Journal of Inclusion Phenomena and Molecular Recognition in Chemistry 7 (1989), 91–97.
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Molecular Recognition of Hydrophobic Ammonium Substrates by a Cationic Octopus Cyclophane Bearing Noncovalently Bound Pyridoxal-5′-phosphate: A Vitamin B_6-dependent Holoenzyme Model

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(Received: 1 February 1988)

Abstract. The inclusion behavior of the octopus cyclophane constructed with a rigid macrocyclic skeleton and eight hydrocarbon chains was studied in aqueous media by means of fluorescence and electronic absorption spectroscopy. Both hydrophobic and electrostatic interactions came into effect in the host–guest complexation process. The cyclophane acted as an effective apoenzyme model for constitution of an artificial vitamin B_6-dependent holoenzyme by simultaneous incorporation of pyridoxal-5′-phosphate and a hydrophobic alkylammonium substrate into the host cavity to give the Schiff-base species, showing the substrate selectivity.

Key words. Cyclophane, pyridoxal-5′-phosphate, Schiff-base, vitamin B_6, hydrophobic interaction, enzyme model.

1. Introduction

Naturally occurring hosts such as enzymes and receptors selectively recognize guest molecules through various interaction modes of both rigid and flexible characters as explained on the basis of the lock-and-key and induced-fit concepts, respectively. In order to mimic extensive functions exerted for molecular recognition in biological systems, cyclophanes having an intramolecular hydrophobic cavity have been widely utilized as artificial host molecules [1]. While most of the cyclophanes previously designed provide a relatively rigid recognition site for hydrophobic guest molecules, we have recently developed so-called octopus cyclophanes, which are capable of providing a large and hydrophobic binding site constructed with a rigid macrocyclic skeleton and flexible hydrocarbon chains in aqueous media [2].

The following characteristic inclusion behavior of the octopus cyclophane has been clarified [2, 3]. (i) The host exercises molecular discrimination toward guests through hydrophobic and electrostatic interactions and strongly binds hydrophobic guest molecules. (ii) The hydrophobic binding site of the octopus cyclophane is highly apolar and acts to repress the molecular motion of guest molecules. (iii) Formation of both 1:1 and 1:2 host–guest complexes is remarkably favored due to the induced-fit binding capability of the host molecule. (iv) The host–guest complex formed with the octopus cyclophane and a hydrophobic vitamin B_{12} derivative acts as an effective vitamin B_{12}-dependent holoenzyme model.

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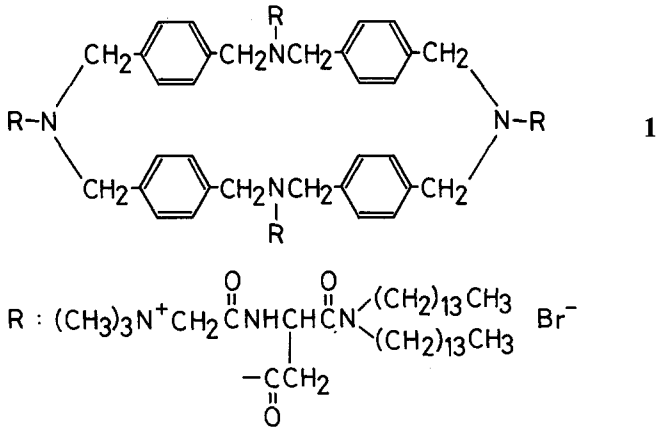
In order to get further insight into the inclusion behavior of the octopus cyclophane, we now prepared a new water-soluble octopus cyclophane and studied its guest-binding behavior for constitution of a vitamin B_6-dependent holoenzyme model.

2. Experimental

2.1. MATERIALS

Preparation of an octopus cyclophane, *N*,*N*′,*N*″,*N*‴-tetrakis[3-(*N*,*N*-ditetradecylcarbamoyl)-3-(trimethylammonio)acetamidopropanoyl]-2,11,20,29-tetraaza[3.3.3.3]paracyclophane tetrabromide (**1**), is to be reported elsewhere [4]; a pale yellow solid, m.p. 136–138°C. Anal. Calcd. for $C_{180}H_{324}Br_4N_{16}O_{12} \cdot 2\,H_2O$: C, 66.31; H, 10.14; N, 6.87%. Found: C, 66.35; H, 9.90; N, 6.85%. All of the guest molecules employed in this work were obtained from commercial sources and used without further purification, as guaranteed reagents. *N*-Phenyl-1-naphthylamine (PNA) was recrystallized from methanol–water (4 : 1 v/v), m.p. 61–62°C.



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2.2. MEASUREMENTS

Elemental analyses were performed at the Microanalysis Center of Kyushu University. Fluorescence and electronic absorption spectra were taken on a Hitachi 650–40 fluorescence spectrometer and a Hitachi 220A spectrophotometer, respectively. The critical aggregate concentration (cac) was determined by surface tension measurements on a Shimadzu ST–1 surface tensometer assembled by the Wilhelmy principle: 2.5×10^{-4} mol dm^{-3} for **1** in aqueous media at room temperature. Thus, the cyclophane concentration was maintained in a range below the cac value for all measurements on the host–guest interactions.

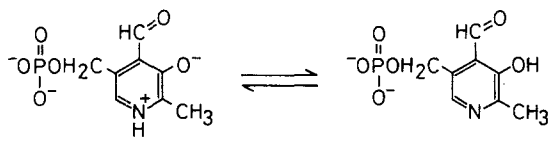
3. Results and Discussion

3.1. CONSTITUTION OF VITAMIN B_6-DEPENDENT HOLOENZYME MODEL

The octopus cyclophane (**1**) showed inclusion behavior toward hydrophobic fluorescent guests in aqueous media, in a similar manner as observed with other octopus cyclophanes previously prepared [2]. For example, the anionic 8-anilinonaphthalene-1-sulfonate (ANS) was incorporated into **1** in a 1:1 stoichiometry with a large binding constant: K_1, 5.3×10^5 dm^3 mol^{-1} in an aqueous 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonate (HEPES) buffer [0.01 mol dm^{-3}, pH 8.0, μ 0.10 (KCl)] at 30.0°C. The microenvironmental polarity of the cyclophane cavity, as evaluated from the fluorescence maximum of ANS (λ_{max}, 467 nm), is equivalent to that provided by 2-propanol; $E_T(30)$, 49 kcal mol^{-1} [2]. While *N*-phenyl-1-naphthylamine (PNA) as a nonionic guest was also incorporated into the hydrophobic cavity of **1** [K_1, 1.0×10^5 dm^3 mol^{-1}; $E_T(30)$, 34 kcal mol^{-1} (λ_{max}, 406 nm)], cationic **1** does not show any binding affinity toward a cationic guest, 1-dimethylaminonaphthalene-5-sulfonamidoethyltrimethylammonium. The results indicate that **1** is a potent hydrophobic host exhibiting molecular discrimination toward guests as originated in the electrostatic effect.

On these grounds, a host–guest complex is expected to be formed between **1** and pyridoxal-5′-phosphate (PLP) under the conditions that PLP behaves as an anionic species. Such a host–guest interaction was examined by electronic absorption spectroscopy in an aqueous HEPES buffer (0.02 mol dm^{-3}, pH 7.0) at 30.0°C.

In the absence of the octopus cyclophane, PLP (1.0×10^{-4} mol dm^{-3}) shows an absorption maximum at 388 nm which originates from the dianionic species (A in Scheme 1) [5]. Addition of **1** to this solution resulted in a red shift of the absorption maximum along with concomitant decrease in its intensity. The isosbestic point was observed at 400 nm by changing the concentration of **1**, and the maximum wavelength was shifted to 392 nm at the host concentration of 2.0×10^{-4} mol dm^{-3}. This spectral change reflects the binding capability of the cationic host toward PLP. We have clarified that an analogous cationic octopus cyclophane is in favor of forming a 1:2 host–guest complex with nonionic guest molecules, but not so with anionic ones. This is due to mutual electrostatic repulsion between the latter guest molecules exercised in the hydrophobic cavity of the host molecule [2]. Thus, a binding constant for the interaction of **1** with PLP was evaluated on the basis of the 1:1 complex formation with reasonable reliability; 6.5×10^4 dm^3 mol^{-1}. Since the complexation was inhibited as the ionic strength of the solution was increased, the electrostatic effect is predominant in the present host–guest interaction. In addition, the octopus cyclophane provides a relatively



Scheme 1.

polar binding site for the hydrophilic PLP molecule because an absorption band due to the uncharged tautomer with respect to the pyridine ring (B in Scheme 1), which is expected to appear at ca. 350 nm [5], was not detected.

3.2. MOLECULAR RECOGNITION THROUGH SCHIFF-BASE FORMATION

We examined the substrate-recognition capability of the present vitamin B_6-dependent holoenzyme model composed of the octopus cyclophane and PLP. Although several types of reactions are catalyzed by vitamin B_6-dependent enzymes, all these reactions are claimed to proceed through formation of a Schiff-base intermediate derived from PLP and a substrate. Thus, our attention was focused here on molecular recognition in the Schiff-base forming process. Alkylamines having various hydrophobic chains were used as substrates in place of α-amino acids for evaluation of the hydrophobic effect on the Schiff-base forming equilibrium. In an aqueous HEPES buffer at pH 7.0, these amines are present as cationic ammonium species. Concentrations of both **1** and PLP were maintained constant, 1.0×10^{-4} mol dm^{-3} each, for the following measurements.

Upon addition of the amine (AA) to the aqueous solution containing **1** and PLP, the extent of the Schiff-base (SB) formation was monitored by electronic absorption spectroscopy. A typical example of the spectral change is shown in Figure 1. Clear isosbestic points were observed for all the measurements. The apparent SB formation constants (K_{SB}) as defined by equation (1) were evaluated according to the Benesi–Hildebrand relationship in a manner that has been reported elsewhere [6]. The K_{SB} values in the absence of the octopus cyclophane were also evaluated. The

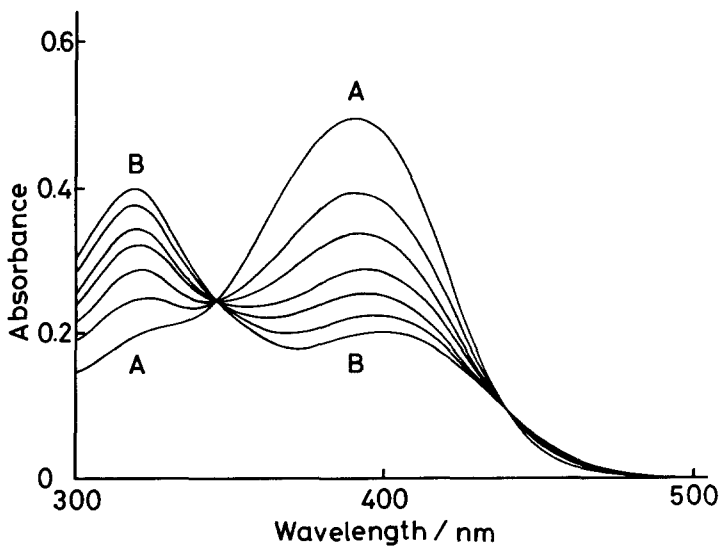


Fig. 1. Electronic absorption spectra for the Schiff-base system derived from PLP (1.0×10^{-4} mol dm^{-3}) and varying amounts of hexylamine in the presence of **1** (1.0×10^{-4} mol dm^{-3}) in an aqueous HEPES buffer (0.02 mol dm^{-3}, pH 7.0) at 30.0°C. Amine concentrations in mmol dm^{-3}: 0, 0.2, 0.4, 0.7, 1.1, 1.7, and 2.4 (read from A to B).

Table I. Apparent formation constants (K_{SB}) for Schiff-bases derived from PLP and alkylammonium species at 30.0°C.[a]

$CH_3(CH_2)_{n-1}NH_3^+$	$K_{SB}/dm^3\ mol^{-1}$	
	Cyclophane system[b]	Without cyclophane
$n = 4$	240	200
$n = 5$	580	200
$n = 6$	2100	290
$n = 8$	120000	230

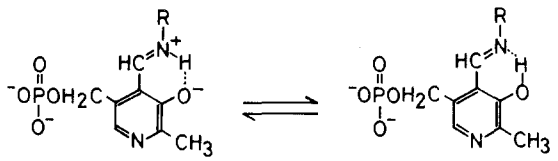
[a] In an aqueous HEPES buffer; 0.02 mol dm^{-3}, pH 7.0.
[b] **1**, 1.0×10^{-4} mol dm^{-3}.

results are listed in Table I. While the K_{SB}

$$K_{SB} = [SB]/([PLP][AA]) \quad (1)$$

values for the various amines are nearly identical with each other in an aqueous phase, the K_{SB} value is markedly dependent on the chain length of AA in the presence of **1**. Thus, the holoenzyme model composed of the octopus cyclophane and PLP clearly discriminates the ammonium substrates through the hydrophobic interaction. In addition, it is noteworthy that the present cationic host shows substrate recognition toward the guests having the same cationic charge when another guest molecule having the opposite charges, i.e. PLP, is concomitantly bound to the octopus cyclophane.

As is apparent from Figure 1, SB shows two absorption bands with maxima at 400 and 318 nm, which are assigned to two tautomeric isomers, C and D in Scheme 2, respectively [7]. Since the relative intensities of these bands sensitively vary depending on the medium polarity, the microenvironmental polarity provided by the octopus cyclophane can be estimated. Figure 2 shows electronic absorption spectra of the SB species formed with PLP and octylamine in the presence and absence of **1**. While SB is present exclusively in form C in an aqueous phase, form D is remarkably favored in the presence of **1**; the microenvironmental polarity in the cavity of **1** is roughly equivalent to that provided by 2-propanol. Accordingly, it is apparent that PLP bound to the hydrophilic site of the octopus cyclophane moves into a more hydrophobic domain of the host molecule via formation of SB with the hydrophobic alkylammonium substrate as schematically illustrated in Figure 3.



C D

Scheme 2.

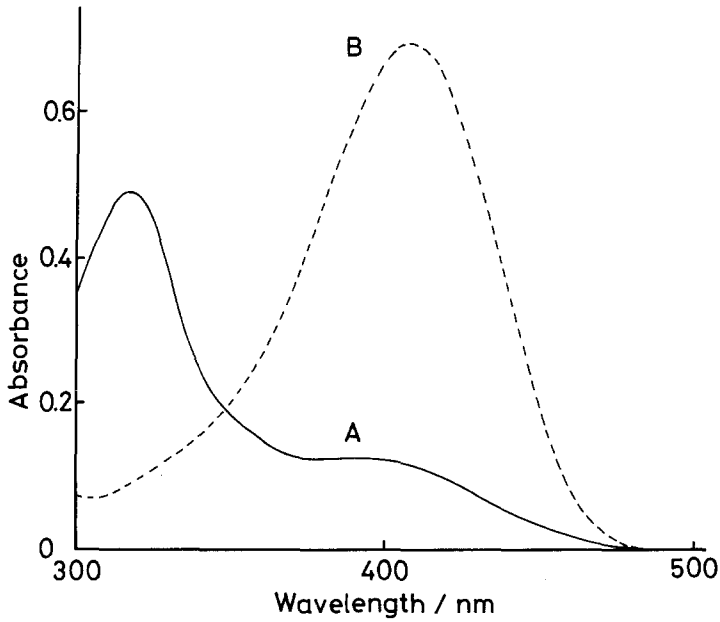


Fig. 2. Electronic absorption spectra of Schiff-base formed with PLP (1.0×10^{-4} mol dm^{-3}) and octylamine in the presence (A) and absence (B) of **1** (1.0×10^{-4} mol dm^{-3}) in an aqueous HEPES buffer (0.02 mol dm^{-3}, pH 7.0) at 30.0°C. Amine concentrations in mol dm^{-3}: A, 2.1×10^{-4}; B, 1.2×10^{-2}.

In conclusion, it became clear that the octopus cyclophane can be utilized as an effective apoenzyme model for constitution of an artificial vitamin B_6-dependent holoenzyme. The ternary complex is formed with **1**, PLP, and a substrate in the initial reaction stage, and then the latter two species bound to **1** undergo Schiff-base formation. Molecular recognition is exercised by the octopus cyclophane in favor of hydrophobic substrates.

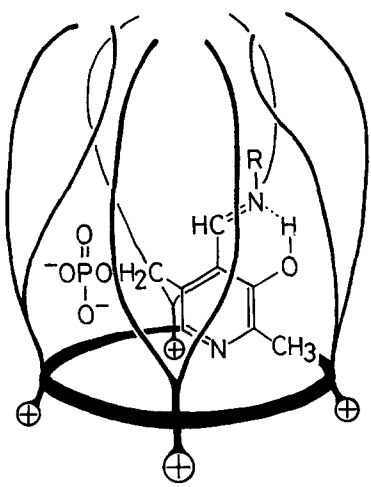


Fig. 3. Schematic representation for hydrophobic incorporation of the PLP Schiff-base into the octopus cyclophane.

Acknowledgement

The present work was partially supported by a Grant-in-Aid for Special Project Research No. 62101002 from the Ministry of Education, Science, and Culture of Japan.

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