ORGANIC LETTERS

2005
Vol. 7, No. 25
5685−5688

Contrasting Effects of Exterior and Interior Hydrophobic Moieties in the Complexation of Amino Acid Functionalized Gold Clusters with α-Chymotrypsin

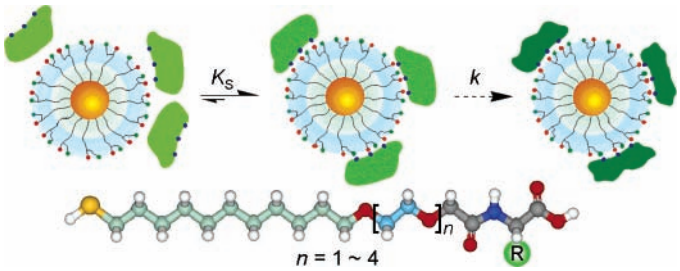
Chang-Cheng You, Mrinmoy De, and Vincent M. Rotello*

Department of Chemistry, University of Massachusetts, 710 North Pleasant Street, Amherst, Massachusetts 01003

rotello@chem.umass.edu

Received September 30, 2005

ABSTRACT



A series of L-amino acid functionalized gold nanoparticles with oligo(ethylene glycol) (OEG) tethers of varying length are prepared. These studies show that the hydrophobic side chains of amino acids facilitate the structural retention of α-chymotrypsin (ChT) but the interior alkyl chains promote its denaturation. An 80-fold range of denaturation rate constants were obtained for ChT in the presence of various nanoparticles. Thus, the tunable denaturation of protein could be achieved by rational combination of amino acid side chains and OEG tethers.

Protein recognition by artificial receptors provides a potent tool to modulate cellular processes such as signal transduction, DNA transcription, and protein enzyme inhibition. To date, a number of synthetic agents such as calixarene derivatives[1] and chemically modified cyclodextrins[2] have been exploited in protein surface recognition and have shown effective modulation of the structure and function of biomacromolecules. Monolayer protected clusters (MPCs) and mixed monolayer protected clusters (MMPCs) are attractive artificial receptors for biomacromolecules such as proteins and DNA.[3] The MMPC motif represents an appealing alternative for generating structurally well-defined bulky organic functional entities with dimensions comparable to biomacromolecules, which are often not easily accessible through conventional organic protocols. Moreover, the functionality of MMPCs is readily tailored by proper design of the organic coating molecules.[4]

(1) (a) Park, H. S.; Lin, Q.; Hamilton, A. D. *J. Am. Chem. Soc.* **1999**, *121*, 8−13. (b) Hamuro, Y.; Calama, M. C.; Park, H. S.; Hamilton, A. D. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 2680−2683.

(2) (a) Otzen, D. E.; Knudsen, B. R.; Aachmann, F.; Larsen, K. L.; Wimmer, R. *Protein Sci.* **2002**, *11*, 1779−1787. (b) Leung, D. K.; Yang, Z. W.; Breslow, R. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 5050−5053.

(3) For recent reviews, see: (a) Verma, A.; Rotello, V. M. *Chem. Commun.* **2005**, 303−312. (b) Pasquato, L.; Pengo, P.; Scrimin, P. *J. Mater. Chem.* **2004**, *14*, 3481−3487. (c) Katz, E.; Willner, I. *Angew. Chem., Int. Ed.* **2004**, *43*, 6042−6108. (d) Shenhar R.; Rotello, V. M. *Acc. Chem. Res.* **2003**, *36*, 549−561. (e) Sastry, M.; Rao, M.; Ganesh, K. N. *Acc. Chem. Res.* **2002**, *35*, 847−855.

(4) (a) Abad, J. M.; Mertens, S. F. L.; Pita, M.; Fernandez, V. M.; Schiffrin, D. J. *J. Am. Chem. Soc.* **2005**, *127*, 5689−5694. (b) Zheng, M.; Huang, X. *J. Am. Chem. Soc.* **2004**, *126*, 12047−12054. (c) Lin, C.-C.; Yeh, Y.-C.; Yang, C.-Y.; Chen, G.-F.; Chen, Y.-C.; Wu, Y.-C.; Chen, C.-C. *Chem. Commun.* **2003**, 2920−2921.

10.1021/ol052367k CCC: $30.25
Published on Web 11/11/2005

Our recent studies[5] revealed that carboxylate-terminated MMPCs are able to target α-chymotrypsin (ChT), a serine protease with a positively charged surface (pI = 8.75) and well-studied enzymatic activity, through surface complementary electrostatic interaction. More interestingly, we found that MMPCs featuring mercaptoundecanoic acid ligands rapidly denatured the bound ChT molecules, while the incorporation of additional tetra(ethylene glycol) spacers onto MMPCs reduced considerably the denaturation extent. Therefore, it is essential to clarify how the linkages between recognition elements and MMPC cores affect the protein-receptor interaction. In this paper, we have synthesized a family of 30 L-amino acid functionalized MMPCs (Figure 1)[6,7] with oligo(ethylene glycol) (OEG) tethers of variable

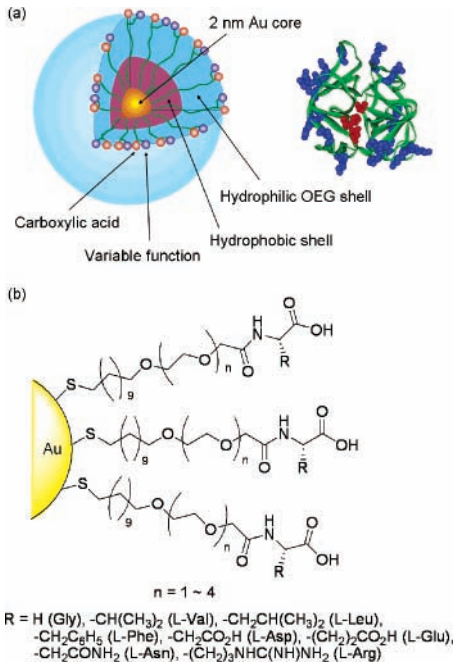


Figure 1. (a) Structural features of amino acid functionalized MMPCs and ChT. The residues in red are in the active site, and residues in blue are cationic. (b) Chemical structure of amino acid functionalized MMPCs.

length and explored their interaction with ChT. OEG units are well-known to resist nonspecific interaction with biomolecules and have been deposited onto various substrates to afford biocompatible monolayers.[8] The varible amino acids at the periphery of MMPCs allow us to detect the side chain effect upon interaction with ChT.[7]

Activity assays were first conducted to evaluate the inhibitory potencies of these MMPCs on the enzymatic activity of ChT. With *N*-succinyl-l-phenylalanine *p*-nitroanilide (SPNA) as substrate, the acitivity of ChT was drastically depressed upon addition of most MMPCs (Figure 2 for **NP_3EG_L-Val**). Only L-Arg functionalized MMPCs

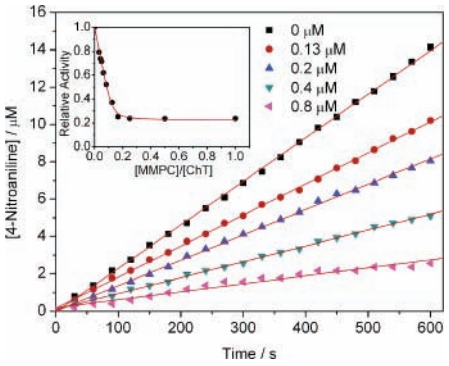


Figure 2. Progress curves for the hydrolysis of SPNA in the presence of ChT and various concentrations of **NP_3EG_L-Val**. [ChT] = 3.2 μM and SPNA [2 mM].[11] (Inset) Normalized activity of ChT in the presence of varying concentrations of **NP_3EG_L-Val**.

exhibited no inhibition due to their positively charged side chains. Consequently, the complementary electrostatic interaction between ChT and MMPCs plays a vital role in the complex formation.[9] The enzymatic activity of ChT typically decreased ca. 60−85% upon incubation with excess MMPCs (Figures S24 and S25). The binding strength between ChT and MMPCs was quantified by analyzing the activity assay data through nonlinear least-squares curve-fitting using an equal *K* model where the MMPCs were assumed to possess *n* identical and independent binding sites.[7] The results show that microscopic binding constants for complexations between ChT and MMPCs are between 10^6 and 10^7 M^{-1} (Table S1).[10] Interestingly, the length of OEG tethers shows considerable influence on the complex stability of ChT and MMPCs (Figure 3). For most MMPCs the affinity increases with decreasing length of OEG tether. One plausible

(5) (a) Hong, R.; Fischer, N. O.; Verma, A.; Goodman, C. M.; Emrick, T.; Rotello, V. M. *J. Am. Chem. Soc.* **2004**, *126*, 739−743. (b) Fischer, N. O.; McIntosh, C. M.; Simard, J. M.; Rotello, V. M. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 5018−5023.

(6) The MMPCs were prepared by place-exchange of 1-pentanethiol-protected nanoparticles ($d \approx 2$ nm) with corresponding amino acid functionalized thiolate ligands in dichloromethane. ^{1}H NMR spectra show that the exchange is accomplished quantitatively. It is estimated that about 100 amino acid functionalized ligands are anchored on each nanoparticle: Hosteler, M. J.; Wingate, J. E.; Zhong, C.; Harris, J. E.; Vachet, R. W.; Clark, M. R.; Londono, J. D.; Green, S. J.; Stokes, J. J.; Wignall, G. D.; Glish, G. L.; Porter, M. D.; Evans, N. D.; Murray, R. W. *Langmuir* **1998**, *14*, 17−30.

(7) You, C.-C.; De, M.; Han, G.; Rotello, V. M. *J. Am. Chem. Soc.* **2005**, *127*, 12873−12881.

(8) (a) Lasseter, T. L.; Clare, B. H.; Abbott, N. L.; Hamers, R. J. *J. Am. Chem. Soc.* **2004**, *126*, 10220−10221. (b) Zheng, M.; Davidson, F.; Huang, X. *J. Am. Chem. Soc.* **2003**, *125*, 7790−7791. (c) Herrwerth, S.; Eck, W.; Reinhardt, S.; Grunze, M. *J. Am. Chem. Soc.* **2003**, *125*, 9359−9366. (d) Kane, R. S.; Deschatelets, P.; Whitesides, G. M. *Langmuir* **2003**, *19*, 2388−2391. (e) *Poly(ethylene glycol) Chemistry: Biotechnical and Biomedical Applications*; Harris, J. M:, Ed.; Plenum Press: New York, 1992.

(9) Control experiments showed that L-Arg-functionalized MMPCs can inhibit the enzymatic activity of negatively charged β-galactosidase (pI = 4.6). The other MMPCs with neutral or negatively charged side chains conversely show no inhibition on this enzyme.

(10) The binding affinity of these MMPCs toward ChT is comparable to the naturally occurring ChT−inhibitor interaction: (a) Chen, C.; Hsu, C.-H.; Su, N.-Y.; Lin, Y.-C.; Chiou, S.-H.; Wu, S.-H. *J. Biol. Chem.* **2001**, *276*, 45079−45087. (b) Fukada, H.; Takahashi, K.; Sturtevant, J. M. *Biochemistry* **1985**, *24*, 5109−5115.

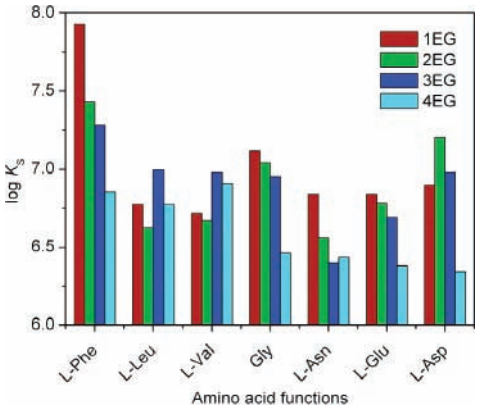


Figure 3. Binding constants (log K_S) between ChT and MMPCs bearing various amino acid side chains and OEG tethers, estimated from activity assays.

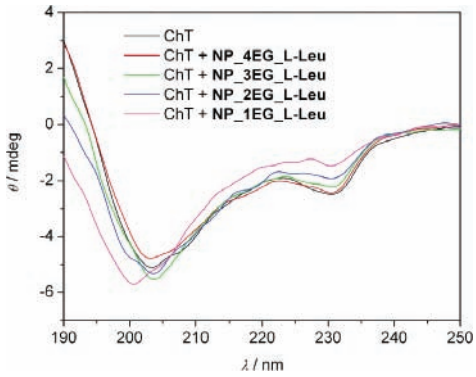


Figure 4. CD spectra of ChT (3.2 μM) in the absence and presence of L-Leu functionalized MMPCs (0.8 μM) with different OEG tethers in 5 mM sodium phosphate buffer (pH 7.4) after 24 h incubation.

interpretation for this phenomenon is that the shorter linkers are more preorganized, decreasing the entropic cost of binding.

Both activity assays and gel electrophoresis studies showed that the binding capacity of ditopic amino acid terminated MMPCs is higher than that of monotopic amino acid functionalized MMPCs, in accordance with our previous studies.[7] For example, the binding stoichiometries of all four L-Asp functionalized MMPCs are ca. 11−13, while the binding stoichiometries of monotopic amino acid (e.g., L-Leu) functionalized MMPCs are ca. 6−8. Surprisingly, the binding capacity is essentially irrespective of the length of OEG tethers for the tested MMPCs. These results suggest that the binding ratios are dependent on the surface "hot spots" (i.e., carboxylates) rather than proportional to the surface area, which is expected to decrease from 300 to 180 nm^2 going from the 4EG to the 1EG spacer. In this context, the association between ChT and these MMPCs is proposed to operate through intermolecular ion pairs, and the highly mobile carboxylates on the MMPC periphery tend to cluster together to realize the maximal electrostatic interaction with ChT through cooperative interactions.

Circular dichroism (CD) spectra of ChT with various MMPCs were subsequently recorded to determine the impact of amino acid side chains as well as the OEG tethers on the protein structure. Figure 4 shows the CD spectra of ChT upon incubation with a series of L-Leu terminated MMPCs for 24 h. Whereas ChT that was incubated with **NP_4EG_L-Leu** showed negligible CD spectral changes, the protein displayed different levels of CD spectral changes upon incubation with L-Leu MMPCs that bear shorter OEG tethers. Among the four MMPCs, **NP_1EG_L-Leu** with the shortest OEG tether induced the most significant spectral changes, i.e., the decrease of the characteristic minimum at 230 nm and the blue shift of the minimum at 204 nm. The spectral changes were found to be time-dependent (as examplified in Figures S26−S29). This observation clearly shows that the initial structure of ChT is efficiently denatured by MMPCs.[12] Deconvolution of the circular dichroism spectra revealed that the contents of α-helices drastically decreased along with the augmentation of β-sheets.[13] The OEG length-related denaturation indicates that OEG tethers can aid in the retention of ChT secondary structure. The amino acid side chains also displayed different levels of influence on the protein structure. Hydrophilic amino acids such as L-Asp, L-Glu, L-Asn, and Gly always induced significant CD spectral changes, whereas hydrophobic amino acids such as L-Phe, L-Leu, and L-Val showed less pronounced effect. Overall, the hydrophilic residues facilitate the loss of protein secondary structure.

The conformational change of ChT in the presence of MMPCs was further quantified using fluorescence spectrometry. The steady-state fluorescence maximum of ChT is 331 nm with a half-height width of 54 nm (Figure 5). Upon incubation with MMPCs for 24 h, significant bathochromic shifts of fluorescence maxima and peak broadening was observed. For **NP_1EG_L-Val**, the fluorescence maximum was red-shifted to 352 nm ($\Delta\lambda = 21$ nm) with concomitant broadening of half-maximal amplitude to 68 nm. These phenomena indicate that the initially buried tryptophan residues are exposed to more polar environment as a result of the unfolding of the protein.[14]

The denaturation of ChT follows a first-order reaction profile. In Figure 6, the logarithm of native ChT concentration is plotted against the incubation period with L-Leu

(11) The absorbance was converted to a concentration scale by a molar absorption coefficient of 9800 M^{-1} cm^{-1} for 4-nitroaniline. Sträter, N.; Sun, L.; Kantrowitz, E. R.; Lipscomb, W. N. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 11151−11155.

(12) This denaturation is partially reversible as evidenced by the enzymatic activity recovery of ChT by addition of sodium chloride into the system to dissociate the complexes.

(13) Sreerama, N.; Woody, R. W. *Anal. Biochem.* **2000**, *287*, 252−260. The deconvolution using CONTINLL protocol showed that the α-helical content in ChT decreased from 16.8% to 6.9%, whereas β-sheet content increased from 30.2% to 36.7% with **NP_1EG_L-Leu**.

(14) Ladokhin, A. S. In *Encyclopedia of Analytical Chemistry*; Meyers, R. A., Ed.; John Wiley & Sons Ltd.: Chichester, U.K., 2000; pp 5762−5779.

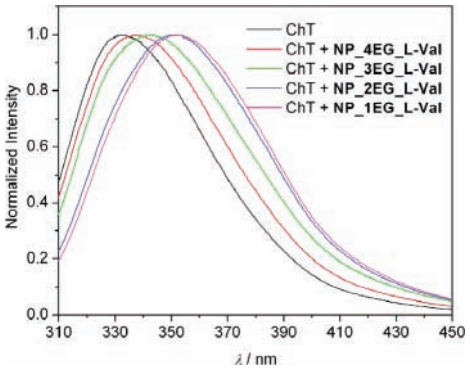


Figure 5. Fluorescence spectra of ChT (3.2 μM) in the absence and presence of L-Val decorated MMPCs with different OEG tethers in 5 mM sodium phosphate buffer (pH 7.4) after 24 h incubation.

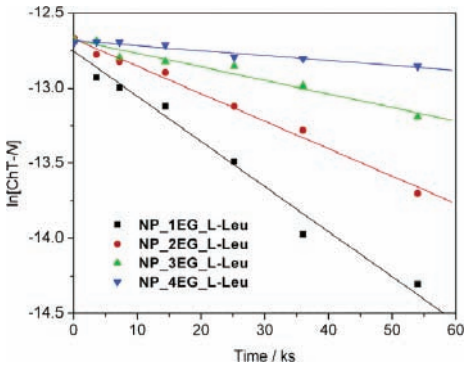


Figure 6. Linear plots for the first-order ChT denaturation in the presence of L-Leu terminated MMPCs with different OEG tethers in 5 mM sodium phosphate buffer (pH 7.4).

functionalized MMPCs. Good linear regressions are obtained, confirming the first-order reaction (see Supporting Information). It can be seen from Figure 6 that the denaturation rates decrease with the length increase of OEG linkage, i.e., 1EG > 2EG > 3EG > 4EG, which is in accordance with the CD study.

The denaturation rate constants of ChT in the presence of various MMPCs were calculated from their kinetic curves (Figure 7; see also Table S2). The denaturation rate constants vary from 4.1×10^{-7} M^{-1} s^{-1} for **NP_4EG_L-Phe** to 3.1×10^{-5} M^{-1} s^{-1} for **NP_1EG_L-Asp**. For the series of 3EG and 4EG, the denaturation rates of ChT decrease with increasing hydrophobicity of the side chains. This trend suggests again that the hydrophilic side chains, in particular

(15) Prime, K. L.; Whitesides, G. M. *J. Am. Chem. Soc.* **1993**, *115*, 10714−10721.

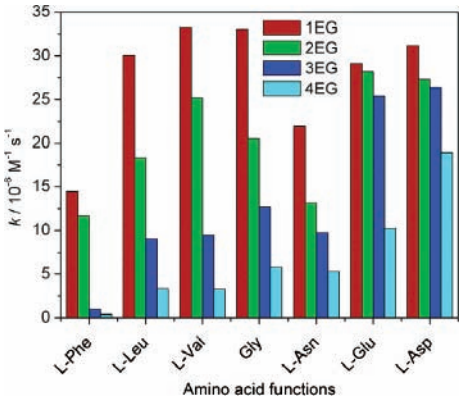


Figure 7. Histograms of first-order denaturation rate constants of ChT upon incubation with MMPCs bearing various amino acid side chains and OEG tethers in 5 mM sodium phosphate buffer (pH 7.4).

anionic, promote the denaturation. For the series of 1EG and 2EG, however, drastic denaturation of ChT was observed for all amino acids. Such results are attributed to the exposure of the interior alkyl chains to the protein surface, accelerating the conformational mutation of the protein through nonspecific hydrophobic interaction.[15] The denaturation rates of MMPCs that bear the same amino acid function but different OEG length all increase in the order 4EG < 3EG < 2EG < 1EG. The spacers of three or more EG units significantly reduce the nonspecific interaction of proteins with interior hydrophobic shell and preserve the native structure of proteins. Therefore, tunable denaturation of proteins can be accomplished by proper combination of the amino acid functions and the OEG tethers in MMPCs.

In summary, we have demonstrated that hydrophobic amino acid side chains favor the retention of the protein structure. Access to the interior alkyl chains, however, denatures the protein as a result of nonspecific hydrophobic interactions. These interactions are essentially eliminated by introducing tethers with three or more EG units. The tunable denaturation of proteins is thus achievable by adjusting either the recognition elements (amino acids) or the OEG linkers.

Acknowledgment. This research was supported by the National Institutes of Health (GM 59249).

Supporting Information Available: Synthesis and characterization of ligands and the preparation and ^{1}H NMR spectra of corresponding MMPCs, representative CD spectra, and plots for the first-order denaturation of ChT. This material is available free of charge via the Internet at http://pubs.acs.org.

OL052367K