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Short Communication

A Calorimetric Study on the Interaction between Vitamin-B_6 and lysozyme

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Abstract

The binding reaction between vitamin B_6 (B_6, pyridoxine) and lysozyme (Lys) was investigated for the first time by isothermal titration calorimetry (ITC), at pH 7 at 27°C in tris buffer (25mmol.L^{-1}). The enthalpies of LYS+B_6 interaction are reported and analysed in term of the extended solvation model. The thermodynamic parameters, enthalpy changes (ΔH) and entropy changes (ΔS) were calculated. These data suggested that hydrophobic interaction was the predominant intermolecular forces stabilizing the complex, which was in good agreement with the results of molecular modeling study. It was found that LYS has one non-cooperative binding site for Vitamin B_6.

Keywords: Vitamin B_6, lysozyme, isothermal titration calorimetry.

Introduction

Lysozyme is abundant in a number of secretions, such as tears, saliva, human milk and mucus. It is also present in cytoplasmic granules of the polymorphonuclear neutrophils (PMN). Large amounts of lysozyme can be found in egg white. In humans, the lysozyme enzyme is encoded by the LYS gene. LYS is a small globular protein, consisting of 129 amino acid residues with four disulfide bonds. The importance of Lys relies on its extensive use as a model system to understand the underlying principles of protein structure, function, dynamics and folding through theoretical and experimental studies[1,2]. High natural abundance is also one of the reasons for choosing LYS as a model protein for studying protein-ligand interaction. Another important aspect of LYS is its ability to carry drug or biological activity substances, and the effectiveness of them depends on their binding ability[3]. Therefore, studies on the interaction between LYS and drugs or biological activity substances are of importance in view of realizing disposition, transportation and metabolism of drugs or biological activity substances as well as efficacy process. Lys contains six tryptophan (Trp) and three tyrosine (Tyr) residues. Three of Trp residues are located at the substrate binding sites, two in the hydrophobic matrix box, while one is separated from the others[4]. Vitamin B_6 (pyridoxine) is one of the compounds that can be called vitamin B_6, along with pyridoxal and pyridoxamine. It differs from pyridoxamine by the substituent at the '4' position. Pyridoxine assists in the balancing of sodium and potassium as well as promoting red blood cell production. It is linked to cardiovascular health by decreasing the formation of homocysteine. Pyridoxine may help balance hormonal changes in women and aid the immune system.Lack of pyridoxine may cause anemia, nerve damage, seizures, skin problems, and sores in the mouth. A very good source of pyridoxine is dragon fruit from South East Asia.

The reports show that Vitamin B_6 improved thermal stability and biological activity of lysozyme. Vitamin B_6 has hydrophobic cavities that prevent direct interactions on the hydrophobic surfaces of proteins and in this way suppress protein aggregation. Hydrophobic interactions are the most important non-covalent forces that are responsible for different phenomena such as structure stabilization of proteins binding of enzymes to substrates and folding of proteins. This kind of interaction appears when non-polar compounds are put into water, and it is an entropy-driven process. we tried to elucidate the effect of Vitamin B_6 on lysozyme stability at 27 °C applying the extended salvation model for the data analysis.

Material and Methods

Chicken egg white LYS (molecular weight (MW) = 14.6 kDa) was purchased from Sigma,Vitamin B_6 (pyridoxine, MW=169.18 gr/mol) was obtained from Merck.and solutions were made in (25 mM, pH 7) tris buffer using doubledistilled water. The isothermal titration calorimetric experiments were carried out on a VP-ITC ultra sensitive titration calorimeter. The microcalorimeter consists of a reference cell and a sample cell of 1.8mL in volume, with both cells insulated by an adiabatic shield. All solutions were thoroughly degassed before use by stirring under vacuum. The sample cell was loaded with lysozyme solution (1 mM) and the reference cell contained buffer solution. The solution in the cell was stirred at 307 rpm by the syringe filled with Vitamin B_6 solutions (15 mmol.L-1) to ensure rapid mixing. The titration of MT with Vitamin B_6 involved 25 consecutive injections of the ligand and each

injection included 50 µL Vitamin B_6. The calorimetric signal was gauged by a digital voltmeter that was part of a computerized recording system. The heat of injection was calculated by the 'Thermometric Digitam 3' software program. The heats of dilution of Vitamin B_6 were evaluated except MT was excluded. The heats of dilution of MT are negligible. The measurements were performed at constant temperatures of 27 °C and the temperature was controlled using a poly-science water bath.

Results and Discussion

As we have shown before the heats of the macromolecules+ligands interactions in the aqueous solvent systems obtained by the following equation[5]:

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

q is the heats of B_6+LYS interaction at certain ligand concentrations and $q_{\max}$ represents the heat value upon saturation of all LYS. The good agreement between the experimental and calculated heats support the extended solvation model (figure-1).

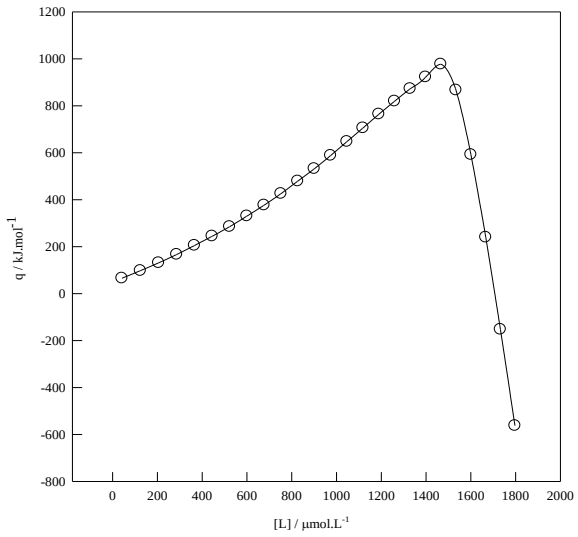

Figure-1
Comparison between the experimental heats, q, for the interaction between Lysozyme and Vitamin B_6 at 27 °C and calculated data (line) and calculated data (○)via Eq. 1.

The parameters δ^{θ}_A and δ^{θ}_B exhibit the LYS stability in the low and high B_6 concentrations respectively. The positive values of δ^{θ}_A and δ^{θ}_B show that Lsozyme is substantially stabilized by vitamin B_6 at 27 °C. x'_B, x'_A can be expressed as follows:

$$x'_B = \frac{px_B}{x_A + px_B} \qquad x'_A = 1 - x'_B \quad (2)$$

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. L_A and L_B can be calculated from heats of dilution of Vitamin B_6 in water, q_{dilut}, as follows:

$$L_B = q_{dilut} - x_A \left(\frac{\partial q_{dilut}}{\partial x_B}\right)$$

$$L_A = q_{dilut} + x_B \left(\frac{\partial q dilut}{\partial x_B}\right) \quad (3)$$

Consider a solution containing ligand L, and a biomacromolecule (M) that contains "g" sites capable of binding the ligand. If the multiple binding sites on a macromolecule are identical and independent, the binding parameters can be reproduced by the following equation[6,7]:

$$\frac{\Delta q}{q_{\max}} M_0 = \left(\frac{\Delta q}{q}\right) L_0 \frac{1}{q} - \frac{K_d}{g} \quad (4)$$

Where $\Delta q = q_{\max} - q$ and q represents the heat value at a certain ligand and biomolecule concentration. q_{max} represents the heat value upon saturation of all biomacromolecule. K_d is the dissociation equilibrium constant for the equilibrium:

$$M + L \Leftrightarrow ML \quad K_d = \frac{[M][L]}{[ML]} \quad (5)$$

Table-1
Binding parameters for Vitamin B_6+LYS interaction recovered from Eqs. 1, 4, 5 and 6. *p*=1 indicates that the binding is non-cooperative in one binding site. The positive values of δ^{θ}_A and δ^{θ}_B show that Vitamin B_6 stabilizes the LYS structure. The binding process for LYS is entropy-driven.

p	1±0.02
g	1.06±0.03
K_a / M^{-1}	201180.4±425
ΔH / kJ.mol^{-1}	1.16±0.05
ΔG / kJ.mol^{-1}	-30.45±0.13
ΔS / kJ.mol^{-1}.K^{-1}	0.10±0.01
δ^{θ}_A	0.03±0.002
δ^{θ}_B	0.09±0.003

If q and q_{max} are calculated per mole of biomacromolecule then the molar enthalpy of binding for each binding site (ΔH) will be $\Delta H = \frac{q_{\max}}{g}$. The changes in the standard Gibbs free energy, ΔG, and in standard entropy of binding, ΔS, could be calculated by using association equilibrium constant, $K_a = \frac{1}{K_d}$, and ΔH value in equations 6 and 7, respectively.

$$\Delta G = -RT \ln K_a \quad (6)$$

$$\Delta S = \frac{\Delta H - \Delta G}{T} \quad (7)$$

All calculated thermodynamic parameters are summarised in

Conclusion

p=1 indicates that the binding is non-cooperative in one binding sites. The positive values of δ^{θ}_{A} and δ^{θ}_{B} show that Vitamin B_6 stabilizes the LYS structure. The binding process is spontaneous which is only entropy driven, indicating that hydrophobic interaction is dominant in the binding.

Acknowledgements

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