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COMMUNICATION

Selective transport of nucleotides mediated by functionalized porphyrin

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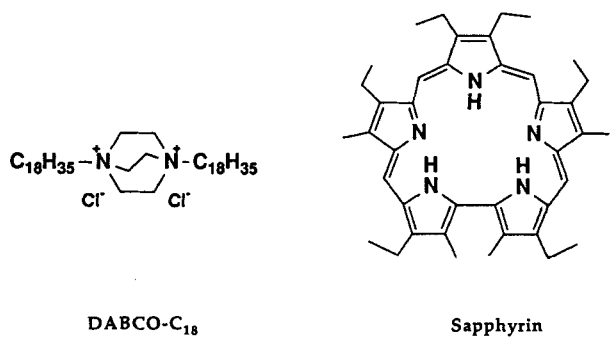
Nucleotide transport through liquid membranes using a zinc(II)-porphyrin having two lipophilic ammonium side chains (1 · Zn) was investigated. Two characteristic features of the present transport system became apparent, i.e. (1) the rate of transport of AMP is much faster than those of other nucleotide, and (2) 1 · Zn is more effective than the corresponding free porphyrin for any nucleotide transport. The selectivity toward AMP remains even under a competitive condition where AMP, GMP, CMP and UMP coexist. The steady state concentration of AMP during the transport is found to be practically same with a maximum concentration observed in a extraction experiment using a simple two phase system. The result strongly suggests that the rate determining step of the present transport system is the substrate releasing step from the membrane $CHCl_3$ phase to the receiver aqueous one.



DABCO-C_{18} Sapphyrin

Figure 1 Carrier molecules for nucleotides.

INTRODUCTION

Molecular recognition of nucleotides and their related compounds is one of most interesting fields in host-guest chemistry. In the course of our efforts to develop artificial receptors, we found that the porphyrin framework is suitable for the nucleobase receptor which shows unique recognition of the substrate through multiple interactions.[1] Based on these results, we have attempted to prepare a new type of a carrier molecule for nucleotide transport system by using a porphyrin framework. To our knowledge, there have been only two examples of nucleoide transport systems where the carrier molecules are designed based on **DABCO**[2] and sapphyrin.[3] Herein we report that a dicationic porphyrin Zn complex acts as the effective carrier of dianionic *nucleotides* in a liquid membrane system.[4] The present third system is more effective than the **DABCO** system and effective under a neutral condition.[5]

RESULTS AND DISCUSSION

Design and syntheses of the carrier

In general, a carrier molecule for nucleotides should satisfy following two requirements, i.e. a) the carrier should extract the nucleotide from an aqueous phase and solubilize it in a hydrophobic membrane phase, b) both of 'on' and 'off' rates in complexation between the carrier and the nucleotide on the membrane surface should be fast enough to carry out the overall transport in a reasonable rate. Thus we designed a new carrier porphyrin **1 · Zn**, in which two lipophilic ammonium side chains may neutralize and solubilize phosphate anions and a central Zn may reversibly interact with the nucleobase unit of the nucleotide in organic solution as described in our previous report.[1b]

The carrier porphyrin was synthesized from mesoporphyrin IX according to the scheme shown in Fig 2. Thus, mesoporphyrin IX was converted to the corresponding mixed anhydride by treatment with acetyl chloride and the resultant product was directly used for the next reaction with the aqueous dimethyl-

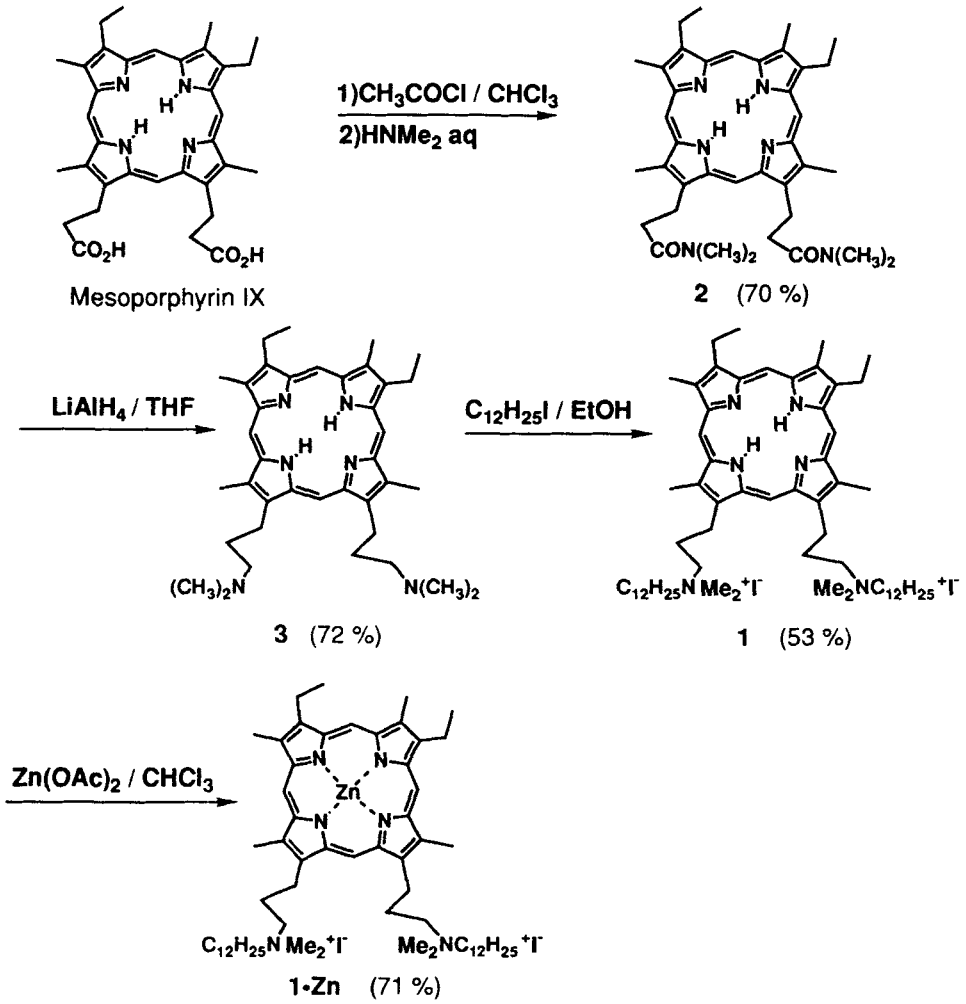


Figure 2 Synthetic route of nucleotide carrier molecule based on mesoporphyrin IX.

amine solution to give the diamide, **2**. Reduction of **3** with lithium aluminium hydride in THF gave the corresponding diamine, **3**, in good yield. Although alkylation of **3** with lauroyl iodide yielded a protonated compound as an initial product, the target molecule **1** was easily obtained by treatment of this initial product with a basic aqueous solution (see experimental section). Since **1** is soluble in the usual organic solvent in spite of existence of two ammonium groups, Zn metalation was able to be carried out under usual conditions using an organic solvent. Both of products, **1** and **1 · Zn**, are practically insoluble in the aqueous solution and soluble in the organic solvent such as chloroform.

Transport of nucleotides

Both **1** and its Zn complex, **1 · Zn**, are highly lipophilic and able to extract nucleotides in an aqueous solution into an organic solvent such as $CHCl_3$. For example, the extraction experiment using the two phase system, H_2O (1.5 mL, 20 mM of AMP·Na_2 salt)—$CHCl_3$ (2.0 mL, 0.1 mM of **1 · Zn**), indicates that, at the equilibrium state, AMP of which amount corresponds to 20% of used **1 · Zn** is transferred from water into $CHCl_3$. Interestingly, the extraction ability of **1** is significantly lower than that of **1 · Zn**, i.e. amount of transferred AMP is only 4% of used **1** under the same condition. During the extraction experiments, no appreciable amount of **1 · Zn** or **1** leaks out the aqueous solution and no precipitation in organic solution is observed.

In order to examine characteristics of nucleotide transport mediated by these dicationic lipophilic porphyrins, following three types of transport experiments were performed, a) usual transport of a single nucleotide, b) competitive transport of AMP, GMP, CMP and UMP, and c) active transport of AMP driven by salt gradient. Thus, the transport rate constant for each nucleotide was determined by using a U-tube type, H_2O (1.5 mL)—$CHCl_3$ (4.0 mL)—H_2O

(1.5 mL), liquid membrane system. Typical time courses of transport of the individual nucleotides are shown in Fig 3 and the transport rate constants are summarized in Table 1 together with those obtained by using other carriers. Two characteristic features of the present transport system are clear, i.e. a) carrier molecules reported here are highly selective for AMP, b) in all cases, **1·Zn** is more effective than **1** and transport efficiency of **1·Zn** is over 10 times higher than that of the **DABCO** derivative.[3] It should be also noted that cis-5,15-bis(2-hydroxyl-1-naphthyl)octaethyl porphyrin Zn complex, **cisBINAPZn**, shows only limited ability as a nucleotide carrier, in spite of its high affinity toward nucleoside such as adenosine in organic medium.[1b] The results indicate that both of the diammonium and porphyrin Zn complex parts of the present carrier play important roles in the present AMP transport. This conclusion is supported by inspection of a space-filling model of the molecule as well as by our previous studies on porphyrin-nucleobase interactions (see Fig 4).[6] Another interesting feature is that, as seen in Fig 3, no significant induction period is observed under the present condition and

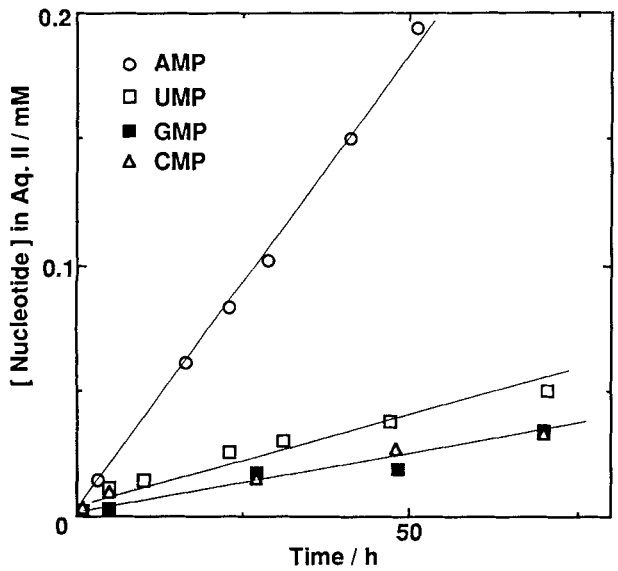


Figure 3 The typical time courses of independent nucleotide transport using **1·Zn** (0.1 mM) as the carrier. In each experiment, the amount of transported nucleotide was measured by the electronic spectra of Aq. II.

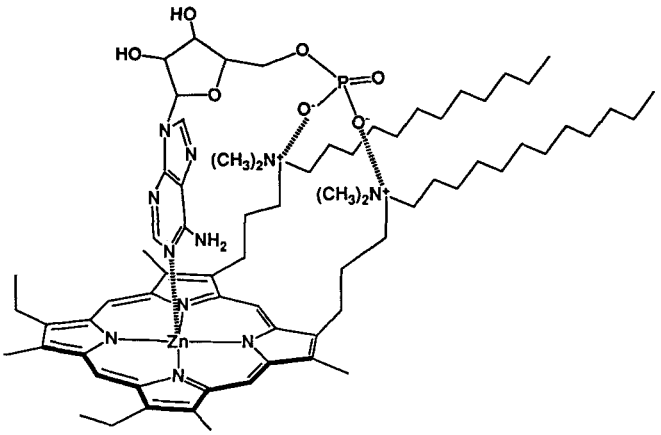


Figure 4 Possible two point recognition between **1·Zn** and AMP.

Table 1 Transport rates of nucleotides across a $CHCl_3$ liquid membrane[a]

Aq.I	*$CHCl_3$*		*Aq.II*	*Transport rate*
Nucleotide (mM)	*Carrier, mM*		*Salt, mM*	*$\mu M/h \cdot cm^2$*
AMP (20)	**1·Zn**	(0.1)	NaBr (20)	3.3
(20)	**1·Zn**	(0.02)	NaBr (20)	0.75
(20)	**1**	(0.1)	NaBr (20)	1.0
(0.5)	**1·Zn**	(0.1)	NaBr (500)-AMP (0.5)[b]	1.3
GMP (20)	**1·Zn**	(0.1)	NaBr (20)	0.41
(20)	**1**	(0.1)	NaBr (20)	0.19
CMP (20)	**1·Zn**	(0.1)	NaBr (20)	0.34
(20)	**1**	(0.1)	NaBr (20)	0.23
UMP (20)	**1·Zn**	(0.1)	NaBr (20)	0.54
(20)	**1**	(0.1)	NaBr (20)	0.29
AMP (20)	Aliquat 336	(1.0)	NaBr (20)	0.12
(10)	**cisBINAPZn**[c]	(3.1)	NaBr (20)	0.11
(10)	**DABCO**	(0.25)	NaBr (10)	0.27[d]
GMP (10), pH 2.5	Sappyrin	(0.1)	pH 10.0	86.9[e]

[a] Initial transport rate at 26 ± 0.5 °C. For details of the experimental apparatus, see ref. 3.
[b] Active transport experiment driven by salt gradient.
[c] See ref. 1b.
[d] See ref. 3.
[e] See ref. 4.

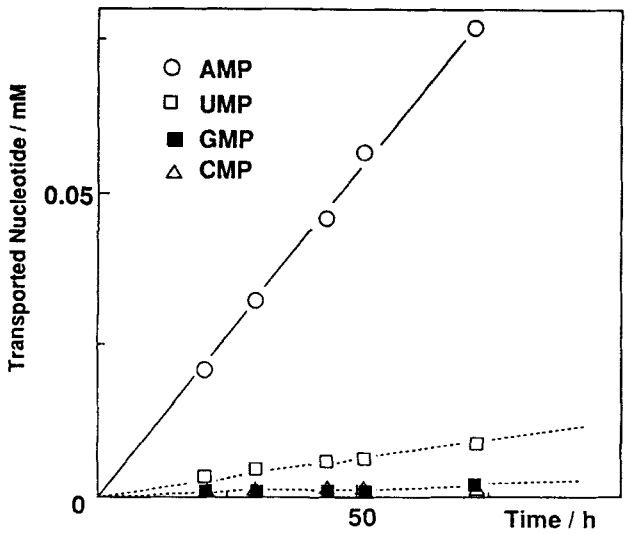


Figure 5 The time course change of the concentration of nucleotides in Aq.II under competitive conditions using **1 · Zn** as the carrier.

HPLC analyses of the $CHCl_3$ phase indicate that, within 2 h, the concentration of AMP attains to the steady state one (ca. 0.02 mM) which is practically the same with that observed for the above mentioned extraction experiment. These observations strongly suggest that the rate determining step of the present transport system is the substrate releasing step from the $CHCl_3$ phase to the receiver one (Aq.II). Furthermore, it is demonstrated by a competitive transport experiment that the observed selectivity of **1 · Zn** toward AMP is retained even in the presence of other nucleotides. Since there are possibilities that kinetic processes other than those involved in the individual nucleotide transport, such as a nucleotide exchange reaction, are operating in the competitive transport, it is important to confirm the selectivity under a competitive condition. Under the condition where AMP, UMP, GMP and CMP (each 20 mM) in Aq.I (1.5 mL), **1 · Zn** (0.1 mM) in the $CHCl_3$ phase (4.0 mL) and NaBr (20 mM) in Aq.II (1.5 mL) are employed, the relative transport rate for AMP/UMP/GMP/CMP is determined to be 41/5/1/1, which shows that, in fact, the present carrier, **1 · Zn**, is selective for AMP transport (see Fig 5).

Finally, we attempt to carry out active transport of AMP where the necessary chemical potential is supplied only by the concentration gradient of NaBr between Aq.I and Aq.II (see Table 1). Although the observed rate of initial active transport ($1.3\ \mu M/h \cdot cm^2$) is satisfactorily fast, after ca. 30 h, the system shows significant depression of transport accompanying the changes of pH of Aq.I and Aq.II which indicate a symport of OH^- and/or antiport of H^+.

In conclusion, the present dicationic porphyrin is the promising nucleotide carrier, because it is easily expected to control the selectivity and efficiency by changing the central metal and functionalizing with additional recognition interactions such as hydrogen bonds.[1]

EXPERIMENTAL

General procedures

^1H NMR spectra were recorded either on a JEOL FX-90Q (90 MHz) or on a JEOL GX-400 (400 MHz). Infrared spectra were recorded on a BIO-RAD FTS-7R. Electronic absorption spectra were recorded on a Hewlett-Packard HP-8452A. HPLC analyses were performed on a Waters Model 600E multisolvent delivery system equipped with a Waters 991J photodiode array detector.

Preparation of 2

A suspension of 4.0 g (7.1 mmol) of mesoporphyrin IX in 50 mL of $CHCl_3$ was stirred at room temperature as 50 mL (0.7 mol) of acetyl chloride was added. The mixture was stirred for 1 h at room temperature and carefully added to 500 mL of 50% $NH(CH_3)_2$ aqueous solution at 0 °C. The mixture was extracted with three 500 mL portions of chloroform. The combined organic layer was washed with four portions of diluted acetic acid (10%) and a saturated $NaHCO_3$ solution and dried over $MgSO_4$. After evaporation of solvent in vacuo, the residual crude product was chromatographed on silica gel (4.5 × 21 cm, elution with 50% acetone-$CHCl_3$ followed by 10% methanol-$CHCl_3$) to afford 3.07 g of **2** as a dark violet solid (70% yield): ^1H NMR ($CDCl_3$, 90 MHz) δ 10.11 (s, 4H), 4.44 (t, J = 9 Hz, 4H), 4.08 (q, J = 9 Hz, 4H), 3.63 (s, 12H), 3.27 (t, J = 9 Hz, 4H), 2.89 (d, J = 3 Hz, 6H), 2.49 (d, J = 4.5 Hz, 6H), 1.75 (t, J = 9 Hz, 6H), −3.75 (bs, 2H); IR (KBr, cm^{-1}) 3313, 2964, 2928, 2869, 1638, 1495, 1454, 1400, 1262, 1225, 1194, 1138, 1100, 1061, 984, 964, 905, 834, 805, 742, 680.

Preparation of 3

To a slurry of 1.2 g (31.6 mmol) of $LiAlH_4$ in 100 mL of dry THF was added a solution of 1.01 g (1.62 mmol) of **2** in 160 mL of THF dropwise at room temperature under N_2 atmosphere. The reaction was followed by disappearance of **2** (Rf 0.42) on TLC (silica gel, 50% acetone-$CHCl_3$). The excess $LiAlH_4$ was quenched with 10 mL of ethyl acetate and the solvent was evaporated to dryness. The residual material was suspended in 100 mL of $CHCl_3$ with vigorous stirring and insoluble inorganic materials are filtered off. After washing the organic layer with 6N aq. NaOH, the

solvent was evaporated in vacuo and the residue was applied on a basic alumina column (5 × 27 cm, elution with $CHCl_3$ followed by 5% methanol-$CHCl_3$) to afford 690 mg of **3** (72% yield): ^1H NMR ($CDCl_3$, 90 MHz) δ 10.10 (s, 4H), 4.05 (m, 8H), 3.65 (s, 12H), 2.60 (m, 8H), 2.30 (s, 12H), 1.85 (t, J = 8 Hz, 6H), −3.90 (bs, 2H). IR (KBr, cm^{-1}) 3316, 2961, 2926, 1461, 1378, 1262, 1104, 1038, 835, 803, 740, 679.

Preparation of 1

A solution of 0.69 g (1.2 mmol) of **3** and 6.9 g (23 mmol) of lauryl iodide in 20 mL of ethanol was refluxed for 24 h. The reaction was monitored with a neutral alumina TLC (5% methanol-$CHCl_3$, R_f 0.87 for **3** and 0.03 for a product). The mixture was evaporated to dryness and the residual product was recrystallized from 40% CH_2Cl_2-hexane to afford 1.33 g of the initial product which was identified mainly as monoprotonated compound, **1**·HI_3. This protonated product (0.15 g, 0.99 mmol) was dissolved in 50 mL of $CHCl_3$ and vigorously stirred with 50 mL of saturated Na_2CO_3 aq. for overnight at room temperature. The organic layer was separated and evaporated to dryness. The residue was recrystallized from 40% CH_2Cl_2-hexane to afford 84.8 mg of **1** which corresponded to 53% yield based on **3**: protonated **1**; 1H NMR ($CDCl_3$, 90 MHz) δ 11.64 (s, 1H), 10.53 (s, 3H), 5.03 (m, 4H), 4.02 (m, 4H), 3.75 – 3.45 (s × 4, 12H), 3.10 (m, 4H), 2.95 – 2.35 (m, 12H), 2.00 – 1.66 (m, 6H), 1.36 – 0.60 (m, 46H), −2.89 (b, 1H), −3.74 (b, 2H). UV-VIS ($CHCl_3$) λ_{max} (log ε) 402(5.23), 500(3.97), 536(4.08), 562(4.18), 606(3.90). Anal. Calcd. for $C_{62}H_{102}N_6I_2 \cdot (HI_3)_{0.8} \cdot (HI)_{0.2}$: C, 49.11; H, 6.85; N, 5.54. Found: C, 48.86; H, 7.02; N, 5.55. **1**; ^1H NMR ($CDCl_3$, 90 MHz) d 9.90 – 9.81 (s × 4, 4H), 4.24 – 3.59 (m, 12H), 3.51 – 3.30 (s × 4, 12H), 2.78 – 2.29 (m, 16H), 2.29 – 1.94 (m, 4H), 1.79 (m, 6H), 1.39 – 0.54 (m, 46H), (ca. 1.5 (bs, this signal disappears on addition of D_2O and is assigned to water protones)). UV-VIS (MeOH) λ_{max} (log ε) 396(5.13), 498(4.05), 530(3.91), 568(3.61), 622(3.58). FAB-MASS m/e 1057 (M-I), 930 (M-2I). Anal. Calcd. for $C_{62}H_{102}N_6I_2 \cdot (H_2O)_{1.5}$: C, 61.42; H, 8.73; N, 6.93. Found: C, 61.28; H, 8.57; N, 6.94.

Preparation of 1 · Zn

To a solution of 100 mg (0.085 mmol) of **1** in 10 mL of $CHCl_3$ was added 10 mL of methanol saturated with $Zn(OAc)_2$. The solution was stirred for 2 h at room temperature. The mixture was passed through short neutral alumina column (2.8 × 10 cm, elution with 10% methanol-$CHCl_3$) and the product was recrystallized from 40% CH_2Cl_2-hexane to afford 74.4 mg of **1 · Zn** (71% yield): ^1H NMR ($CDCl_3/CD_3OD$, 400 MHz) δ 9.99 – 9.89 (s × 4, 4H), 4.18, 4.17 (t × 2, J = 7 Hz, 4H), 4.09 – 3.95 (m, 8H), 3.60, 3.59, 3.57, 3.55 (s × 4, 12H), 3.03, 3.01 (t × 2, J = 8 Hz, 4H), 2.93, 2.91 (m, 12H), 2.60 – 2.47 (m, 4H), 1.83, 1.82 (t × 2, J = 7.8 Hz, 6H), 1.29 – 0.78 (m, 46H). UV-VIS (MeOH) λ_{max} (log ε): 408(5.46), 538(4.18), 574(4.20). Anal. Calcd. for $C_{62}H_{100}N_6I_2Zn$: C, 59.64; H, 8.07; N, 6.73. Found: C, 59.74; H, 8.19; N, 6.72.

Nucleotide transport

Nucleotide transport experiment was conducted by the use of U-form glass tube. Two aqueous phases of which volume were 1.50 mL (Aq.I as source phase and Aq.II as receiver phase) were separated by 4.0 mL of $CHCl_3$ liquid membrane. Each interfacial cross section were 1.13 cm^2. The $CHCl_3$ phase was gently stirred at 900 rpm with a magnetic bar during the experiments. In the experiments of single nucleotide transport, the concentration of nucleotide in Aq.II was determined from UV absorption intensity. In the competitive transport experiments, concentration of each nucleotide was determined by HPLC analyses of 10 μL of Aq.II phase using a reverse phase column (YMC Co., Ltd., AQ-312, S-5 120A ODS, elution with 0.1 M phosphate buffer (pH 6.0) at flow rate 0.7 mL/min).

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