On-and-Off Control of Allosteric Affinity toward Flavin Mononucleotide by the Use of a Pseudocyclophane Formed with Cu(I) as an Effector

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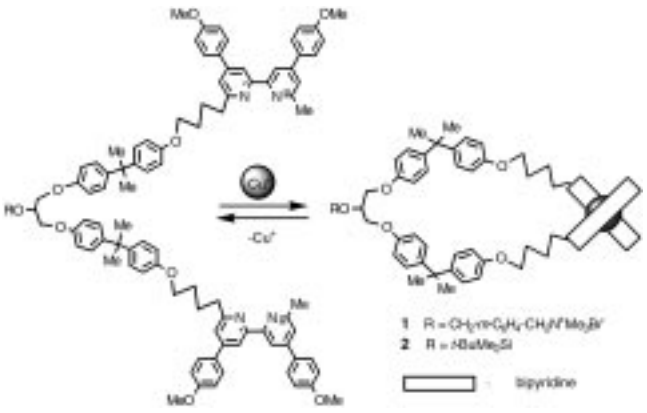
Molecular functions of biological systems such as the catalytic activity of enzymes, molecular recognition of receptors, etc., are often controlled by allostery to maintain the material balance necessary for life.[1] The allostery of the molecular functions is also very important and useful in the construction of artificial functional systems[2] regulated by molecular information.[3,4a]

We have designed artificial ionophores that contain two bipyridine moieties to bind a Cu(I) ion as an effector.[5] Quantitative conversion from its less selective form to the more selective one was performed. Recently, control of molecular recognition by metal coordination has been reported.[6] However, on−off action of the recognition ability in situ has not been achieved by the systems utilizing a metal ion. Here, we wish to report the first example of an allosteric system *reversible* in situ by the use of host **1** for binding flavin mononucleotide (FMN), which is an important cofactor of various redox enzymes.[7] Host **1** consists of two dimethyldiphenylmethane skeletons derived from 4,4′-isopropylidenediphenol, two 2,2′-bipyridine moieties, and an ammonium group (Scheme 1). Upon complexation of **1** with a Cu(I) ion, the formation of *pseudocyclophane* bearing a binding cavity with the ammonium moiety in close proximity results in a considerable *positive allosteric effect* on extraction and transport of FMN.

Hosts **1** and **2** were prepared according to a synthetic route shown in Scheme 2. A bathochromic shift and a MLCT band (λ_{max} = 479 nm in $CHCl_3$−CH_3CN (95:5, v/v)) characteristic of a tetrahedral complex of bipyridines with Cu(I)[5] were observed in the absorption spectrum of **1**·Cu(I). On addition of CuCl, resonances of the picolyl methyl and methylene protons of **1** were shifted upfield (3.01 → 2.71 ppm and 2.69 → 2.48 ppm, respectively, in $CDCl_3$−CD_3CN−CD_3OD (47:3:50, v/v)) by an anisotropic effect of the bipyridine moieties due to the tetrahedral geometry because the two bipyridine parts are forced into a close and orthogonal geometry.[5] Downfield shifts were also observed in protons of the bipyridine moieties. This result supports coordination of the ligands to Cu(I), too. Both UV−vis and ^{1}H NMR spectroscopic titrations using the spectral changes described above suggested the exclusive formation of a 1:1 complex between **1** (or **2**) and Cu(I) with a tetrahedral geometry. A FABMS spectrum of Cu(I) complex of **1** ascertained a 1:1 stoichiometry (*m*/*z* 1645, $[M - Br]^+$, M calcd for **1**·CuCl). We call such a framework *pseudocyclophane* because the cyclic structure is maintained by metal coordination instead of covalent bonding, as seen in the pseudocrown ethers.[5]

Allosteric control of FMN recognition was achieved in an extraction experiment using a 1,2-dichloroethane−water biphasic system. After mixing with an aqueous solution of FMN sodium salt (**10**) and a 1,2-dichloroethane solution containing the host, changes of concentrations of **10** in the aqueous phase were determined from the decrease in the

Scheme 1. Formation and Decomposition of the Multirecognition System



Scheme 2. Synthesis of the Artificial Allosteric Hosts (1 and 2)

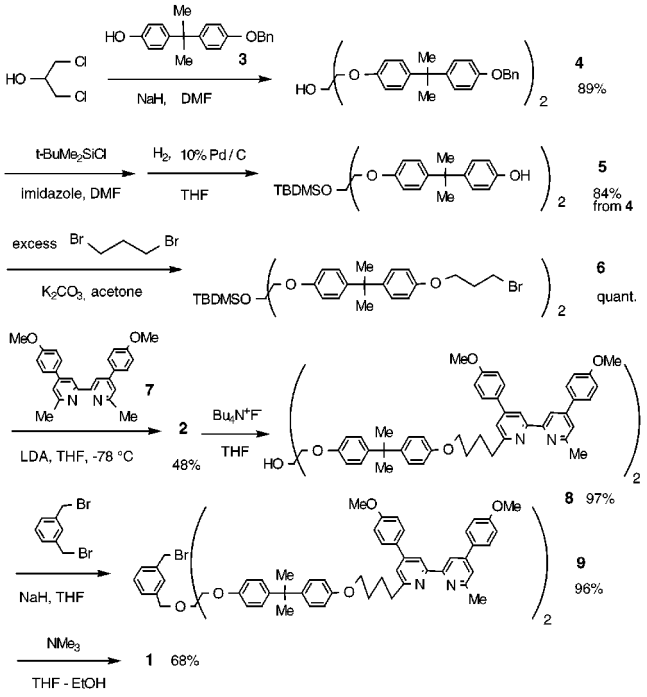


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Table 1. Solvent Extraction of Flavin by the Multirecognition System[a]

host	decrease of $[flavin]_{aq}$[b] (%) $[\mathbf{10}]_{aq}$	$[\mathbf{11}]_{aq}$	host	decrease of $[flavin]_{aq}$[b] (%) $[\mathbf{10}]_{aq}$	$[\mathbf{11}]_{aq}$
1	20 ± 2	3 ± 1	**2** + Cu^+	3 ± 1	<1
1 + Cu^+	56 ± 5	1	**7** + Cu^+	2 ± 1	1
2	5 ± 1	<1	none	1	0

[a] Organic phase; CH_2ClCH_2Cl/CH_3CN = 10:0.1 (v/v), [**1**] = [**2**] = 6.0×10^{-5} M, [**7**] = 1.2×10^{-4} M, $[Cu^+] = 6.6 \times 10^{-5}$ M; aqueous phase; $[flavin]_0$: the initial concentration of flavins in the aqueous phase, 6×10^{-5} M, monitored at 445 nm. [b] Decrease of $[flavin]_{aq}$ (%) = $([flavin]_0 - [flavin]_{aq})/[flavin]_0(100)$, where $[flavin]_{aq}$ is the concentration of flavins in the aqueous phase after mixing.

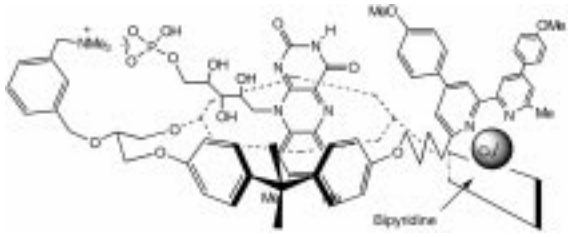

Figure 1. Plausible structure of **1**·Cu(I)·**10**.

absorbance of **10** at 445 nm in the aqueous phase (Table 1). As reflected by such a decrease for **1**, a considerable allosteric effect was observed using CuCl as an effector. In the absence of Cu(I), the degree of decrease is small (20 ± 2%). However, the concentration of **10** in the aqueous phase is decreased drastically (56 ± 5%) in the presence of Cu(I). Furthermore, a mixture of **7** and Cu(I) did not affect the amount of **10** in the aqueous phase significantly (2 ± 1%). Thus, the large decrease observed in the mixture of **1** and Cu(I) results from the interaction between the pseudocyclophane and **10**. In contrast, **2** bearing a *t*-$BuMe_2Si$ group instead of an ammonium moiety does not exhibit an effective allostery (5 ± 1% without Cu(I), 3 ± 1% with Cu(I)). Absorption spectra of the organic phases suggested that under these conditions both **1** and **2** are converted to the corresponding 1:1 complexes with Cu(I). A large enhanced decrease of guest concentrations did not occur in the case of riboflavin (**11**), since it does not contain a phosphate group. These results clearly indicate that presence of the cavity and the ammonium group is necessary to recognize **10**. Namely, the pseudocyclophane with the ammonium moiety in close proximity to the cavity exhibits a very good affinity toward **10**. This affinity may be explained by cooperative binding with the two sites in a fashion as illustrated in Figure 1, which is also suggested by CPK model inspection.

Moreover, reversal of the recognition of **10** by **1**·CuCl was easily achieved. The absorbance of **10** in the aqueous phase is highly recovered (77 ± 2% of the initial value of **10**, i.e., 23 ± 2% decrease) by the addition of bathocuproine (**12**, 4 equiv to **1**) into the biphasic system. This recovery is caused by formation of the very stable tetrahedral **12**·Cu(I) complex. From ^1H NMR spectroscopy, the Cu(I) ion bound in **1** is completely removed by 4 equiv of the highly Cu(I) selective reagent **12**[8] in $CDCl_3-CD_3CN-CD_3OD$ (47:3:50, v/v). Instead of **12**, $Et_3NH^+Cl^-$ was also employed as an additive to recover **10** into the aqueous phase (94 ± 2% of the initial value of **10**, i.e., 6 ± 2% decrease). This inhibition suggests the importance of electrostatic interaction between the ammonium moiety of **1**·Cu(I) and the phosphate anion of **10**. Hence, the allosteric recognition is switched on with Cu(I) and off with **12** and prohibited by $Et_3NH^+Cl^-$. As far as we know, there is no example of an artificial allosteric recognition system switchable in situ, to date.

Table 2. Transport of Flavins by 1 through a Liquid Membrane[a]

host	optical density of flavins in the receiving phase after 4 d FMN Na (**10**)	riboflavin (**11**)
1	0.05	0.02−0.03
1 + Cu^+	0.22	0.03
2	0.04	0.02
2 + Cu^+	0.07	0.02
7 + Cu^+	0.07	0.02
none	0.03	0.01−0.02

[a] Organic phase (1% $CH_3CN-CH_2ClCH_2Cl$) 50 mL: [host] = 1.2×10^{-4} M, [**7**] = 2.4×10^{-4} M, $[Cu^+] = 1.3 \times 10^{-4}$ M. Source phase (dist H_2O) 4 mL: [**10**] = 1.2×10^{-3} M, [**11**] = 3×10^{-4} M. Receiving phase (dist H_2O) 40 mL monitored at 445 nm.

Transport ability of **1** toward **10** through a 1,2-dichloroethane layer as a liquid membrane using a dual cylindrical cell[5] is regulated allosterically with Cu(I). Amounts of **10** (after 4 days) in the receiving phase determined by absorption spectroscopy are summarized in Table 2. A small amount of **10** was carried in the absence of **1**. The amount of the guest transported by **1** was about twice as much as that of the control. In the presence of Cu(I), the rate of the transport of **10** is increased by ca. 4-fold compared to **1** alone. If the control experiment is taken into account, the overall enhancement by **1** and Cu(I) together is 10-fold. In contrast, host **2** does not transport **10** efficiently, even when the complexation with Cu(I) occurs. The interaction between **1** and **10** was not examined in the same solvent system due to the very low solubility of **10**. However, the complexation of **1** with Cu(I) does not enhance transport ability toward **11**, although a half amount of the guest is transported without **1**. Thus, these results also indicate the importance of multirecognition by the pseudocyclophane cavity and the ammonium moiety in close proximity for a high performance of the FMN binding. However, all data obtained here are consistent with the fact that the multiple recognition of the **1**·Cu(I) complex toward **10** is caused by the ammonium group and the cavity of the pseudocyclophane produced upon the complexation with Cu(I). In addition, ESIMS spectroscopy strongly suggests the complexation of **1**·Cu(I)·**10** because the molecular ion, $[\mathbf{1}\cdot Cu\cdot\mathbf{10} - Na - Br]^+$, was observed and the isotope patterns are in good accordance with the theoretical values.

Accurate structural analysis of the interactions between **1**·Cu(I) and **10** was not carried so far. Further study is necessary to elucidate the binding ability in detail. Nonetheless, switchable allostery for the solvent extraction and transport was observed in the present system. The concept described here is essential for the construction of molecular systems with sophisticated functions whose activities are controlled by information at the molecular level. Further extension using the present framework for allosteric regulation of molecular recognition for other biologically important molecules and of catalytic activities is in progress.

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Supporting Information Available: Details of the synthetic procedures, ^1H and ^{13}C NMR data for all new compounds, ESIMS data of **1**·Cu·**10**, and spectroscopic titrations for complexation with Cu(I) (14 pages).

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