Size-modulated catalytic activity of enzyme–nanoparticle conjugates: a combined kinetic and theoretical study†

Chung-Shu Wu,† Cheng-Che Lee, Chia-Tien Wu, Yuh-Shyong Yang and Fu-Hsiang Ko*

Received 22nd February 2011, Accepted 4th May 2011
DOI: 10.1039/c1cc11020a

A series of experiments were performed to systematically analyze the effect of nanoparticle (NP) size on the catalytic behavior of enzyme–NP conjugates, and a shielding model based on diffusion–collision theory was developed to explain the correlation between the size effects and the kinetic responses.

Many biomolecules, such as membrane proteins, perform their specific biorecognition or biocatalytic events while immobilized on the surfaces of cells or organelles. Several artificial technologies (e.g., bioconversion, bioremediation, biosensing) also take advantage of immobilized biomolecules and bio-species. In this regard, the modification and immobilization of enzymes has been studied widely for the generation of biocatalysts exhibiting enhanced stability and selectivity. Several synthetic scaffolds and supports, including gels, macromolecules, planar surfaces, and nanocomposites, have been used to immobilize enzymes. Among them, nanoparticles (NPs) provide an almost ideal mix of properties—minimum diffusional limitation, maximum surface area and high effective enzyme loading—to optimize the performance of immobilized enzymes while harnessing the fluorescent, magnetic and interferential behavior of the resulting nanomaterials. However, studies of the effects of NPs size, shape and material properties on both the kinetic experiments and theoretical model of the catalytic reactions of its enzyme–NP conjugates still remain rare.

In a previous paper, we demonstrated that adsorbing lipase onto gold NPs (AuNPs) significantly increased its enzymatic activity. In further investigations, we observed that the size of the NPs affected the catalytic activity; based on a diffusion–collision theory, we have attempted to develop a length-scale-dependent model to explain the effect of NP size on the modulation in the activity of the enzyme–AuNP conjugate systems. In Nature, controllable modulation of enzyme activity is a potent means of regulating several cellular processes (e.g., signal transduction, biosynthesis, metabolism). The modulation of biocatalytic behavior is an attractive feature for exploitation in the field of nano-biotechnology. For extended studies of biocatalysis, lipases are highly suitable esterases because of their well-defined structures, properties, and applications. Lipases are used industrially in detergents, in paper and food technology, in the preparation of specialty fats, in various clinical studies, and for drug delivery. Therefore, in this study we selected Candida rugosa lipase (CRL, E.C. 3.1.1.3) to construct our enzyme–NP conjugates. As illustrated schematically in Scheme 1, we reported a series of experiments and a theoretical model designed to analyze the effect of AuNP size on the modulation of enzyme activity.

We selected AuNPs as the adsorption materials for CRL in this study because they can be synthesized in a variety of diameters (13–100 nm) and readily characterized using UV-Vis spectrophotometry and scanning electron microscopy (SEM). In these experiments, we used a standard chemical reduction method to prepare spherical AuNPs having mean diameters of 13.1, 25.2, 37.5, 50.8, and 69.6 nm, under conditions that ensured their stability and lack of aggregation (see ESI†). For the preparation of enzyme-functionalized AuNPs with their colloidal stability, we performed the salt-induced colloidal tests to analyze the enzyme coverage and concentration of the protection factor (Cpr; see ESI††), determined by measuring the ratios of the absorbance at 620 nm (A620) to those of the absorption signals (Apeak) of the differently sized AuNPs; Fig. S5 (ESI††) displays these progress plots. To analyze the enzyme coverage, we define R as the ratio Apeak/A620. When the enzyme concentration was sufficient to cap the AuNPs...
## Table 1

<table>
<thead>
<tr>
<th>AuNP size/nm</th>
<th>$k_{\text{cat}}$/s$^{-1}$</th>
<th>$K_m$/μM</th>
<th>$k_{\text{cat}}/K_m$/μM$^{-1}$ s$^{-1}$</th>
<th>μcP</th>
<th>$k_{\text{cat}}$/μM$^{-1}$ s$^{-1}$</th>
<th>$k_{\text{cat}}$/μM$^{-1}$ s$^{-1}$</th>
<th>η</th>
<th>$k_{\text{cat}}$/μM$^{-1}$ s$^{-1}$</th>
<th>exp(−$E_{\text{cat}}$/RT) at 303 K</th>
<th>μcP</th>
</tr>
</thead>
<tbody>
<tr>
<td>NP size → 0</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.38</td>
<td>0.07</td>
<td>—</td>
</tr>
<tr>
<td>13.1</td>
<td>18.0 ± 0.56</td>
<td>9.5 ± 1.02</td>
<td>1.89 ± 0.26</td>
<td>1.14 ± 0.016</td>
<td>15.11</td>
<td>1.22</td>
<td>1.37</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25.2</td>
<td>18.3 ± 0.26</td>
<td>14.1 ± 0.68</td>
<td>1.30 ± 0.08</td>
<td>1.14 ± 0.012</td>
<td>14.97</td>
<td>0.85</td>
<td>0.12</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>37.5</td>
<td>19.6 ± 0.43</td>
<td>15.7 ± 1.11</td>
<td>1.25 ± 0.12</td>
<td>1.14 ± 0.016</td>
<td>14.90</td>
<td>0.81</td>
<td>1.06</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50.8</td>
<td>19.1 ± 0.30</td>
<td>17.1 ± 0.84</td>
<td>1.12 ± 0.07</td>
<td>1.12 ± 0.016</td>
<td>15.11</td>
<td>0.78</td>
<td>1.03</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>69.6</td>
<td>17.9 ± 0.38</td>
<td>18.1 ± 1.20</td>
<td>0.99 ± 0.09</td>
<td>1.13 ± 0.025</td>
<td>14.92</td>
<td>0.75</td>
<td>0.98</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immobilized on a planar surface (NP size → ∞)</td>
<td>18.6 ± 0.39 (22.6 ± 1.41)$^a$</td>
<td>0.82 ± 0.07</td>
<td>1.52 ± 0.021</td>
<td>10.95</td>
<td>0.50</td>
<td>0.48</td>
<td>0.0880 ± 0.0058</td>
<td>0.0880 ± 0.0058</td>
<td>0.0880 ± 0.0058</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ Data measured using the Lee–Yang approach from ref. 6. $^b$ Average viscosity (μ) for the reaction solutions of various CRL–AuNP conjugate systems.

An obtained lower value of $K_m$, $k_{\text{cat}}$, and $k_{\text{cat}}/K_m$ represented greater kinetic affinity between the enzyme and the substrate; i.e., the conjugated system with the smaller AuNPs exhibited enhanced kinetic affinity, thereby improving the catalytic efficiency $k_{\text{cat}}/K_m$.

To further correlate the NP dimensions (size effect) to the catalytic efficiency ($k_{\text{cat}}/K_m$) of the conjugated enzyme, we developed a shielding model based on diffusion–collision theory (a detailed derivation is given in the ESI†, p. 14, theoretical considerations). We made the following assumptions: (i) the NPs, enzymes, and substrate molecules behaved as “rigid balls” (cf. Fig. 1); (ii) the intrinsic properties of the enzymes conjugated onto the differently sized NPs were similar; and (iii) the orientation of the active site of the enzyme–NP conjugates was random. Without considering multistep reactions, the catalytic reaction of substrate $S$ with the conjugated enzyme $E_{\text{NP}}$ can be approximated as the bimolecular reaction (Fig. 1(a)) in eqn (1):

$$E_{\text{NP}} + S \xrightleftharpoons[k_{-1}^s][k_{1}^s] E_{\text{NP}} + P$$

The reaction rate $v$ (μM s$^{-1}$) can be expressed as the product of the bimolecular rate constant $k_{\text{cat}}$/μM$^{-1}$ s$^{-1}$ and the concentrations of the enzyme ($C_E$) and substrate ($C_S$), as in eqn (3).

$$v = k_{\text{cat}}/C_E C_S$$

Relative to the free enzyme in solution, the $E_{\text{NP}}$ provided additional steric hindrance for the binding of the substrate to the enzyme. We defined the shielding factor $\eta$ as the solid-angle ratio of the enzyme surface exposed to substrate bombardment; the open solid-angle fraction $\Omega$ was displayed in Fig. 1(c) with respect to the full solid angle 4π. Furthermore, combining diffusion–collision theory, the shielding effect (on the modulation of the probability of bombardment) of smaller AuNPs had lower values. In previous studies, we found that the kinetics of the enzyme immobilized on one side of a planar surface could be effectively modeled and measured; in Table 1, when varying the AuNPs’ dimensions, the values of $K_m$, $k_{\text{cat}}$, and $k_{\text{cat}}/K_m$ displayed an increasing trend in the presence of larger AuNP size, even under the extreme condition of AuNP size → ∞. In simple terms, the rate constants for the individual steps are described in eqn (1):

$$E_{\text{NP}} + S \xrightleftharpoons[k_{-1}^s][k_{1}^s] E_{\text{NP}} + P$$

The reaction rate $v$ (μM s$^{-1}$) can be expressed as the product of the bimolecular rate constant $k_{\text{cat}}$/μM$^{-1}$ s$^{-1}$ and the concentrations of the enzyme ($C_E$) and substrate ($C_S$), as in eqn (3).

$$v = k_{\text{cat}}/C_E C_S$$
Fig. 1  Geometric relationships between the substrate S, the enzyme E and the nanoparticle NP. (a) If the distance between the S and E was less than the sum of the radii of two bounding spheres, \( r_S + r_E \), then a collision event occurred. (b) Concerning diffusion effects in a fluid, the effective radius of the enzyme–NP conjugate should increase to \( r_S + 2 r_E \). (c) The solid-angled fraction \( \Omega \) exposed for collisions between the substrate and the enzyme. (d) Geometric relationships of the respective radii.

Substituting the viscosity, absolute temperature, and radii \( r_S, r_E, \) and \( r_NP \) for the radii of the NP, enzyme, and substrate, respectively.


developed a shielding model to explain the correlation between the size effects and the kinetic responses. A simple and efficient method for the preparation of this functional conjugates, with colloidal stability, under retention of enzymatic activity had been reported. The association of CRL with the AuNPs did not influence the values of \( k_C \), but the smaller AuNPs promoted the catalytic efficiency of CRL by increasing its kinetic affinity (i.e., lower \( K_m \) values) toward the substrate \( \rho \).

In summary, we had performed a series of experiments to systematically analyze the modulated NP size-dependent enzymatic activities of CRL–AuNP conjugates, and had developed a shielding model to explain the correlation between the size effects and the kinetic responses. A simple and efficient method for the preparation of this functional conjugates, with colloidal stability, under retention of enzymatic activity had been reported. The association of CRL with the AuNPs did not influence the values of \( k_C \), but the smaller AuNPs promoted the catalytic efficiency of CRL by increasing its kinetic affinity (i.e., lower \( K_m \) values) toward the substrate \( \rho \).

In this study, we found that the sizes of these conjugates acted as a controllable and efficient factor for modulating the activity of the enzyme. We believed that as the integrative field of nano-biotechnology evolves, such studies of the fundamental interactions of nanostructures with biological systems will become, by necessity, more common.

Notes and references


