactions is negetive, indicating that in the low concentration of the metal ions the JBU structure is destabilized. Destabilization by a ligand indicates that the ligand binds preferentially to the unfolded (denatured) enzyme or to a partially unfolded intermediate form of the enzyme. Such effects are characteristic of nonspecific interactions, in that the nonspecific ligand binds weakly to partially unfolded species of JBU. The negative δ_A^θ values indicate that the nonspecific interactions are dominant in the low Co^{2+} ion concentration domain. The positive values for δ_B^θ show that the JBU structure is stabilized by the addition of Co^{2+}, indicate that JBU involves specific interactions with Co^{2+} ions in the high Co^{2+} ion concentration region. p values are one (table-1), indicating that there are a set of 12 identical and non-interacting binding sites for JBU + Co^{2+} interaction.

According to the recently data analysis method, using equation 6, 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.

$$\frac{\Delta q}{q_{\max}} M_0 = (\frac{\Delta q}{q})L_0 \frac{1}{g} - \frac{K_d}{g} \qquad (6)$$

Where g is the number of binding sites, K_d is the dissociation equilibrium constant, M_0 and L_0 are total concentrations of biomacromolecule and ligand, respectively, $\Delta q = q_{\max} - q$, q represents the heat value at a certain L_0 and q_{max} represents the heat value upon saturation of all biomacromolecule. If q and q_{max} are calculated per mole of biomacromolecule then the molar enthalpy of binding for each binding site (ΔH) will be ΔH= q_{max}/g. The linearly of the plot has been examined by different estimated values for q_{max} to find the best value for the correlation coefficient (near to one). The best linear plot with the correlation coefficient value of 0.999 was obtained using amount of -1425.6μJ (equal to -198 kJ mol^{-1}) for q_{max} at 300 K and -1382.4μJ (equal to -192 $kJmol^{-1}$) for q_{max} at 310 K. The amounts of g and K_d, obtained from the slope and vertical-intercept plot, are 12 and 234.78 μM, M at 300 and 310 K, respectively. Dividing the q_{max} amounts of -198 $kJmol^{-1}$, –192 $kJmol^{-1}$ by g=12, therefore, gives ΔH= -16.5 $kJmol^{-1}$at 300 K and ΔH= -16 $kJmol^{-1}$ at 310 K. Binding parameters have been listed in table-1.

Conclusion

The agreement between the calculated and experimental results (figure-1) is striking, and gives considerable support to the use of equation 2. δ_A^θ value for Co^{2+}+JBU interactions is negetive, indicating that in the low concentration of the metal ions the JBU structure is destabilized. The positive values for δ_B^θ show that the JBU structure is stabilized by the addition of Co^{2+}.

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Table-1
Binding parameters for JBU+ Co^{+2} interaction in 10 mM [Co $(No_3)_2$] solution, *p*=1 suggests that Co^{2+} ion binds to JBU non-cooperatively

Parameters	JBU +Co^{2+} (T=300 K)	JBU +Co^{2+} (T=310 K)
δ_A^θ	-0.051±0.010	-0.108±0.017
δ_B^θ	1.674±0.014	1.431±0.012
K_a / M^{-1}	4259.30±50	3438.32±42
g	12	12
p	1±0.04	1±0.04
$\Delta H / kJmol^{-1}$	-16.5	-16
$\Delta G / kJmol^{-1}$	-20.08	-20.98
$\Delta S / kJmol^{-1}K^{-1}$	0.02	0.016

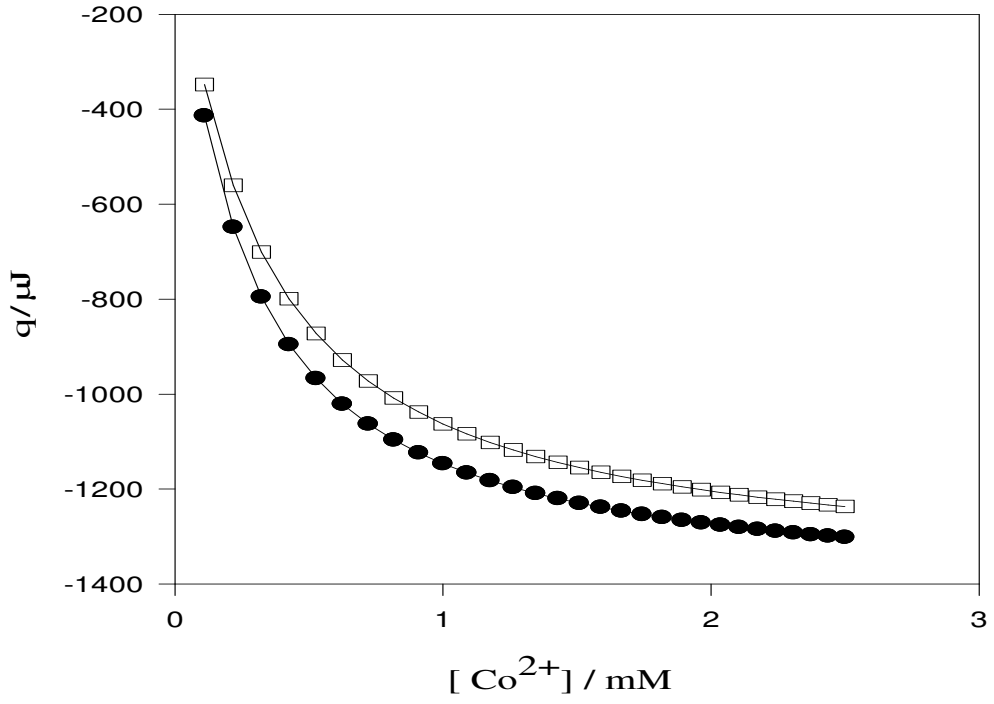

Figure-1
The heats of Co^{2+} ions binding with JBU at 300K(•) and 310K(ϒ) for 30 automatic cumulative injections, each of 20 µL, 10 mM of the cations solutions, into sample cell containing 1.8 ml of 4µM JBU solution vs. total concentration of Co^{2+} ions