HOST-GUEST INTERACTIONS WITH HYDROPHOBIC CYCLOPHANES IN AQUEOUS MEDIA

Yukito Murakami
Department of Organic Synthesis
Faculty of Engineering, Kyushu University
Hakozaki, Higashi-ku, Fukuoka 812
Japan

ABSTRACT. Hydrophobic cyclophanes containing amide nitrogens in a rigid macrocyclic skeleton and flexible hydrocarbon chains, branched out at such nitrogen atoms, were prepared and their substrate-binding behavior was studied in aqueous media. The fluorescence spectroscopy was primarily adopted for the investigation of host-guest interactions by the aid of various hydrophobic probes. These host molecules provide cavities that are deep and hydrophobic enough to incorporate hydrophobic substrates of various bulkiness through an induced-fit mechanism originated from the flexible character of the alkyl branches. In addition to the hydrophobic interaction, the roles of electrostatic and charge-transfer interactions in molecular recognitions were clarified. Much hydrophobic, less polar, and highly viscous binding sites for hydrophobic guest molecules were provided by the octopus azaparacyclophane bearing eight hydrocarbon chains and the tetraazacyclotetradecane-capped azaparacyclophane having four flexible hydrocarbon chains connecting both macrocycles.

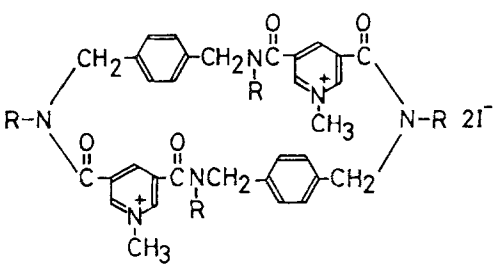
INTRODUCTION

Macrocyclic compounds with a sizable internal cavity have been extensively investigated from the viewpoint of host-guest chemistry. The most important driving force for molecular recognition in aqueous media is the hydrophobic interaction, and cyclophanes are typical artificial hosts capable of providing such hydrophobic binding sites. Moreover, when additional functional sites are introduced into appropriate positions within host molecules in terms of non-covalent interactions (electrostatic, charge-transfer, and so on) as well as metal-coordination interactions, the host-guest interaction is expected to be much more enhanced. The pseudo-intramolecular interactions within inclusion complexes can be readily examined by the aid of CPK molecular models.

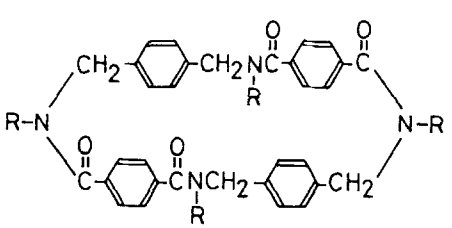
In the past decade, we have prepared various [20]- and [10.10]paracyclophanes to explore novel host-guest interaction systems and found that these host molecules demonstrate two different incorporation modes, penetration and face-to-face, toward various hydrophobic guest mole-

Journal of Inclusion Phenomena **2**, 35–47. 0167-7861/84.15.

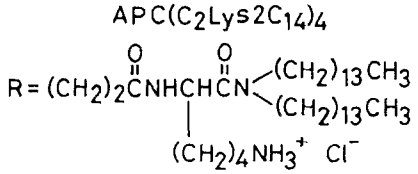
cules [1]. We have also prepared azaparacyclophanes containing amide nitrogens in the cyclic skeleton and four long alkyl chains as branches of the skeleton to provide deeper and efficient binding sites for various hydrophobic guest molecules by cooperation of flexible alkyl chains in aqueous media [2,3]. In order to enhance the binding ability of azaparacyclophanes, we now prepared the following three types of novel cyclophanes: (i) an azacyclophane having two pyridinium moieties in the macrocyclic skeleton in addition to four hydrophobic chains, $APy^{+}C(C_{10}CO_2H)_4$; (ii) an azaparacyclophane having eight long alkyl chains (octopus cyclophane), $APC(C_2Lys2C_{14})_4$; (iii) an azaparacyclophane constructed with two rigid macrocyclic skeletons, a large macrocycle of [3.3.3.3]paracyclophane and a small one of cyclotetradecane, both macrocycles being connected with four flexible hydrocarbon chains, capped-APC. The pyridinium moiety of $APy^{+}C(C_{10}CO_2H)_4$ is a good acceptor for the charge-transfer interaction, so that this molecule may act as a potent host for various guests having electron-donating character. On the other hand, $APC(C_2Lys2C_{14})_4$ and capped-APC, both providing a hydrophobic binding site with a large cavity size, may behave as effective host molecules for bulky and hydrophobic guests. This article describes the characteristic substrate-binding behavior of these azacyclophanes in aqueous media in comparison with that of the related azacyclophanes, $APC(C_nCO_2H)_4$, $APC(C_{10}CO_2Me)_4$, $APC(C_{10}N^{+})_4$, $APC(C_{10}N)_2(C_{10}N^{+}Im)_2$, and $APyC(C_{10}CO_2H)_4$

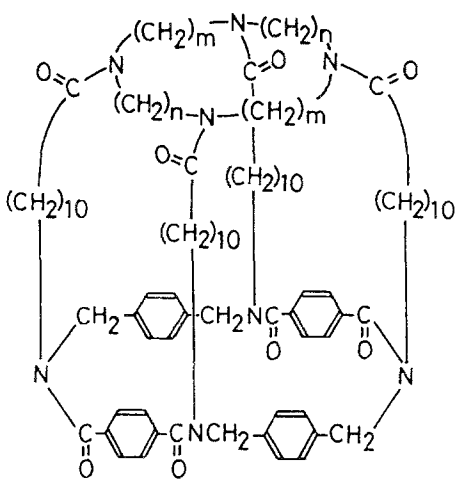


$APy^{+}C(C_{10}CO_2H)_4$
$R=(CH_2)_{10}CO_2H$

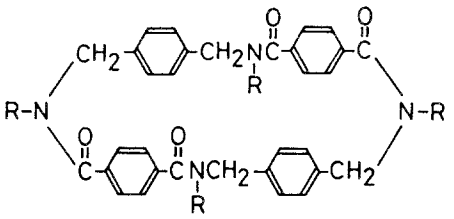


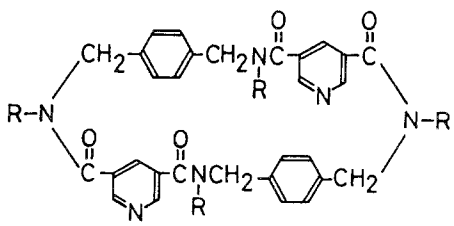
$APC(C_2Lys2C_{14})_4$





capped-APC
(m=3,n=2 ; m=2,n=3)





$APC(C_nCO_2H)_4$ (n=2,10) : $R=(CH_2)_nCO_2H$

$APC(C_{10}CO_2Me)_4$: $R=(CH_2)_{10}CO_2CH_3$

$APC(C_{10}N^+)_4$: $R=(CH_2)_{10}N^+(CH_3)_3\ I^-$

$APC(C_{10}N)_2(C_{10}N^+Im)_2$:

$R=2/4(CH_2)_{10}N(CH_3)_2$, $2/4(CH_2)_{10}N^+(CH_3)_2(CH_2Im)\ Cl^-$

$APyC(C_{10}CO_2H)_4$: $R=(CH_2)_{10}CO_2H$

MATERIALS AND METHODS

Preparation of Azacyclophanes

N,N',N'',N'''-Tetrakis(10-carboxydecyl)-2,6,11,20,24,29-hexaaza[3.3.3.3]-metaparametaparacyclophane-3,10,21,28-tetraone, $APyC(C_{10}CO_2H)_4$, was prepared by the condensation of N,N'-bis(10-methoxycarbonyldecyl)-p-xylylenediamine [2] with pyridine-3,5-dicarbonyl dichloride under high dilution conditions and subsequent alkaline hydrolysis. The pyridyl nitrogens of $APyC(C_{10}CO_2H)_4$ were quaternized with methyl iodide to give N,N',N'',N'''-tetrakis(10-carboxydecyl)-6,24-dimethyl-2,6,11,20,24,29-hexaaza[3.3.3.3]metaparametaparacyclophane-3,10,21,28-tetraone diiodide, $APy^+C(C_{10}CO_2H)_4$. N,N',N'',N'''-Tetrakis(2-carboxyethyl)-2,11,20,29-tetraaza[3.3.3.3]paracyclophane-3,10,21,28-tetraone, $APC(C_2CO_2H)_4$, was prepared according to a procedure similar to that used for the synthesis of N,N',N'',N'''-tetrakis(10-carboxydecyl)-2,11,20,29-tetraaza[3.3.3.3]-paracyclophane-3,10,21,28-tetraone, $APC(C_{10}CO_2H)_4$ [2]. The synthetic procedure for N,N',N'',N'''-tetrakis[2-[N-[1-(N,N-ditetradecylcarbamoyl)-5-ammonio-1-pentyl]carbamoyl]ethyl]-2,11,20,29-tetraaza[3.3.3.3]paracyclophane-3,10,21,28-tetraone tetrachloride, $APC(C_2Lys2C_{14})_4$, is shown in Scheme 1. The tetraazacyclotetradecane-capped azaparacyclophane,

Scheme 1

$$PhCH_2OC(O)NH(CH_2)_4CHCO_2H \xrightarrow[(ii)\ HCO_2H]{(i)HN(C_{14}H_{29})_2/DCC} PhCH_2OC(O)NH(CH_2)_4CHC(O)NR_2$$

$$(CH_3)_3COC(O)NH \qquad\qquad NH_2$$

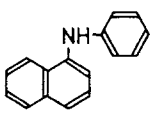
$$A \quad (R = C_{14}H_{29})$$

$$APC(C_2CO_2H)_4 \xrightarrow[(iii)\ HBr/CF_3CO_2H,\ (iv)ion\text{-}exchange]{(i)SOCl_2,\ (ii)A/N(C_2H_5)_3} APC(C_2Lys2C_{14})_4$$

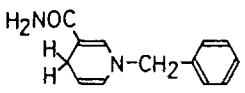
capped-APC, was synthesized by the condensation of the acid chloride form of $APC(C_2CO_2H)_4$ with 1,4,8,11-tetraazacyclotetradecane under high dilution conditions. All the products were purified by gel-filtration chromatography and identified by spectral and chemical analyses. The synthetic procedures for other cyclophanes, $APC(C_{10}CO_2Me)_4$, $APC(C_{10}N^+)_4$, and $APC(C_{10}N)_2(C_{10}N^+Im)_2$, have been reported previously [2,3].

Substrates

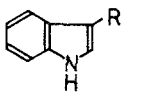
The following fluorescent probes were chosen as substrates: N-phenyl-1-naphthylamine (PNA), 1-benzyl-1,4-dihydronicotinamide (BNAH), indole, indolyl-3-acetic acid, 8-anilinonaphthalene-1-sulfonate (ANS), 6-p-toluidinylnaphthalene-2-sulfonate (TNS), and 1-dimethylaminonaphthalene-5-sulfonamidoethyltrimethylammonium (DASP).



PNA

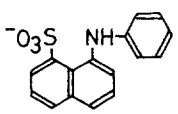


BNAH

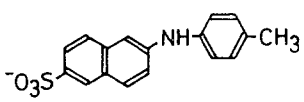


Indole : R=H

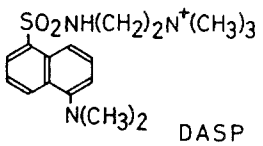
Indolyl-3-acetic acid : $R=CH_2CO_2H$



ANS



TNS



DASP

Measurements of Binding Constants

The substrate-binding behavior of the azaparacyclophanes was examined by fluorescence spectroscopy in the following aqueous buffers (0.01 mol dm^{-3}, μ 0.10 with KCl) containing 5 - 10%(v/v) organic solvent at 30.0 °C: 2-(N-morpholino)ethanesulfonate (MES), 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonate (HEPES), and 3-(cyclohexylamino)propanesulfonate (CAPS) at pH 6.0, 8.0, and 10.0, respectively. In general, fluorescence spectra of the guest molecules were measured by changing concentrations of the host molecules. Binding constants for the inclusion complexes were calculated on the basis of the Benesi-Hildebrand-type relationship (eq. 1) [4] derived under an assumption that only the 1:1 host-guest interaction takes place.

$$1/\Delta I = 1/(\Delta I_c[G]_0) + 1/(\Delta I_c K[G]_0[H]_0) \qquad (1)$$

Here, ΔI is the extent of fluorescence intensity change upon addition of a host, ΔI_c stands for the difference in fluorescence intensity between bound and free guest molecules, and $[G]_0$ and $[H]_0$ are the total concentrations of guest and host molecules, respectively. Good linear correlations of $1/\Delta I$ vs. $1/[H]_0$ were obtained for all the measurements.

Fluorescence Polarization Spectroscopy

The fluorescence polarization (P) was calculated by eq. 2, where I is the fluorescence intensity, and the subscripts v and h refer to the orientations, vertical and horizontal, respectively, for the excitation and analyzer polarizers in this sequence. C_f is the grating correction factor, given by I_{hv}/I_{hh}.

$$P = (I_{vv} - C_f I_{vh})/(I_{vv} + C_f I_{vh}) \quad (2)$$

The P value is also given by eq. 3, where τ is the fluorescence lifetime of a probe, ρ is the relaxation time for rotation of a probe, and P_0 refers to the maximal value of P in the absence of any rotational motion of a probe [5].

$$1/P - 1/3 = (1/P_0 - 1/3)(1 + 3\tau/\rho) \quad (3)$$

RESULTS AND DISCUSSION

Induced-Fit Incorporation of Octopus-Like Azacyclophanes [2,3]

Multiple hydrophobic alkyl chains of azacyclophanes may be arranged in the same direction due to their mutual hydrophobic interactions in aqueous media, while in non-aqueous media they are presumably extended to minimize their mutual interactions. Thus, such hydrophobic branches may provide a deep hydrophobic cavity in aqueous media. We have studied the substrate-binding ability of octopus-like azacyclophanes, $APC(C_{10}CO_2H)_4$ and $APC(C_{10}N^+)_4$, spectrophotometrically using several organic dyes as guest molecules. The appearance of isosbestic points is consistent with the formation of inclusion complexes in 1:1 stoichiometry, and the spectral data were analyzed according to the Benesi-Hildebrand equation [4]. Cationic dyes such as Rhodamine 6G and Quinaldine Red as well as a suitably bulky neutral dye, 1-(2-pyridylazo)-2-naphthol (PAN), undergo complex formation with an anionic host, $APC(C_{10}CO_2H)_4$, while an anionic dye, Orange G, and PAN do so with a cationic host, $APC(C_{10}N^+)_4$. The binding constants lie in a range of 10^2 - 10^3 mol^{-1} dm^3. On the other hand, cationic and anionic dyes do not show any spectral change upon addition of $APC(C_{10}N^+)_4$ and $APC(C_{10}CO_2H)_4$, respectively. Furthermore, dyes having a charge opposite to the host molecule are incorporated into the hydrophobic cavities of azacyclophanes more tightly than neutral ones of similar sizes. These results indicate that both electrostatic and hydrophobic interactions contribute effectively to the overall substrate-binding process of these octopus-like

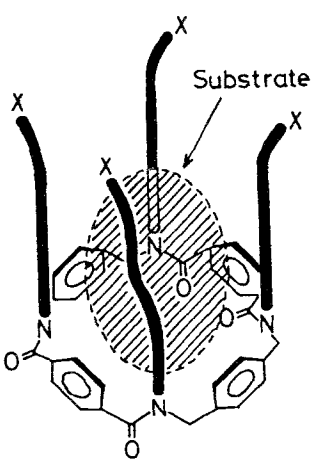


Figure 1. Schematic representation of the substrate-binding mode of octopus-like azacyclophane.

cyclophanes. It is interesting to note that the incorporation of the above dyes into the cavities in 1:1 stoichiometry is not disturbed by the aggregate formation of host molecules. The substrate-binding mode is schematically shown in Figure 1. Since these azaparacyclophanes can incorporate substrates of various molecular sizes, the induced-fit mechanism must be operative in the binding process. In addition, we found that the octopus-like azacyclophane bearing two imidazolyl groups at the end of two hydrophobic chains, $APC(C_{10}N)_2(C_{10}N^+Im)_2$, acts as an effective esterase model showing the induced-fit catalysis [3].

Charge-Transfer Effect on Substrate-Binding Behavior of Octopus-Like Azacyclophanes [6]

It is well-known that the charge-transfer (CT) interaction is one of the intrisically important interactions for molecular recognition. We examined the contribution of such a CT effect on the overall host-guest interaction in aqueous media by employing the following azacyclophanes as host molecules; $APC(C_nCO_2H)_4$ (n = 2, 10), $APyC(C_{10}CO_2H)_4$, and $APy^+C(C_{10}CO_2H)_4$. The acceptor efficiency increases in this sequence. The substrates employed are classified into three categories as follows: PNA as a nonionic, bulky, and poor donor, ANS as an anionic, bulky, and poor donor; BNAH as a nonionic, medium-sized, and good donor; indole as a nonionic, small, and excellent donor, indolyl-3-acetic acid as an anionic, small, and excellent donor.

The fluorescence intensity of PNA increases upon complex formation with the azacyclophanes. The binding constant increases in the following order: $APC(C_{10}CO_2H)_4 > APyC(C_{10}CO_2H)_4 > APy^+C(C_{10}CO_2H)_4 \simeq APC(C_2CO_2H)_4$ (TABLE I). The results give a useful piece of information in two aspects: (i) the deep hydrophobic cavity constructed with four hydrocarbon chains

TABLE I. BINDING CONSTANTS FOR THE INCLUSION COMPLEXES OF AZACYCLOPHANES

Substrate[a]	$10^{-3}K$[b] / mol^{-1} dm^3			
	$APC(C_2CO_2H)_4$	$APC(C_{10}CO_2H)_4$	$APyC(C_{10}CO_2H)_4$	$APy^+C(C_{10}CO_2H)_4$
PNA	c)	1.6	0.73	c)
BNAH	c)	2.0	1.4	5.8
Indole	2.5	2.5	6.7	13
Indolyl-3-acetic acid	1.2	1.2	3.0	13

a) Excitation and emission wavelengths (nm) were 340 and 460 for PNA, 375 and 515 for ANS (no complex formation), 361 and 465 for BNAH, 277 and 347 for indole, and 280 and 363 for indolyl-3-acetic acid, respectively. b) In an aqueous CAPS buffer (pH 10.0) containing 5%(v/v) dimethylsulfoxide at 30.0 °C. Concentrations in mol dm^{-3}: guests, 1.0 x 10^{-5}; hosts, 5.0 x 10^{-5}- 5.0 x 10^{-4}. c) Any spectral change due to complex formation was not detected.

and one macrocyclic skeleton of the octopus-like cyclophanes is required for the strong binding interaction with bulky substrates such as PNA; (ii) as hydrophobicity of a macrocyclic skeleton is reduced, the host-guest interaction is weakened. In addition, the polarity parameters for the microenvironments where the PNA molecule is incorporated were evaluated from the maximal wavelengths of fluorescence spectra. As shown in Figure 2, the fluorescence maximum of PNA is shifted to lower wavelength with decrease in medium polarity. The microenvironments for the substrate-binding sites of these cyclophanes are nearly equivalent to that in water: polarity parameter $E_T(30)$ [7], 61.0 and 62.6 for the inclusion complexes with $APC(C_{10}CO_2H)_4$ and $APyC(C_{10}CO_2H)_4$, respectively. No spectral changes due to complex formation were detected when the anionic ANS was used as a guest for these anionic hosts. This substrate-binding behavior is consistent with that observed by the electronic absorption spectroscopy for the interaction between $APC(C_{10}CO_2H)_4$ and various organic dyes.

When BNAH was used as a guest, an increase in fluorescence intensity was observed upon complex formation with $APC(C_{10}CO_2H)_4$ and $APyC(C_{10}CO_2H)_4$. However, the host-guest interaction of $APy^+C(C_{10}CO_2H)_4$ with BNAH decreased the fluorescence intensity as shown in Figure 3. It is well

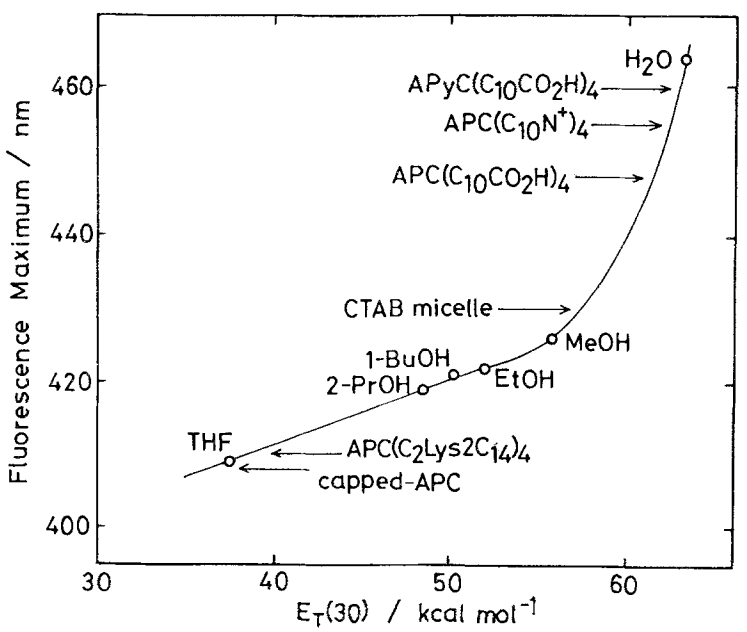


Figure 2. Solvent effect on fluorescence of PNA: in an aqueous MES buffer (pH 6.0) containing 5%(v/v) ethanol for $APC(C_2Lys2C_{14})_4$ and $APC-(C_{10}N^+)_4$; in an aqueous HEPES buffer (pH 8.0) containing 10%(v/v) ethanol for capped-APC; in an aqueous CAPS buffer (pH 10.0) containing 5%(v/v) dimethylsulfoxide for $APC(C_{10}CO_2H)_4$, $APyC(C_{10}CO_2H)_4$, and $APy^+C(C_{10}CO_2H)_4$.

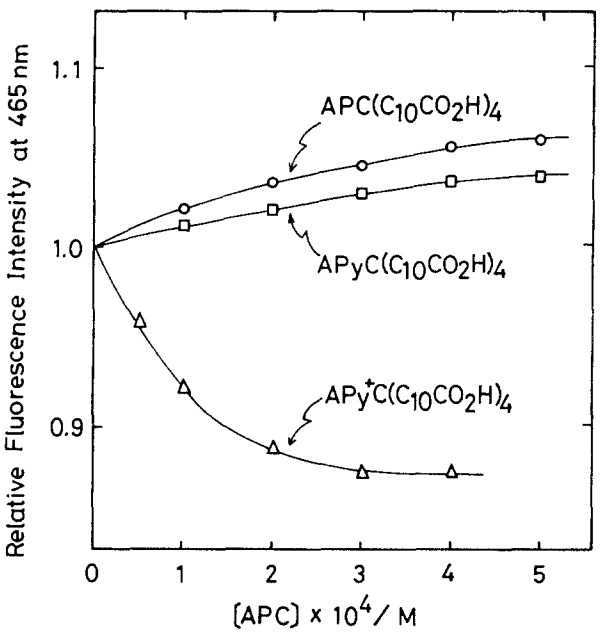


Figure 3. Correlations between azacyclophane concentration ([APC]) and fluorescence intensity of BNAH (1.0×10^{-5} mol dm^{-3}) in an aqueous CAPS buffer (pH 10.0) containing 5%(v/v) dimethylsulfoxide; excitation wavelenth at 361 nm.

known that 1,4-dihydronicotinamides form CT complexes with their oxidized forms and related electron-deficient pyridinium derivatives, and that the formation constants for such CT complexes are relatively small in aqueous media [8]. The fluorescence from BNAH is quenched by the CT interaction with its oxidized form, 1-benzylnicotinamide chloride, and the formation constant (4 mol^{-1} dm^3) is in good agreement with the value evaluated by the measurements of electronic absorption spectra (3.76 mol^{-1} dm^3) [8]. Thus, the decrease in fluorescence intensity observed for the $APy^+C(C_{10}CO_2H)_4$ – BNAH system is caused by the CT interaction between the pyridinium moieties of the host and the dihydropyridine ring of the guest. The fluorescence spectra for the guest was also measured in the presence of potassium iodide. Any detectable effect of the iodide ion on the fluorescence phenomenon was observed under the conditions employed ($[I^-] \leqq 1 \times 10^{-3}$ mol dm^{-3}). The contribution of such a CT interaction gives rise to the stabilization of the inclusion complex (TABLE I).

The indolyl moiety is also a good donor for the CT interaction [9], and quenching of its fluorescence upon formation of the CT complex with the nicotinamide group has been reported [10]. It is noteworthy that the fluorescence intensities of indole and indolyl-3-acetic acid decrease as any of the present azacyclophanes was added. The binding constant with respect to the host molecules increases in the following order:

$APC(C_2CO_2H)_4 \simeq APC(C_{10}CO_2H)_4 < APyC(C_{10}CO_2H)_4 < APy^+C(C_{10}CO_2H)_4$. This sequence seems to reflect the extent of CT interaction between the electron-deficient acceptor groups in the macrocyclic skeletons and the guest molecules. In this case, the small donor molecule may be completely incorporated into the cavities formed with the macrocyclic skeletons, and the hydrophobic and electrostatic interactions with the long chain segments, which have been observed for the host-guest interaction between the azacyclophanes and larger guest molecules, are not so much important for complexation.

The binding modes of octopus-like azacyclophanes toward various guest molecules are divided into two categories. For small molecules which can be completely incorporated into the macrocyclic cavity, the CT interaction is the most important one for the host-guest association, and the hydrophobic interaction with the long chain segments makes much less contribution to the overall substrate-binding behavior. On the other hand, for guest molecules larger than the macrocyclic cavity size, the induced-fit function exercised by the four hydrophobic chain segments is the predominant factor controlling the substrate incorporation and the CT interaction is only an additional one for substrate recognition.

Substrate-Binding Behavior of Octopus Azaparacyclophane, $APC(C_2Lys2C_{14})_4$ [11]

In order to enhance further the substrate-binding ability that was observed for the octopus-like azacyclophanes, we now prepared an azacyclophane having eight hydrophobic chains, $APC(C_2Lys2C_{14})_4$, and investigated its performance as a hydrophobic host molecule. The CPK molecular model study strongly suggests that this host molecule incorporates hydrophobic substrates of various bulkiness into the hydrophobic cavity which is well shielded from the bulk aqueous phase (Figure 4).

In the light of the critical aggregate concentration (CAC) of $APC(C_2Lys2C_{14})_4$ obtained by the surface tension method based on the Wilhelmy principle (1×10^{-4} mol·dm^{-3}), all the spectroscopic measurements were carried out below its CAC. $APC(C_{10}N^+)_4$ was used as a reference host, and the following fluorescent probes were chosen as guest molecules: ANS and TNS as anionic ones, PNA as a nonionic one, and DASP as a cationic one. The fluorescence intensity for anionic and nonionic guests increased upon addition of the host, and the binding constants thus obtained are summarized in TABLE II. The binding constants for the host-guest interactions of $APC(C_2Lys2C_{14})_4$ with the anionic and nonionic guest molecules are much greater than the corresponding values for $APC(C_{10}N^+)_4$, whereas both of the cationic hosts do not exhibit any binding affinity toward the cationic guest. Thus, the octopus azaparacyclophane, $APC(C_2Lys2C_{14})_4$, is the potent hydrophobic host showing the substrate selectivity due to the electrostatic interaction.

The microscopic polarities of environments around the incorporated guest molecules were evaluated from their fluorescence maxima. As shown in Figure 2, $APC(C_2Lys2C_{14})_4$ provides a microenvironment equivalent to that provided by THF, which is less polar than those provided by the hexadecyltrimethylammonium bromide (CTAB) micelle and the azacyclophanes bearing four hydrophobic chains such as $APC(C_{10}N^+)_4$ and $APC(C_{10}CO_2H)_4$.

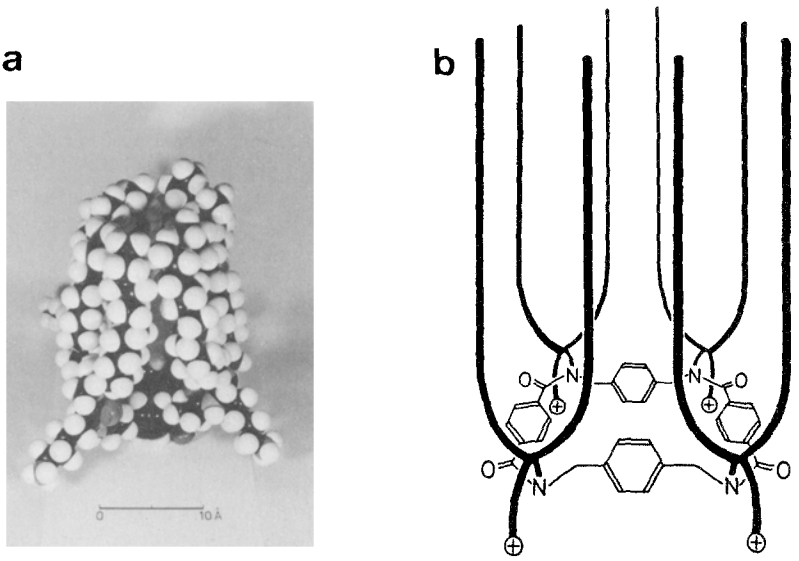


Figure 4. CPK molecular model of APC(C_2Lys2C_{14})$_4$, showing the most efficient hydrophobic association of its eight hydrophobic chains in aqueous media, (a) and its schematic representation (b).

TABLE II. BINDING CONSTANTS (K/mol^{-1} dm^3) AND MICROENVIRONMENTAL POLARITY PARAMETERS (E_T(30)/kcal mol^{-1}) FOR THE INTERACTIONS OF AZACYCLOPHANES WITH VARIOUS GUESTS[a)]

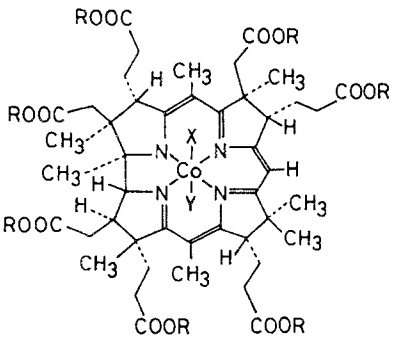
Guest	Host			
	APC(C_2Lys2C_{14})$_4$		APC($C_{10}N^+$)$_4$	
	K	E_T(30)[b)]	K	E_T(30)[b)]
ANS	2.8 x 10^5	40 (461)	1.1 x 10^4	55 (481)
TNS	3.0 x 10^5	52 (425)	7.5 x 10^3	55 (440)
PNA	1.3 x 10^6	38 (410)	4.6 x 10^3	62 (455)

a) In an aqueous MES buffer (pH 6.0) containing 5%(v/v) ethanol. Concentrations in mol dm^{-3}: guests, 1.0 x 10^{-6}; APC(C_2Lys2C_{14})4, 5.0 x 10^{-6}- 3.0 x 10^{-5}; APC($C_{10}N^+$)$_4$, 5.0 x 10^{-5}- 3.0 x 10^{-4}. Temp: 30.0 °C. No complex formation was detected with DASP as a guest. b) Fluorescence maxima (in nm) are given in parentheses.

The microscopic polarity parameters evaluated with some guest molecules are listed in TABLE II, and APC(C_2Lys2C_{14})$_4$ provides less polar microenvironments for these guests than APC($C_{10}N^+$)$_4$. Relatively large fluorescence polarization (P) values were obtained for the probes incorporated into APC(C_2Lys2C_{14})$_4$: 0.29, 0.31, and 0.21 for ANS, TNS, and PNA, respectively. Meanwhile, the P values are much smaller in ordinary media:

e.g., 0.02, 0.01, 0.006, and 0.002 for PNA in water, 1-butanol, 2-propanol, and THF, respectively. The fluorescence intensity undergoes variation commensurate with the fluorescence lifetime (τ) and was found to increase with a decrease in medium polarity. While ANS and PNA incorporated into APC(C_2Lys2C_{14})$_4$ exhibit intensities nearly identical with those measured in 1-butanol, TNS bound to the host shows an intermediate value between those measured in 1-butanol and water. These results clearly indicate that the large P value observed in the presence of the host primarily reflect the high microscopic viscosity in the hydrophobic cavity of APC(C_2Lys2C_{14})$_4$.

Accordingly, the octopus azaparacyclophane behaves as an effective cationic host for the anionic and nonionic guest molecules, providing a highly apolar and viscous binding site. In addition, the present host molecule incorporate relatively large coenzyme models such as a hydrophobic vitamin B_{12}, heptapropyl dicyanocobyrinate [$(CN)_2$Cob(III)-C3ester] [12], into its large hydrophobic cavity constructed with the rigid macrocyclic skeleton and the eight flexible hydrocarbon chains so that it is promising to utilize the octopus azaparacyclophane as an effective apoenzyme model.



$(CN)_2$Cob(III)C3ester
X=Y=CN, R=C_3H_7

Substrate-Binding Behavior of Capped Azaparacyclophane, capped-APC, [13]

The capped azaparacyclophane, capped-APC, is constructed with two rigid macrocyclic skeletons, a large macrocycle of tetraaza[3.3.3.3]paracyclophane and a small one of tetraazacyclotetradecane, and four flexible hydrocarbon chains connecting both macrocycles.

In an aqueous HEPES buffer (pH 8.0), the binding constant for the inclusion complex of capped-APC with a nonionic guest, PNA, is 5.1×10^4 mol^{-1} dm^3, which is one order larger than that for the complex of APC-($C_{10}N^+$)$_4$ with the same guest (4.6×10^3 mol^{-1} dm^3). Ionic guest molecules are also incorporated into capped-APC with smaller binding constants compared with that for the complex with PNA: 1.7×10^4 and 9.1×10^3 mol^{-1} dm^3 for ANS and TNS, respectively.

The microscopic environment for the substrate-binding site was evaluated from the fluorescence maximum of a selected probe. For guests such as PNA and ANS, whose molecular shapes are similar to each other, capped-APC provides a microenvironment nearly identical in polarity with those provided by THF and the octopus azaparacyclophane [APC(C_2Lys2C_{14})4]: $E_T(30)$ = 37 (see Figure 2). However, capped-APC provides a microenvironment with higher polarity for more slender molecules such as TNS: $E_T(30)$ = 53. The results imply that the hydrophobic cavity size of capped-APC is more suitable for the former guests as compared with the latter. Figure 5 shows a plausible structure of capped-APC complexed with PNA, in which the hydrophobic interaction between the host and guest molecules becomes most effective. The tight molecular interaction tends to

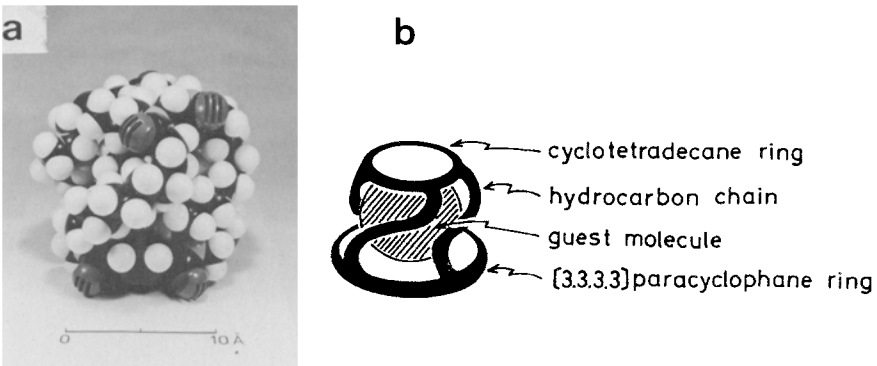


Figure 5. CPK molecular model of the inclusion complex formed with capped-APC and PNA, having the conformation favorable for effective hydrophobic interaction between the host and guest molecules in aqueous media, (a) and its schematic representation (b).

repress the molecular motion of the guest molecule as indicated by a large value of fluorescence polarization (P = 0.33 for PNA). The flexibility of four hydrocarbon chains linking the two macrocycles seems to allow the induced-fit host-guest interaction.

CONCLUSION

We have shown here some novel aspects in designing hydrophobic macrocycles which may undergo effective host-guest interactions in aqueous media. The sterically adjustable three-dimensional space constructed with some hydrophobic and rigid macrocyclic skeleton(s) and flexible hydrocarbon chains can provide a deep and efficient cavity for guest molecules of various bulkiness. The molecular recognition primarily originated from the hydrophobic effect is further advanced by additional non-covalent interactions such as electrostatic and CT modes. The observed multifunctional features of the present host-guest interactions may give us a useful piece of information for manipulation of molecular discrimination. In addition, the substrate-binding sites provided by APC(C_2Lys2C_{14})$_4$ and capped-APC are highly apolar and act to repress the molecular motion of guest molecules. Since these characteristics are accompanied with the desolvation effect, both the octopus and capped azaparacyclophanes can be utilized as models for simulation of enzyme functions.

ACKNOWLEDGMENTS

I wish to express my gratitude to Dr. Jun-ichi Kikuchi and other collaborators and students who have contributed to the work described in this article. The present work was partially supported by research funds from the Ministry of Education, Science, and Culture of Japan.

REFERENCES

[1] Y. Murakami, "Cyclophanes II," Topics in Current Chemistry No. 115, ed by F. Vögtle, Springer-Verlag, Berlin (1983), pp. 107 - 155; and references cited therein.
[2] Y. Murakami, A. Nakano, R. Miyata, and Y. Matsuda, J. Chem. Soc., Perkin Trans. 1, 1979, 1669.
[3] Y. Murakami, A. Nakano, K. Akiyoshi, and K. Fukuya, J. Chem. Soc., Perkin Trans. 1, 1981, 2800.
[4] H. A. Benesi and J. H. Hildebrand, J. Am. Chem. Soc., 71, 2703 (1949).
[5] G. Weber, Adv. Prot. Chem., 8, 415 (1953).
[6] Y. Murakami, J. Kikuchi, and H. Tenma, Chem. Lett., submitted for publication.
[7] C. Reichardt, "Solvent Effects in Organic Chemistry," Verlag Chemie, Weinheim (1979), pp. 270 - 272.
[8] G. Cilento and S. Schreier, Arch. Biochem. Biophys., 107, 102 (1964).
[9] G. Cilento and P. Tedeschi, J. Biol. Chem., 236, 907 (1961); S. Shinkai, K. Tamaki, and T. Kunitake, Bull. Chem. Soc. Jpn., 48, 1918 (1975).
[10] S. F. Velick, J. Biol. Chem., 233, 1455 (1958); S. Shifrin, Biochim. Biophys. Acta, 81, 205 (1964); Y. Murakami, Y. Aoyama, J. Kikuchi, K. Nishida, and A. Nakano, J. Am. Chem. Soc., 104, 2937 (1982).
[11] Y. Murakami, J. Kikuchi, M. Suzuki, and T. Takaki, Chem. Lett., submitted for publication.
[12] Y. Murakami, Y. Hisaeda, and T. Ohno, Bull. Chem. Soc. Jpn., 57, 2091 (1984).
[13] Y. Murakami, J. Kikuchi, and H. Tenma, Chem. Lett., submitted for publication.