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The Effect of Colloidal Silica Nanoparticles on the Activity of α-Amylase

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ABSTRACT
The effect of silica nanoparticles on the activity of α-amylase is determinations using isothermal titration calorimetry. It was found that the immobilized enzyme activity increased as evidenced by the stability parameters recovered from the extended solvation theory. The stability indexes of the immobilized α-amylase was less than of the free enzyme, thereby the activity of the enzyme was increased as a result of its interaction with silica nanoparticles.
The present report shows that silica nanoparticles are activator of α-amylase, as the complexes of silica+α-amylase are less stable than the free enzyme.

Keywords: α-amylase, silica nanoparticles, extended solvation model, immobilized, activator

1. INTRODUCTION
Silica and laponite nanoparticles are used as effectors on α-amylase activity. The results show that, if the nanoparticles and enzyme are added simultaneously, laponite enhances the enzyme performance toward starch soil removal, whereas silica imposes a small effect on the enzymatic activity towards the same soil substrates. However, when nanoparticles are added first, the enzyme activity is not affected much by laponite but is hindered significantly by silica nanoparticles. Sequential addition of the enzyme followed by silica nanoparticles improves soil removal. Electron microscopic analyses, measurements of the enzyme activity in suspensions of nanoparticles, and particle size characterization suggest that dense coverage of soil surface by the silica nanoparticles be likely a mechanism for the experimentally observed hindrance of soil removal when silica nanoparticles are added before enzyme[1-3].

The amylase activity was reduced or, in most cases, was abolished in media of pH higher than 7.0. The amylase activity requires neutral or slightly acid pH values. Since pHs higher than 8.0 caused the precipitation of Mg^{2+}, the loss of amylase activity could be associated also with the absence of this cation in the solution[5-6].

In the present work, the effect of silica nanoparticles on the activity of α-amylase is determinations using isothermal titration calorimetry. It was found that the immobilized enzyme activity increased as evidenced by the binding parameters recovered from the extended solvation model.

2. EXPERIMENTAL
α-amylase concentration was determined from absorbance measurements at 277 nm in 1 cm quartz cuvette. All other materials and reagents were of analytical grade, and solutions were made in 50 mM buffer phosphate (pH=7) using double-distilled water.

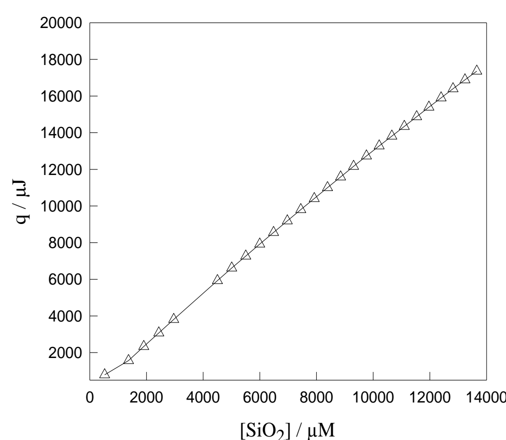

Fig-1: Comparison between the experimental heats (in μJ), q, (△), for α-amylase + silica interactions and calculated data (lines) via Eq. 1. $[SiO_2]$ are concentrations of colloidal silica nanoparticles solutions in μM at pH=7.

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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 α-amylase solution (15.90 μM) and the reference cell contained buffer solution. The solution in the cell was stirred at 307 rpm. Injections were started after baseline stability had been achieved. The titration of α-amylase with silica nanoparticles colloidal solution involved 28 consecutive injections of the ligand solution, with 10 μL per injection. To correct the thermal effects due to silica nanoparticles dilution, control experiments were done in which identical silica nano particles were injected into the buffer solution with the exception of α-amylase. In the ITC experiments, the heat changes associated with processes occurring at a constant temperature are measured. The measurements were performed at a constant temperature of 27.0±0.02 °C and the temperature was controlled using a Poly-Science water bath. The obtained heats were shown graphically in Figure-1.

3. RESULTS AND DISCUSSION

We have shown previously that the heats of the macromolecules + ligands interactions in the aqueous solvent systems can be reproduced by Eq. 1[7-10].

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

q is the heat of α-amylase+nanosilica interaction at certain ligand concentrations and $q_{\max}$ represents the heat value upon saturation of all α-amylase. x'_B, can be expressed as follows:

$$x'_B = \frac{p x_B}{x_A + p x_B} \quad (2)$$

x'_B is a fraction of bound colloidal silica nanoparticles with α-amylase molecule and $x'_A = 1 - x'_B$ is the fraction of unbound silica. Where x_B can be defined as follows:

$$x_B = \frac{[silica]}{[silica]_{\max}}$$

[*silica*] is the concentration of colloidal silica nanoparticle after every injection and $[silica]_{\max}$ is the maximum concentration of the nanoparticle upon saturation of all α-amylase. The heats of α-amylase+silica interactions, q, were fitted to Eq. 1 over the whole silica nanoparticle compositions. In the fitting procedure, p was changed until the best agreement between the experimental and calculated data was approached. If the binding of ligand at one site increases the affinity for ligand at another site, the macromolecule exhibits positive cooperativity ($p>1$). Conversely, if the binding of ligand at one site lowers the affinity for ligand at another site, exhibits negative cooperativity ($p<1$). If the ligand binds at each site independently, the binding is non-cooperative ($p=1$). L_A and L_B are the relative unbound and bound ligand contributions to the heats of dilution in the absence of α-amylase. L_A and L_B can be calculated from heats of dilution of silica nanoparticles in water (q_{dilut}) as follows:

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

The optimized δ_A^θ and δ_B^θ values are recovered from the coefficients of the second and third terms of Eq. 1. The small relative standard coefficient errors and the high r^2 values (0.99999) support the method. The binding parameters for α-amylase+silica interactions recovered from Eq. 1 were listed in Table 1.

The negative values of δ_A^θ and δ_B^θ show that weak interaction of α-amylase+nanosilica complex is more immobilized than the free enzyme. For silica nanoparticles having higher pore diameter, α-amylase seems to be immobilized inside the pores and the observed high activity of immobilized α-amylase was proved by the negative δ_A^θ and δ_B^θ values. In other words, δ_A^θ and δ_B^θ values show that the immobilized enzyme activity is much more than that of the free enzyme. Immobilization efficiency of α-amylase revealed that the enzyme activity was increased, as evidenced by the negative δ_A^θ and δ_B^θ values.

For a set of identical and independent binding sites (g), it is possible to use Eq. (5) for calculation of K_d and "g" in a very simple way as follows:

$$\frac{\Delta q}{q_{\max}} M_0 = \left(\frac{\Delta q}{q}\right) L_0 \; \frac{1}{g} - \frac{K_d}{g} \quad (5)$$

Where $\Delta q = q_{\max} - q$. q represents the heat value at a certain ligand and biomolecule concentration. M_0 and L_0 are the concentrations of α-amylase and silica nanoparticles, respectively. q_{max} represents the heat value upon saturation of all α-amylase molecule. K_d is the dissociation equilibrium constant for α-amylase+silica complex
If q and q_{max} are calculated per mole of biomacromolecule then the molar enthalpy of binding for each binding site (ΔH) will be:

$$\Delta H = \frac{q_{\max}}{g}$$

A non-linear least squares computer program has been developed to fit data in Eq. 5. The best correlation coefficient $(R^2 \approx 1)$ good support for the use of Eq. 5.

The standard Gibbs free energy, $\Delta G°$, can be calculated from association constant $(K_a = \frac{1}{K_d})$ as follows:

$$\Delta G^\circ = -RTLnK_a \quad (6)$$

Where K_a is the association equilibrium constant of α-amylase+silica complex. All thermodynamic parameters of ligand binding to α-amylase are summarized in Table-1.

Table-1: Binding parameters for α-amylase+silica interaction recovered from Eq. 1at pH=7. p=1 indicates that the binding is non-cooperative in around 136 binding sites. The negative value of δ_A^θ and δ_B^θ show that silica nanoparticles immobilized the α-amylase structure in the low and high concentration domains, resulting in higher activity of the enzyme.

p	**1.00±0.01**
g	136.56±0.2.33
K_a / M^{-1}	1469.08±6.18
$\Delta \mathrm{H} / \mathrm{kJmol}^{-1}$	0.92±0.06
$\Delta G / \mathrm{kJmol}^{-1}$	-18.19±0.03
$\Delta S / \mathrm{kJmol}^{-1}\mathrm{K}^{-1}$	0.06±0.01
δ_A^θ	-7.89±0.04
δ_B^θ	-7.93±0.0

4. CONCLUSION

The thermodynamic parameters for the interaction of nanosilica particles with α-amylase (Table-1) indicate that the α-amylase+silica complex can spontaneously. p=1 indicates that the binding is non-cooperative in around 136 binding sites. The negative value of δ_A^θ and δ_B^θ show that silica nanoparticles imobilized the α-amylase structure in the low and high concentration domains. The obtained results indicate that there are 136 independents and identical binding sites for α-amylase+silica interactions and the positive value of the molar enthalpy (0.9 kJ mol^{-1} at 300 K) and entropy suggest that the binding process is entropy-driven (Table-1).

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