

Investigation on the effects of nanoAg on the enzyme lysozyme at the molecular level

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1. Supporting Information Available

1.1 Size Distribution of Lysozyme

Malvern Zetasizer Nano-ZS was used to measure the size distribution of lysozyme in ultrapure water. The results were shown in Fig.9. Both the size distribution results of lysozyme by volume and number proved the size of lysozyme was around 1 nm which was much smaller than nanoAg.

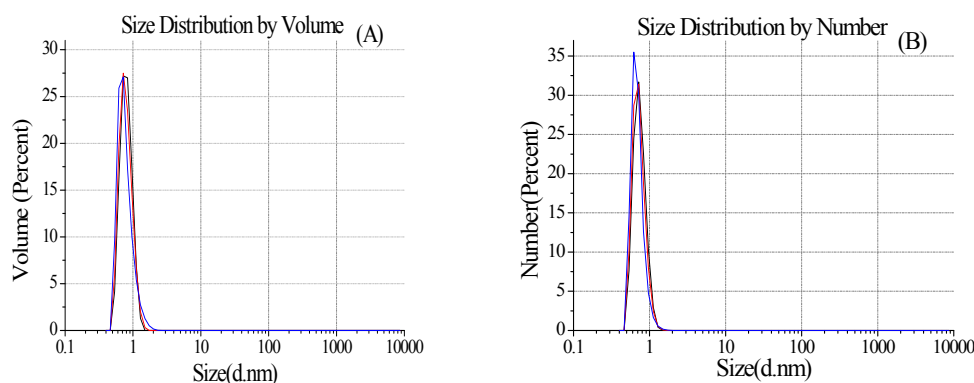


Fig.9 The size distribution by volume and number of lysozyme

1.2 Isothermal Titration Calorimetry Measurement

Isothermal titration calorimetry (ITC) is a direct and powerful method to study the complete thermodynamic parameters, including the binding affinity constant (K_a), binding stoichiometry (n), enthalpy changes (ΔH), entropy changes (ΔS), and Gibbs energy changes (ΔG)¹. The Gibbs free energy (ΔG) was calculated using the equation: $\Delta G = \Delta H - T\Delta S = -RT \ln K$, where T is the thermodynamic temperature and R is the gas constant. ITC results recorded during titration of lysozyme solution with nanoAg solution were shown in Fig.10 after

correction for dilution effects. The upper part of Fig.10 showed that a plot heat flow as time went by, and the lower part showed the results of this system analyzed. The derived thermodynamic parameters that were analyzed with the single set of binding site model were listed in Table 3. The negative value of ΔH suggested that the interaction between nanoAg and lysozyme was an exothermic reaction. The negative values of ΔH and ΔS revealed that van der Waals and hydrogen bonding interaction played the major roles in the binding reaction^{2, 3}. Additionally, the binding affinity constant K (10^7) suggested that the binding of nanoAg and lysozyme was a strong ligand-protein interactions with K values ranging from 10^7 to 10^8 .⁴

Table 3: Thermodynamic parameters for interaction of nanoAg and lysozyme

Thermodynamic parameters	nanoAg-lysozyme
K (mol^{-1})	$6.06(\pm 0.662) \times 10^7$
ΔH (cal mol^{-1})	$-165.8(\pm 1.406) \times 10^4$
ΔS ($\text{cal mol}^{-1} \text{K}^{-1}$)	-5.52×10^3
ΔG (cal mol^{-1})	$-1.304(\pm 1.406) \times 10^4$

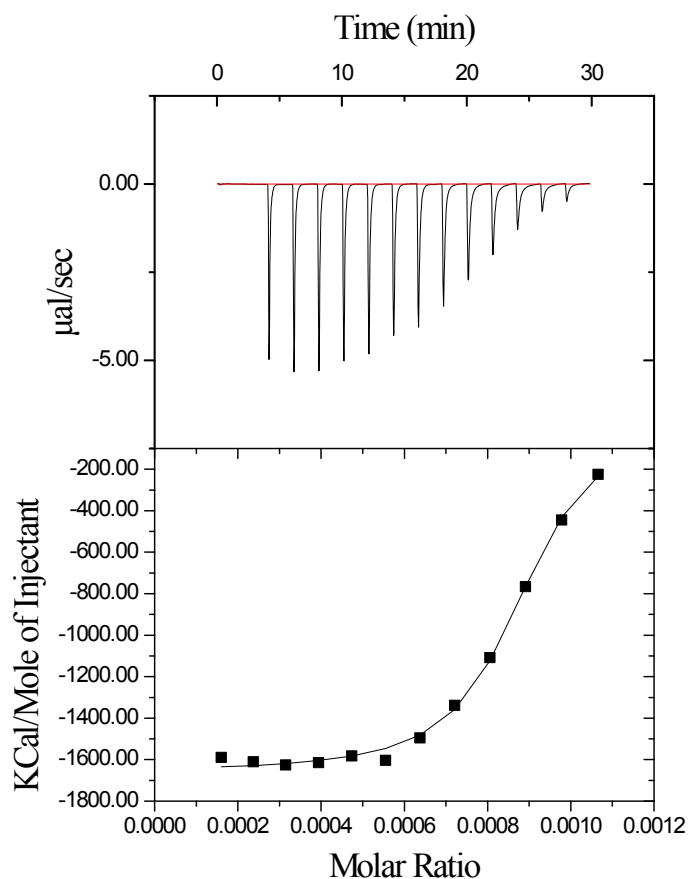


Fig.10 Isothermal titration calorimetry of nanoAg-lysozyme. $c(\text{lysozyme})=1.0 \times 10^{-5} \text{ mol L}^{-1}$, $c(\text{nanoAg})=1.0 \times 10^{-3} \text{ mol L}^{-1}$; pH=5.4, T=298 K.

References

1. A. K. Dutta, J. r. R02sgen and K. Rajarathnam, *Using Isothermal Titration Calorimetry to Determine Thermodynamic Parameters of Protein–Glycosaminoglycan Interactions*, Springer New York, 2015.
2. B. Yang, H. Fang, J. Li, D. Chen and R. Liu, *Journal of Photochemistry & Photobiology B Biology*, 2013, **128**, 35–42.
3. Q. Wu, Z. Hao, S. Tao, B. Zhang and R. Liu, *Spectrochimica Acta Part A Molecular & Biomolecular Spectroscopy*, 2015, **151**, 124-130.
4. D. M. Charbonneau and T. R. Heidar-Ali, *Journal of Physical Chemistry B*, 2010, **114**, 1148-1155.

