J|A|C|S
COMMUNICATIONS

Published on Web 11/22/2006

Highly Enantioselective Recognition of Dicarboxylic Acid Substrates by the Control of Nonlinear Responses

Tomohiro Ikeda, Osamu Hirata, Masayuki Takeuchi,* and Seiji Shinkai*

Department of Chemistry and Biochemistry, Graduate School of Engineering, Kyushu University, Fukuoka 819-0395, Japan

Received September 29, 2006; E-mail: taketcm@mbox.nc.kyushu-u.ac.jp

The design of synthetic allosteric systems is of great significance not only to regulate the complexation ability or the catalytic activity of synthetic receptors but also to attain high selectivity in a nonlinear fashion.[1−7] The pivotal feature of positive homotropic allostery is a nonlinear sigmoidal response to outside information, for example, the effector and/or substrate concentration, which then generates bistable OFF/ON states.[8] The control of nonlinear sigmoidal responses (binding isotherms) would allow the generation of high selectivity and specificity toward the effectors and/or substrates for the precise processing of molecular information as those found in nature.

We previously reported that a cerium(IV) bis(porphyrinato) double-decker complex, **DDPy8**, exhibits positive homotropic allostery on binding cyclohexane-(1*R*,2*R*)-dicarboxylic acid (RR-**CHDA**) to form the 1:4 **DDPy8**·(RR-**CHDA**)$_4$ complex[9,10] (Figure 1). If we could synthetically input the structural information of RR-**CHDA** into such an allosteric host so that the conformation of host is complimentary to RR-**CHDA**, the host should then display different responses toward each enantiomer. The change in the resulting binding isotherms generates the different kind of OFF/ON states and allows opening of a concentration window within which highly enantioselective recognition is expected (see Figure S2). To confirm the hypothesis and demonstrate the high selectivity, here we designed **DDPy7-R**, which was a compound analogous to the 1:1 **DDPy8**·RR-**CHDA** complex. The structural information of RR-**CHDA** was introduced into the host molecule via a covalent amide bond and intra-hydrogen-bonding interaction (Figure 1). We used computational methods (Insight II and Discover) to evaluate whether **DDPy7-R** could form a complex with both enantiomers. We confirmed that **DDPy7-R** could recognize three equivalents of RR-**CHDA** via seven hydrogen bonds without significant conformational changes; by contrast, one of the porphyrin planes of **DDPy7-R** should rotate or oscillate[11,12] with dissociation of the internal hydrogen bond in order to form a complex with SS-**CHDA** through six hydrogen bonds (Figure S3).

We evaluated the formation of the **DDPy7-R**−**CHDA** complexes in a tetrachloroethane (TCE)−tetrahydrofuran (THF) 30:1 (v/v) mixed solvent at 298 K using the circular dichroism (CD) spectral change that is induced upon the successive addition of **CHDA** (Figure S4). The value of the CD intensity at 310 nm increased for RR-**CHDA** with tight isosbestic points, whereas it decreased for SS-**CHDA**. These changes and the shape of the CD signals are consistent with those observed for the **DDPy8**−**CHDA** complexation systems.[9,10] It is important to note that a plot of the CD intensity at 310 nm versus [**CHDA**] displayed a sigmoidal curvature for SS-**CHDA**, whereas saturation-type behavior was observed for the RR-**CHDA** binding isotherm (Figure 2a). In order to analyze these binding isotherms and evaluate the association constants, we estimated the stoichiometries of the complexes formed between **DDPy7-R** and RR-**CHDA** or SS-**CHDA** using continuous-variation

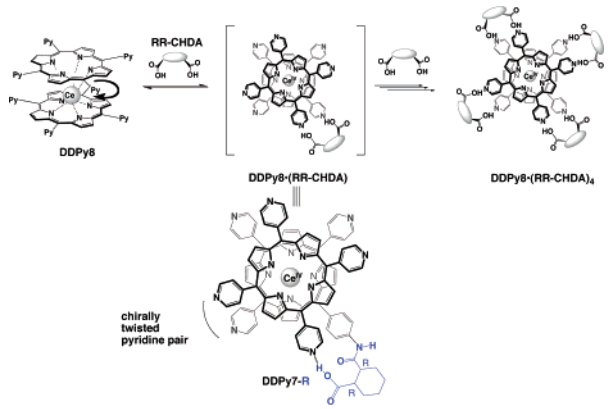

Figure 1. Schematic illustration of the allosteric binding system for **DDPy8** and the chemical structure of **DDPy7-R**. The presence of the internal hydrogen bond in **DDPy7-R** was confirmed by the disappearance of the hydrogen-bond-induced circular dichroism spectrum upon addition of triethylamine (Figure S1).

plots. The results clearly indicate the formation of the 1:3 **DDPy7-R**·(**CHDA**)$_3$ complex (Figure S5). Initially, these guest bindings were analyzed using the Hill equation.[13] From the slope and the intercept of the linear (Hill) plots, we obtained n_H and log$K$; n_H values were calculated as 1.6 for the **DDPy7-R**·(RR-**CHDA**)$_3$ complex and 2.9 for the **DDPy7-R**·(SS-**CHDA**)$_3$ complex (Figure S6). The n_H values of 1.6 and 2.9 imply that **CHDA** binding takes place cooperatively, as a higher value of n_H (>1.0) is related to a higher degree of cooperativity. For RR- and SS-**CHDA**, we reanalyzed the binding isotherm by a nonlinear curve-fitting method assuming the stepwise association scheme; we determined the stepwise association constants for RR-**CHDA** (K_n/M^{-1}) to be logK_1 = 3.1, logK_2 = 3.4, logK_3 = 2.9, and logK_{total} = 9.4 (ΔG^{RR} = −53.3 kJ·mol^{-1}; correlation coefficient R = 0.996) and for SS-**CHDA** (K_n/M^{-1}) to be logK_1 = 0.7, logK_2 = 2.7, logK_3 = 4.7, and logK_{total} = 8.1 (ΔG^{SS} = −46.1 kJ·mol^{-1}; R = 0.994). In the case for the SS-**CHDA** binding to the host, almost no intermediate 1:1 and 1:2 species exists under the equilibrium (Figures S7 and S8); in fact, we also evaluated the association constant logK_{total} to be 8.1 assuming the direct 1:3 complex formation (R = 0.994).

The ratio of this first binding constant for SS-**CHDA** to that for the RR-**CHDA** is large (227), and the ratios of K_1·K_2 and K_1·K_2·K_3 between them were also calculated to be 1260 and 18, respectively. We infer that the multiple equilibrium would amplify the ratio (the energy difference) in the initial recognition process because, within the highlighted concentration window of 0−0.6 mM in Figure 2a, the meaningful amount of 1:1, 1:2, and 1:3 complexes of **DDPy7-R**·RR-**CHDA** form, whereas more than 99 and 97% of the host is uncomplexed under the conditions of [SS-**CHDA**] = 0.4 and 0.6 mM, respectively (see Figure S7). We can thus expect that extremely high enantioselectivity toward RR-**CHDA** should be

10.1021/ja067001x CCC: $33.50

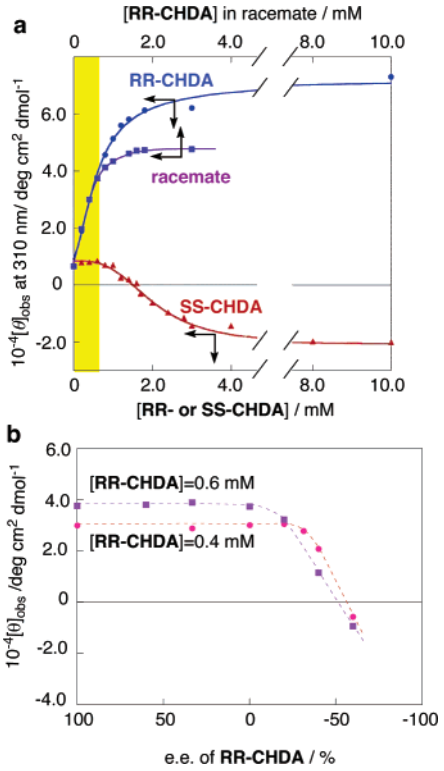

Figure 2. CD spectroscopic titrations and schematic illustrations of this cooperative binding of **CHDA**. (a) Plots of the CD intensity change at 310 nm of **DDPy7-R** (0.10 mM) versus [RR-**CHDA**] (blue line), [SS-**CHDA**] (red line), and [RR-**CHDA**] in the racemate (purple line). The blue and red lines represent the fitted theoretical curve assuming the stepwise association scheme (see text). (b) Competitive titration experiments under the conditions of [RR-**CHDA**] = 0.40 and 0.60 mM and [**DDPy7-R**] = 0.10 mM in a 30:1 TCE−THF (v/v) mixed solvent at 298 K (cell length = 1.0 mm).

attained, although the energy difference between the 1:3 RR-**CHDA** and 1:3 SS-**CHDA** complexes is just 7.2 kJ·mol^{-1} at 298 K.

Titration using a racemate provides information regarding how **DDPy7-R** exhibits high enantioselectivity toward RR-**CHDA** over SS-**CHDA**. In Figure 2a, we superimposed the result for the titration of the racemate onto those for the titration of enantiomerically pure RR-**CHDA** and SS-**CHDA**, where the upper *x*-axis shows the concentration of RR-**CHDA** in the racemic substrate. It is important to note that the change in the complexation-induced CD intensities upon the addition of the racemic substrate was not the sum of the differences between the single enantiomers; indeed, it traced the change of RR-**CHDA** until the concentration of RR-**CHDA** in the racemate reached 0.6 mM ([racemate] = 1.2 mM). In the concentration window from 0 to 0.6 mM, **DDPy7-R** ([**DDPy7-R**] = 0.10 mM) recognizes only RR-**CHDA** in the racemate to produce **DDPy7-R**·RR-**CHDA** complexes, as further supported by ^{1}H NMR experiments; the chemical shifts of **DDPy7-R**·**CHDA** ([racemate] = 0.8 mM) complex were almost the same as that of **DDPy7-R**·RR-**CHDA** ([RR-**CHDA**] = 0.4 mM), whereas SS-**CHDA** (0.4 mM) caused no change in the chemical shift of **DDPy7** (Figure S9).

To demonstrate the extremely high enantioselectivity toward RR-**CHDA**, we conducted competitive titration experiments. Upon the addition of SS-**CHDA**, the CD signal of the **DDPy7-R**−RR-**CHDA** complex ([**DDPy7-R**] = 0.10 mM and [(RR-**CHDA**)$_3$] = 0.40 or 0.60 mM) was not affected at all (error is within 2%) until [SS-**CHDA**] reached 0.6 mM (the ee values of RR-**CHDA** are −20 and 0% in the cases of 0.40 and 0.60 mM RR-**CHDA**, respectively) and then gradually decreased because of the competitive formation of the **DDPy7-R**·(SS-**CHDA**)$_3$ complex (Figure 2b). Apparently, **DDPy7-R** exhibits extremely high enantioselectivity toward RR-**CHDA** even under the conditions of −20% ee, whereas the information of an error molecule (SS-**CHDA**) is precisely filtered off by the control of the nonlinear responses ("error filtering"[6]). This is effected by proper incorporation of the structural information of one enantiomer into the host and utilization of multiple equilibrium in allosterism. The concept that we have described in this paper complements the existing techniques in the field of analytical chemistry by providing a new general means of displaying high selectivity toward a target analyte even when the selectivity expected from the energy difference would be low under the conventional 1:1 stoichiometric system.

Acknowledgment. M.T. and T.I. thank Dr. Masato Ikeda for discussion. This study was supported partially by a Grant-in-Aid for Scientific Research B, and the 21st Century COE Program, "Functional Innovation of Molecular Informatics," of the Ministry of Education, Culture, Science, Sports, and Technology (Japan).

Supporting Information Available: Experimental details and spectral data of this system (Figures S1−S9). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) For reviews on synthetic allosteric systems, see: (a) Kovbasyuk, L.; Kramer, R. *Chem. Rev.* **2004**, *104*, 3161. (b) Takeuchi, M.; Ikeda, M.; Sugasaki, A.; Shinkai, S. *Acc. Chem. Res.* **2001**, *34*, 865. (c) Nabeshima, T. *Coord. Chem. Rev.* **1996**, *148*, 151. (d) Rebek, J., Jr. J. *Acc. Chem. Res.* **1984**, *17*, 258.
(2) For recent synthetic allosteric recognition systems, see: (a) Kawai, H.; Katoono, R.; Nishimura, K.; Matsuda, S.; Fujiwara, K.; Tsuji, T.; Suzuki, T. *J. Am. Chem. Soc.* **2004**, *126*, 5034. (b) Gianneschi, N. C.; Cho, S.-H.; Nguyen, S. T.; Mirkin, C. A. *Angew. Chem., Int. Ed.* **2004**, *43*, 5503. (c) Chang, S.-Y.; Jang, H.-Y.; Jeong, K.-S. *Chem.—Eur. J.* **2004**, *10*, 4358. (d) Nabeshima, T.; Saiki, T.; Iwabuchi, J.; Akine, S. *J. Am. Chem. Soc.* **2005**, *127*, 5507. (e) Thordarson, P.; Coumans, R. G. E.; Elemans, J. A. A. W.; Thomassen, P. J.; Visser, J.; Rowan, A. E.; Nolte, R. J. M. *Angew. Chem., Int. Ed.* **2004**, *43*, 4755. (f) Ercolani, G. *Org. Lett.* **2005**, *7*, 803. (g) Jones, P. D.; Glass, T. E. *Tetrahedron* **2004**, *60*, 11057. (h) Hirata, O.; Takeuchi, M.; Shinkai, S. *Chem. Commun.* **2005**, 3805. (i) Wakabayashi, R.; Kubo, Y.; Hirata, O.; Takeuchi, M.; Shinkai, S. *Chem. Commun.* **2005**, 5742. (j) Baylies, C. J.; Riis-Johannessen, T.; Harding, L. P.; Jeffery, J. C.; Moon, R.; Rice, C. R.; Whitehead, M. *Angew. Chem., Int. Ed.* **2005**, *44*, 6909. (k) Katoono, R.; Kawai, H.; Fujiwara, K.; Suzuki, T. *Chem. Commun.* **2005**, 5154. (l) Sessler, J. L.; Tomat, E.; Lynch, V. M. *J. Am. Chem. Soc.* **2006**, *128*, 4184. (m) Heo, J.; Mirkin, C. A. *Angew. Chem., Int. Ed.* **2006**, *45*, 941. (n) Botta, B.; Caporuscio, F.; Subissati, D.; Tafi, A.; Botta, M.; Filippi, A.; Speranza, M. *Angew. Chem., Int. Ed.* **2006**, *45*, 2717.
(3) (a) Changeux, J.-P.; Edelstein, S. J. *Science* **2005**, *308*, 1424. (b) Changeux, J.-P.; Edelstein, S. J. *Neuron* **1998**, *21*, 959.
(4) Perutz, M. *Mechanisms of Cooperativity and Allosteric Regulation in Proteins*; Cambridge University Press: Cambridge, 1990.
(5) Wyman, J.; Gill, S. J. *Binding and Linkage*; University Science Books: Mill Valley, CA, 1990.
(6) Lehn, J.-M. *Supramoleculer Chemistry*; VCH: New York, 1995.
(7) Kern, D.; Zuiderwegy, E. R. P. *Curr. Opin. Struct. Biol.* **2003**, *13*, 748.
(8) Shinkai, S.; Ikeda, M.; Sugasaki, A.; Takeuchi, M. *Acc. Chem. Res.* **2001**, *34*, 494.
(9) Takeuchi, M.; Imada, T.; Shinkai, S. *Angew. Chem., Int. Ed.* **1998**, *37*, 2096.
(10) Ikeda, M.; Takeuchi, M.; Sugasaki, A.; Robertson, A.; Imada, T.; Shinkai, S. *Supramol. Chem.* **2000**, *12*, 321.
(11) Tashiro, K.; Konishi, K.; Aida, T. *J. Am. Chem. Soc.* **2000**, *122*, 7921.
(12) Ikeda, M.; Takeuchi, M.; Shinkai, S.; Tani, F.; Naruta, Y.; Sakamoto, S.; Yamaguchi, K. *Chem.—Eur. J.* **2002**, *8*, 5541.
(13) Connors, K. A. *Binding Constants*; John Wiley & Sons: New York, 1987.

JA067001X