Adsorption Reaction Constants between Nanosilica/Nanodiamond and Lysozyme Molecule at pH = 11.0

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Adsorption reaction constants of chicken egg lysozyme of 0–1000 nM prepared with potassium phosphate buffer solution (PPBS) of 7.0 mM and at pH = 11.0 on the surfaces of nanosilica and nanodiamond of diameter 100 nm by fluorescence method have been determined as 4.0×10^7 and 1.5×10^8 nM^{-1}, respectively. The Langmuir isotherm can be well followed under the assumption of monolayer adsorption on both surfaces. The available surface areas per individual lysozyme molecule adsorbed on nanosilica and nanodiamond within the linear regions of Langmuir isotherms can be approximated with 2 and 10 nm^2, respectively.

Immobilization of proteins on solid surfaces constitutes a research area of considerable importance in emerging technologies employing biocatalytic and biorecognition events, e.g., biosensors for pathogen detection, investigation of conformational reaction dynamics on membrane, the following chain reactions, microarrays for proteomic analysis, etc. Ability to quantitatively immobilize functionally stable proteins is paramount importance in achieving high sensitivity and sustained operating efficiency in these applications.

Lysozyme is one of the proteins, which has been mostly investigated and reported. The chicken egg white lysozyme having 129 amino acids is one of the lysozymes and contains 6 tryptophanes, which are useful for the spectroscopic detection, and was chosen for this experiment. Traditionally, UV absorbance[1] has been applied, whatever can resolve the lysozyme concentration up to ca. 50–100 μM via Soret band at 409 nm. The adsorption capabilities as well as adsorption mechanisms of proteins, e.g., lysozyme[2–7] on the surfaces of nanosilica,[2–6] nanodiamond,[4–6,8] and other materials, can not be differentiated by this method under such concentration.

Because of the unimaginable adsorption capabilities of nanosilica and nanodiamond, nearly all kinds of proteins in a solution can be completely adsorbed. In order to determine the rest concentration after adsorption as well as the extremely diluted protein concentration lower than 1 μM, to differentiate the adsorption capability and to investigate the respective adsorption mechanism on the surface of the nanoparticle, fluorescence measurement with Xe-lamp (ORC, XM 2500H/VC, L. P. Associates, U. S. A.) as light source at 285 nm and PMA-11 from Hamamatsu, Japan for detection is found very powerful. Nanosilica (VP OX 10, degussa, Germany) and nanodiamond (KDM, Kay Diamond Products, U. S. A.) of diameter 100 nm as substrates and lysozyme (Sigma Chemicals, U. S. A.) as adsorbate have been chosen for this experiment. Nanosilica was used directly for the experiment; nanodiamond was carboxylated[1,6,9] (No. I-21) and contained carboxyl group under 10% on the surface. The sensitivity to determine the concentration of lysozyme can be followed down to 10 nM, whichever has been increased with over 5000–10000 times. The surface adsorption capabilities–coverages by lysozyme on the surfaces and adsorption constants of nanosilica and nanodiamond can be parallely obtained. pH = 11.0 (pH meter Beckman, Φ390, U. S. A.) has been chosen, because lysozyme is approximately neural around this value, and the interactions between surface and nanoparticle can be simplified.

Reaction between a lysozyme (Adsorbate) molecule and a nanoparticle or agglomerate (Substrate) can be treated as:

$$A + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} AS. \tag{1}$$

The equilibrium reaction constant is equal to the quotient of the forward and backward reaction rate constants k_1 and k_2,

$$K_{eq} = K_{ad} = \frac{k_1}{k_2} = \frac{k_{adsorption}}{k_{escape}} = \frac{[\omega]}{[A-\omega][S-\omega]} \tag{2}$$

where A, S are the start molar concentrations of lysozyme and nanoparticle; ω is that of AS. This classical description of a chemical reaction can be rewritten as "Langmuir isotherm" equation,

$$K_{ad} = \frac{\theta}{X(1-\theta)}\,[nM]^{-1} \tag{3}$$

where θ represents the coverage of the nanoparticle, X concentration of lysozyme or the free lysozyme left in PPBS. X and θ correspond to $S - \omega$ and ω/A, respectively. At the situation of $\theta = 1$, X is the saturated concentration. The coverage can then be expressed as

$$\theta = \frac{K_{ad}X}{1 + K_{ad}X}.$$

Simulation of the coverage of our experimental data according to such Langmuir isotherm equation can be done with LangmuirEXT1: $y = abx/(1 + bx)$ available in Origin6.0, where x is the real or rest concentration of lysozyme corrected after the reference curve; "a" is equal to the experimental fluorescence differences before and after treatment with nanosilica (Figure 1a) and nanodiamond (Figure 1b) and normalized to 1.0; "b" is obtained and equivalent to the equilibrium as well as adsorption constant of lysozyme on the surface of the respective nanoparticle. Adsorption reaction constants (Table 1) for lysozyme on the surfaces of nanosilica and -diamond can then be obtained.[4–6,8]

The negative fluorescence counts after the treatment with nanoparticles were caused by the detection noises and set to zero before they were subtracted by the respective values before the treatment (Figures 1a and 1b) and for the further simulation of coverage (Figures 2a and 2b).

The BET (Brunauer-Emmett-Teller) surface area of 20 and 55 m^2/g for nanosilica and -diamond, respectively, were obtained by static volumetric methods (Gemini V series, Micromeritics, Norcross U. S. A.). Every lysozyme solution of different concentrations was 2.0 mL. Those of nanoparticles were 5 μg in 20 μL PPBS. Total 2.02 mL were put together for the reaction. The linear region of Langmuir isotherm can be approximated within the concentration range of lysozyme 0–40/50 (Figure 2a) and 0–20/30 nM (Figure 2b) for nanosilica and -diamond, which correspond

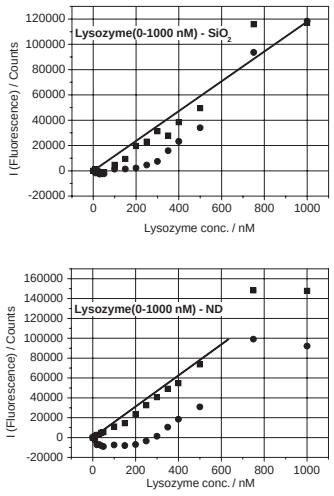

Figure 1. a (above) and b (below). Typical results for nanosilica (Figure 1a)/nanodiamond (Figure 1b) and lysozyme, evaluated with allometric1 of origin 6.0. *x*, and *y* axses are for the prepared lysozyme solutions of 0–1000 nM at pH = 10.91 (Figure 1a) and 11.0 (Figure 1b) and the fluorescence, respectively. The cubics for the linearity and rhombuses are the fluorescence intensities with respect to those before and after the treatment with nanoparticles.

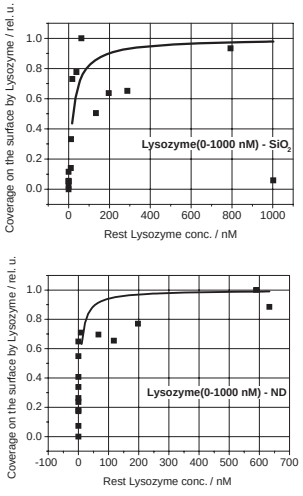

Figure 2. a (above) and b (below). Typical coverages for systems of nanosilica (Figure 2a)/nanodiamond (Figure 2b) and lysozyme. *x*, and *y* axses are for the rest lysozyme concentrations 0–1000 in nM, in PPBS = 7.0 mM at pH = 10.91 (Figure 2a) and 11.0 (Figure 2b), and coverages by lysozyme, respectively. The cubics represent the data after evaluation of the raw experimental data.

Table 1. Adsorption reaction constants of system nanosilica/nanodiamond and lysozyme at pH = 11.0

Nanoparticle Protein	Nanosilica Lysozyme	Nanodiamond Lysozyme
Adsorption constant	4.0×10^7 nM^{-1}	1.5×10^8 nM^{-1}

to 5×10^{13} and 3×10^{13} lysozyme molecules, respectively. The available surface areas per individual lysozyme molecule adsorbed on nanosilica and -diamond within this linear region can be roughly approximated as 2 and 10 nm^2, respectively (Table 2). Whatever corresponds well that the adsorption capability of the surface of nanoparticle is proportional to one of the possible factors available surface area contributed by the nanoparticle for each adsorbed lysozyme.

During a biomolecular reaction[10,11] or an enzymatic action, a specific conformation or helicity for combination or association is required. How far the adsorbed lysozyme molecule can optimize its helicity,[3,12–14] and what kind of conformational change is undertaken, as well as the respective activities of lysozyme under the same experimental conditions before and after adsorption must be further investigated.

It has been found in this experiment that system lysozyme–nanodiamond shows approximately 10 times higher adsorption capability than lysozyme–nanosilica within the linear Langmuir Isotherm region, and each adsorbed lysozyme on the surface of nanodiamond has approximately 5 times larger free space than that on nanosilica. The surface of nanodiamond may be much more rough or "porous" than that of nanosilica. The adsorbed lysozyme molecule can, therefore, better adjust its optimal helicity as well as do its appropriate orientation during the mutual adsorptive action.

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Table 2. Approximation of the available surface areas during the adsorption reactions for nanosilica and nanodiamond

Nanoparticle	Nanosilica	Nanodiamond
BET surface	20 m^2/g	55 m^2/g
Nanoparticle treated	5.0 µg	5.0 µg
Linear region	40–50 nM	20–30 nM
Total volume	2.02 mL	2.02 mL
Molecules involved	$4.9–6.1 \times 10^{13}$	$2.4–3.6 \times 10^{13}$
Available surface	2 nm^2	10 nm^2

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