A Fluorescent Molecule-Recognition Sensor with a Protein as an Environmental Factor

Juan Wang, Asao Nakamura, Keita Hamasaki, Hiroshi Ikeda, Tsukasa Ikeda, and Akihiko Ueno*
Department of Bioengineering, Faculty of Bioscience and Biotechnology, Tokyo Institute of Technology, 4259 Nagatsuta-cho, Midori-ku, Yokohama 226

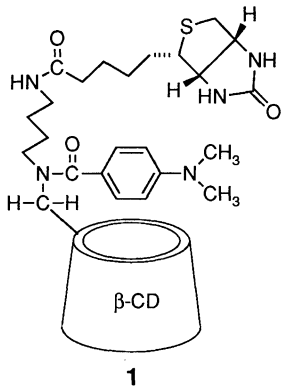
(Received December 1, 1995)

Modified cyclodextrin (**1**), which has p-N,N-dimethylaminobenzoyl and biotin units as a fluorophore and a protein-binding site, respectively, exhibits promoted sensing ability for various organic compounds in aqueous solution in the presence of avidin.

Cyclodextrins (CDs) form inclusion complexes with various organic compounds in aqueous solution. Although they are spectroscopically inert, they can be converted into spectroscopically active hosts by modification with chromogenic or fluorescent units. We found that various spectroscopically active CDs change their spectral intensities associated with guest binding and act as sensors or indicators for molecules.[1,2] On this basis, much attention is currently focused to the effects of the environmental moiety on the sensing abilities of the sensors as shown by an example of monensin-incorporated fluorescent β-CD.[3]

We report here the remarkable effects of a protein (avidin) as an environmental factor in a triad β-CD system (**1**), in which biotin is a protein-binding site, p-N,N-dimethylaminobenzoyl unit is a fluorophore, and β-CD is a receptor site for a guest molecule.

We synthesized **1** by reacting 6-O-tosyl-β-CD with 1,4-diaminobutane, followed by the reactions with biotin at the end of the diaminobutane pendant and then with p-N,N-dimethylaminobenzoic acid, both using dicyclohexyl-carbodiimide as a condensation reagent. The purification of **1** was performed by column chromatography with CM-50 and the obtained product was identified by elemental analysis and 500 MHz ^{1}H-NMR spectra.[4]



1

Figure 1 shows fluorescence spectra of **1** alone (25 μM, (M= mol dm^{-3})) and in the presence of avidin in aqueous solution. Avidin exists as tetramer of the same subunit and here the concentration of **1** is the same as that of the subunit. We previously reported that the fluorophore, p-N,N-dimethylaminobenzoyl moiety, exhibits dual emissions from nonpolar planar (NP) and twisted intramolecular charge transfer (TICT) excited states[2,5] The fluorescence spectrum of **1** alone shows a predominant NP fluorescence peak around 375 nm with an indication of the presence of the TICT fluorescence around 500 nm. The spectrum of **1** in the presence of avidin also shows the predominant NP fluorescence and it is remarkable that its intensity is markedly enhanced (8.4-fold) by avidin. This result demonstrates that the environment of avidin is effective in increasing the NP fluorescence intensity. One possible explanation for this effect of avidin is the insertion of the fluorophore moiety of **1** into the same hydrophobic pocket of avidin as the pocket where biotin is included. In this case, the fluorophore unit is located either in the CD cavity or in the hydrophobic pocket of avidin and the situation is different from that of **1** alone where the fluorophore is located either in the CD cavity or in the bulk water solution.

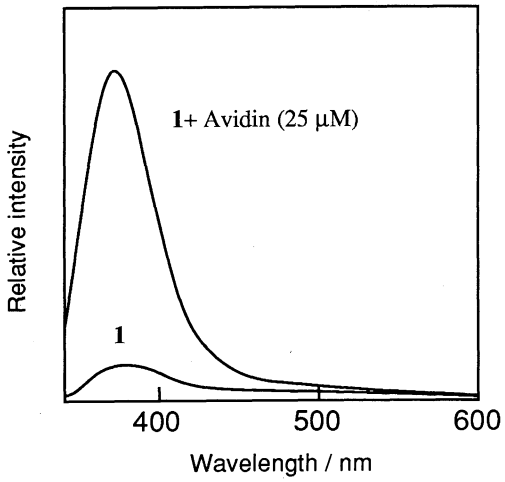
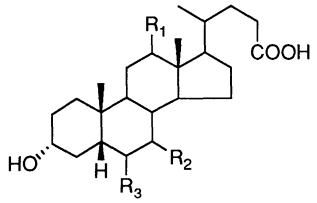


Figure 1. Effect of avidin on fluorescence of **1** (25 μM) in phosphate buffer (pH 7.0) at 25 °C.

The value of $\Delta I_{NP}/I^0_{NP}$ can be used as the sensitivity value where I^0_{NP} and I_{NP} are intensities of NP fluorescence of **1** alone and in the presence of guest, respectively, and $\Delta I_{NP} = I_{NP} - I^0_{NP}$. Figure 2 shows fluorescence variations of **1** alone as a function of the concentration of five steroid compounds such as hyodeoxycholic acid (HDCA), ursodeoxycholic acid (UDCA), chenodeoxycholic acid (CDCA), deoxycholic acid (DCA) and cholic acid (CA). Among these steroidal compounds, the



	HDCA	UDCA	CDCA	DCA	CA
R_1	—H	—H	—H	···OH	···OH
R_2	—H	—OH	···OH	—H	···OH
R_3	···OH	—H	—H	—H	—H

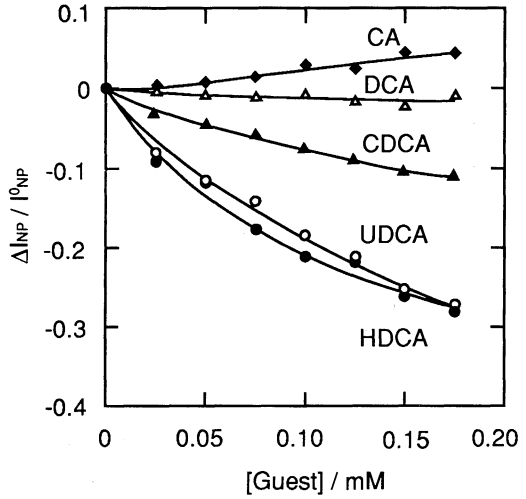


Figure 2. Variations of the fluorescence sensitivity value of **1** (25 μM) as a function of the steroid concentration in phosphate buffer (pH 7.0) at 25 °C. λ_{EX} : 340 nm, λ_{EM} : 375 nm.

compounds except for CA are isomers with one hydroxyl group at different positions or with the opposite orientation of the hydroxyl group at the same carbon. The NP fluorescence intensity decreases with increasing concentration of the guest for all the compounds except for CA, more remarkably in the order of DCA<CDCA<UDCA<HDCA. The binding constants obtained by the analysis of the concentration dependency of the fluorescence intensity are 2650, 3370, and 7150 M^{-1} for CDCA, UDCA, and HDCA, being consistent with the above order.

Figure 3 shows fluorescence variations of **1** in the presence of avidin as a function of the concentration of the guest. It is surprising that the NP fluorescence intensity increases with increasing concentration of the guests although the concentration dependencies for DCA and CA are almost negligible.

The sensitivity order of the avidin-incorporated system is CDCA<UDCA<HDCA, being consistent with the binding constants 14100, 30100, and 43000 M^{-1} for CDCA, UDCA, and HDCA. It is noted that the degrees of the fluorescence variations and the binding constants are remarkably enlarged in this system.

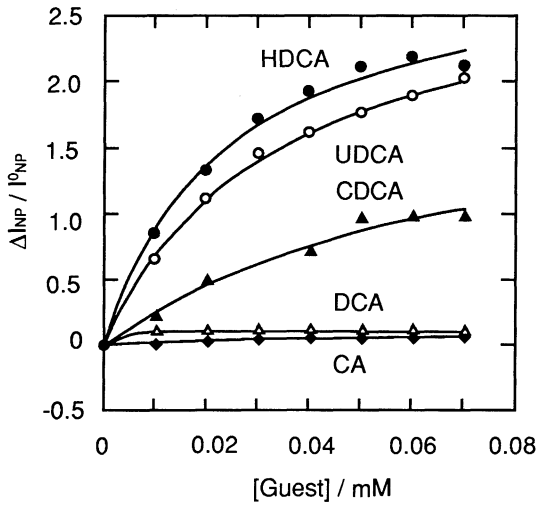


Figure 3. Variations of the fluorescence sensitivity value of **1** (10 μM) in the presence of avidin (subunit: 10 μM) as a function of the steroid concentration in phosphate buffer (pH 7.0) at 25 °C. λ_{EX} : 340 nm, λ_{EM}: 380 nm.

The above data might be related with the locational change of the fluorophore moiety from the CD cavity to the hydrophobic pocket of avidin, occurring associated with guest accommodation in the cavity of **1**. Although further study is needed to clarify the location of the fluorophore moiety in avidin, these results suggest that proteins are very useful as the environment that leads to highly responsive and selective sensors for molecules in aqueous solution.

This research was supported by the Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture, Japan.

References and Notes

1 A. Ueno, I. Suzuki, and T. Osa, *J. Am. Chem. Soc.*, **111**, 6391 (1989); A. Ueno, T. Kuwabara, A. Nakamura, and F. Toda, *Nature*, **356**, 136 (1992); A. Ueno, *Adv. Mater.*, **5**, 132 (1993); M. Nakamura, T. Ikeda, A. Nakamura, H. Ikeda, A. Ueno, and F. Toda, *Chem. Lett.*, **1995**, 343.

2 K. Hamasaki, H. Ikeda, A. Nakamura, A. Ueno, and F. Toda, *J. Am. Chem. Soc.*, **115**, 5035 (1993); K. Hamasaki, A. Ueno, F. Toda, I. Suzuki, and T. Osa, *Bull. Chem. Soc. Jpn.*, **67**, 516 (1994).

3 M. Nakamura, A. Ikeda, N. Ise, T. Ikeda, H. Ikeda, F. Toda, and A. Ueno, *J. Chem. Soc., Chem. Commun.*, **1995**, 721.

4 The structure of **1** was fully characterized by the spectroscopic analysis. The selected data are as follows. ^1H NMR (D_2O) δ 7.27 (d, 2H), 6.76 (d, 2H), 5.15-4.88 (m, 7H), 4.40 (q, 1H), 4.20-3.40 (m, 47H), 3.08 (br, 6H), 2.76 (d, 1H), 2.25 (t, 2H), 1.80-1.38 (m, 10H).

5 The effects of native CDs on TICT emission were reported in the following papers; G. S. Cox, P. J. Hauptman, and N. J. Turro, *Photochem. Photobiol.*, **39**, 597 (1984); A. Nag, and K. Bhuttacharyya, *Chem. Phys. Lett.*, **151**, 474 (1988); A. Nag, R. Dutta, N. Chattopadhyay, and K. Bhuttacharyya, *Chem. Phys. Lett.*, **157**, 83 (1989); A. Nag, and K. Bhuttacharyya, *J. Chem. Soc., Faraday Trans.*, **86**, 53 (1990).