J|A|C|S
COMMUNICATIONS

Published on Web 09/29/2004

Monolayer-Controlled Substrate Selectivity Using Noncovalent Enzyme−Nanoparticle Conjugates

Rui Hong,[†] Todd Emrick,[‡] and Vincent M. Rotello*,[†]

Department of Chemistry, Department of Polymer Science and Engineering, University of Massachusetts Amherst, Amherst, Massachusetts 01003

Received June 30, 2004; E-mail: rotello@chem.umass.edu

Enzyme modification and immobilization have been extensively studied and utilized to generate biocatalysts with improved stability and selectivity.[1] Enhanced or altered enzyme selectivity not only affords novel biocatalysts directly but also finds application in sensing and enzyme-related biotechnology.[2] Given the highly specific interaction between enzymes and substrates, genetic mutation or chemical modification of enzymes represents the primary method for alteration of substrate selectivity.[3] An alternative strategy for altering enzyme selectivity, however, is through noncovalent surface interaction with protein using synthetic scaffolds.[4] The large surface area and ample surface functionalization chemistry of nanometer-scale materials, especially monolayer-protected nanoparticles,[5] have made them promising scaffolds to manipulate enzyme activities.[6]

In our previous work,[7] we have shown control over chymotrypsin (ChT) structure and function by using surface-functionalized CdSe nanoparticles. The interaction between ChT and nanoparticle ligands is electrostatically driven. Binding of ChT to nanoparticles functionalized with alkanethiol-tetra(ethylene glycol)acetic acid ligands, TCOOH (Figure 1), inhibits the ChT-catalyzed hydrolysis of succinyl-Phe-pNA (SPNA) without protein denaturation. The inhibition was attributed to the spatial blocking of the ChT active site by the nanoparticle scaffold. However, a substrate-selective behavior of the protein−nanoparticle complex was observed in further investigations, which could not be explained by pure steric effects. We report in this communication that the surface-bound protein (i) retains activity and (ii) exhibits pronounced substrate chemoselectivity.

Anionic TCOOH-functionalized Au nanoparticles (**Au-TCOOH**) were prepared by standard place exchange chemistry[8] (see Supporting Information (SI)). Binding of ChT to **Au-TCOOH** was achieved through electrostatic attraction as its CdSe analogue. Distinguishing our approaches from previously reported protein−nanoparticle complexes in which the cysteine−gold interaction was the driving force,[3,9] here the interacting sites were built into the ligand structure, rather than directly on the gold surface. We consider this an important alternative to control protein−nanoparticle interactions, allowing variation of the surface functionalities and the reduction of nonspecific interactions.[10] The adsorption of ChT onto **Au-TCOOH** nanoparticles was confirmed as in our previous studies[7] by activity assays and fluorescence quenching of Trp residues in ChT (see SI).

A series of commercially available oligopeptides was tested as substrates for ChT, including SPNA, *N*-benzoyl-tyrosine-pNA (BTNA), glutaryl-Phe-pNA (GPNA), and succinyl-Ala-Phe-pNA (SAPNA) (Figure 2). The ChT-catalyzed hydrolysis reaction was followed spectroscopically by monitoring the formation of *p*-nitroaniline (pNA). Interestingly, we found a substantially different

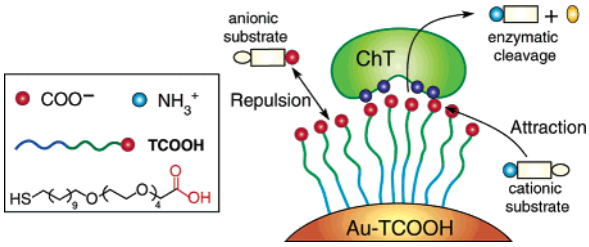

Figure 1. Chemical structure of the TCOOH ligand and schematic depiction of substrate−monolayer interaction-induced enzyme selectivity.

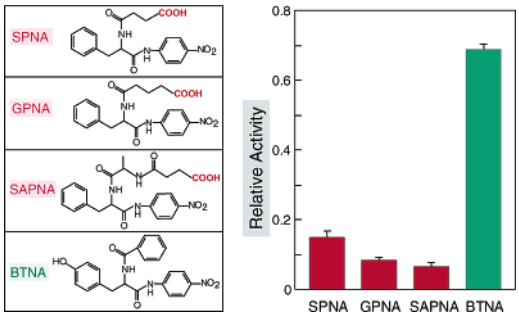

Figure 2. Chemical structure of the substrates and the normalized activity of ChT bound to **Au-TCOOH** relative to ChT alone with these substrates. See Supporting Information for activity assay protocol.

degree of relative activity of ChT toward these substrates. With SPNA, GPNA, and SAPNA, the ChT activity decreased dramatically when bound to **Au-TCOOH**, with residual activities of ∼15% for SPNA and below 10% for the other two. When the neutral substrate BTNA was used, however, a ∼70% relative activity to free ChT was observed.

One similarity we noted for all the substrates inhibited was the presence of carboxylic acid groups in the molecules, which gives negative charge to the substrates in neutral pH buffer. With the elimination of charges, such as with BTNA, much less inhibitory effect was observed. Although this observation suggested that electrostatic repulsion between the carboxylate of the substrate and the anionic nanoparticle monolayer may contribute to ChT selectivity, other variables of the substrate structures, such as size and hydrophobicity, made difficult the direct attribution of the observed selectivity to electrostatic effects.

To further elucidate the role of substrate charge on the selectivity, three SPNA derivatives, **1**, **2**, and **3** with different charges, were synthesized and analyzed (see SI for synthesis). Heterofunctionalized tri(ethylene glycol) (EG3) molecules, with a primary amine at one end for conjugation to SPNA, and a charged moiety at the other end, were used to modify SPNA. Carbodiimide coupling reactions, followed by necessary deprotection, successfully afforded desired products **1** with carboxylic acid, **2** with alcohol, and **3** with primary amine end groups in good yields (Figure 3a). The choice of EG3 as the linker between SPNA and the charged groups ensured

[†] Department of Chemistry.
[‡] Department of Polymer Science and Engineering.

10.1021/ja0461163 CCC: $27.50

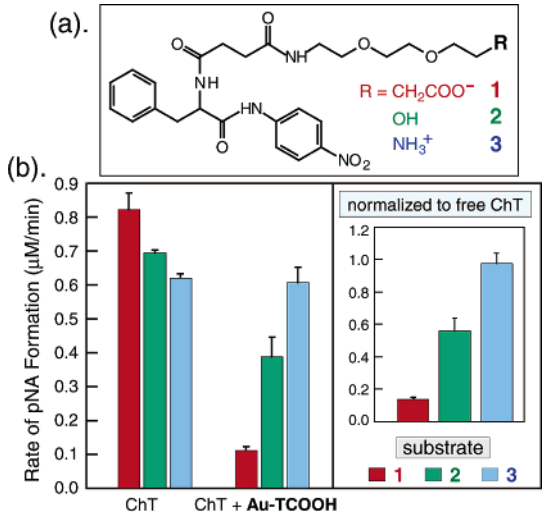

Figure 3. (a) Structures of the modified SPNA substrates **1**−**3**. (b) Initial rates of ChT hydrolysis of these modified substrates. (Inset) Normalized activity of **Au-TCOOH**-bound ChT toward substrates **1**−**3**.

sufficient water solubility of the modified substrates, especially for neutral substrate **2**. EG3 also extended the charged groups away from the binding and catalytic sites of the enzymatic reaction,[11] thereby minimizing secondary effects of the modification. Most importantly, this modification provided molecules of very similar structure, differing only at the EG3 chain ends. This minimized the differences in chain length and hydrophobicity and emphasized the effect of charged groups on the enzymatic activity of surface-bound ChT.

Each of the modified SPNA molecules proved to be good substrates for ChT as shown in Figure 3b. No detectable autohydrolysis was observed for any of the modified SPNA compounds. The higher hydrolysis rate of these modified SPNA molecules relative to that of SPNA itself confirmed the successful substrate design and synthesis.[12] The initial rates of enzymatic hydrolysis by native ChT were similar for all the SPNA derivatives, which directly reflected the similarity in the structures of these molecules.

Enhanced chemoselectivity of ChT was observed when bound to the **Au-TCOOH** surface. The ChT−**AuTCOOH** complex showed very low activity toward negatively charged substrate **1**. However, a ∼50% and a nearly 100% relative activity of bound ChT to free ChT were observed toward the neutral substrate **2** and the positively charged substrate **3**, respectively (Figure 3). In a control experiment, when the activity assays were performed in a solution of elevated ionic strength (200 mM, with NaCl), at which condition the electrostatic attraction was screened as evident in fluorescence assay (see SI), ChT with **Au-TCOOH** displays almost identical activity as ChT alone toward all the modified SPNAs (see SI). Therefore, the observed selectivity can be directly attributed to binding of ChT to the **Au-TCOOH** surface monolayer. Considering the characteristic substrate structure, together with the anionic nature of the nanoparticle monolayer, this chemoselectivity can be explained by a combination of steric hindrance and electrostatic interactions (Figure 1). For negatively charged **1**, the interactions between **1** and the surface-bound ChT were disfavored by steric and electrostatic effects. As a result, the catalytic reaction was dramatically slowed, in accord with anionic substrates SPNA, GPNA, and SAPNA. For neutral substrate **2**, steric effects hindered the substrate hydrolysis, but there were no unfavorable electrostatic interactions. An intermediate inhibitory effect was then observed; BTNA was also in this class as the charge repulsion was not present. In the case of **3**, substrate access to ChT was presumably affected by binding to **Au-TCOOH**. However, the overall charge of the ChT−**AuTCOOH** was negative, thus favoring the adsorption of cationic **3** to the monolayer. The increased local concentration of **3** near the nanoparticle surface in turn increased the accessibility of it to the enzyme. The seemingly unchanged activity can thus be viewed as a cancellation of the unfavorable steric hindrance by favorable electrostatic attraction.

In summary, we have presented control over enzymatic activity at a higher level than a simple "on/off" mode. The bound enzyme retains activity and exhibits enhanced chemoselectivity due to the substrate−monolayer interactions. Our finding has three major implications. First, the monolayer of the protein-binding nanoparticle scaffolds can be used to control the interactions between the protein and nanoparticle. Second, the structure of the monolayer can also be used to control enzyme−substrate or protein−ligand interactions; a useful attribute of surface binding that is difficult to achieve with small molecular recognition units. Finally, the interactions of protein−substrate−3D nanoparticle monolayer[13] studied here also give insight into those interactions on 2D SAMs or on other solid supports, which is important for the fabrication of bioactive surfaces and materials.[14]

Acknowledgment. This research was supported by the National Institutes of Health (GM 62998, V.R.) and a National Science Foundation Career Award (CHE-0239486, T.E.).

Supporting Information Available: Synthesis of **Au-TCOOH** and modified SPNA compounds, activity assay protocol, fluorescence quenching of ChT by **Au-TCOOH**, activity of ChT with Au-TCOOH at elevated ionic strength. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Bornscheuer, U. T. *Angew. Chem., Int. Ed.* **2003**, *42*, 3336−3337 and references therein. Cao, L. Q.; van Langen, L.; Sheldon, R. A. *Curr. Opin. Biotechnol.* **2003**, *14*, 387−394. Hult, K.; Berglund, P. *Curr. Opin. Biotechnol.* **2003**, *14*, 395−400.

(2) Bordusa, F. *Chem. Rev.* **2002**, *102*, 4817−4868.

(3) Wu, X. L.; Chen, S. G.; Petrash, J. M.; Monnier, V. M. *Biochemistry* **2002**, *41*, 4453−4458. Haring, D.; Schreier, P. *Naturwissenschaften* **1999**, *86*, 307−312. Haring, D.; Schreier, P. *Angew. Chem., Int. Ed.* **1998**, *37*, 2471−2473. Davis, B. G. *Curr. Opin. Biotechnol.* **2003**, *14*, 379−386. Huang, Y. F.; Huang, C. C.; Chang, H. T. *Langmuir* **2003**, *19*, 7498−7502.

(4) Peczuh, M. W.; Hamilton, A. D. *Chem. Rev.* **2000**, *100*, 2479−2493.

(5) Templeton, A. C.; Wuelfing, M. P.; Murray, R. W. *Acc. Chem. Res.* **2000**, *33*, 27−36. Niemeyer, C. M. *Angew. Chem., Int. Ed.* **2001**, *40*, 4128−4158.

(6) (a) Fischer, N. O.; McIntosh, C. M.; Simard, J. M.; Rotello, V. M. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 5018−5023. (b) Rege, K.; Raravikar, N. R.; Kim, D. Y.; Schadler, L. S.; Ajayan, P. M.; Dordick, J. S. *Nano Lett.* **2003**, *3*, 829−832.

(7) Hong, R.; Fischer, N. O.; Verma, A.; Goodman, C. M.; Emrick, T.; Rotello, V. M. *J. Am. Chem. Soc.* **2004**, *126*, 739−743.

(8) Hostetler, M. J.; Green, S. J.; Stokes, J. J.; Murray, R. W. *J. Am. Chem. Soc.* **1996**, *118*, 4212−4213.

(9) Gole, A.; Dash, C.; Ramakrishnan, V.; Sainkar, S. R.; Mandale, A. B.; Rao, M.; Sastry, M. *Langmuir* **2001**, *17*, 1674−1679.

(10) Zheng, M.; Davidson, F.; Huang, X. Y. *J. Am. Chem. Soc.* **2003**, *125*, 7790−7791.

(11) Case, A.; Stein, R. L. *Biochemistry* **2003**, *42*, 3335−3348.

(12) The rate of *p*-nitroaniline formation was 0.097 ± 0.004 μM/min when SPNA was used as substrate.

(13) Terrill, R. H.; Postlethwaite, T. A.; Chen, C.-H.; Poon, C.-D.; Terzis, A.; Chen, A.; Hutchison, J. E.; Clark, M. R.; Wignall, G.; Londono, J. D.; Superfine, R.; Falvo, M.; Johnson, C. S., Jr.; Samulski, E. T.; Murray, R. W. *J. Am. Chem. Soc.* **1995**, *117*, 12537−12547.

(14) Hodneland, C. D.; Lee, Y.-S.; Min, D.-H.; Mrksich, M. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 5048−5052. Hong, M.-Y.; Yoon, H. C.; Kim, H.-S. *Langmuir* **2003**, *19*, 416−421. Chen, X.; Ferrigno, R.; Yang, J.; Whitesides, G. M. *Langmuir* **2002**, *18*, 7009−7015. Seong, G. H.; Heo, J.; Crooks, R. M. *Anal. Chem.* **2003**, *75*, 3161−3167.

JA0461163