Catalytic Functions of Artificial Enzyme Composed of Simple Vitamin B_{12} Model and Synthetic Bilayer Membrane†

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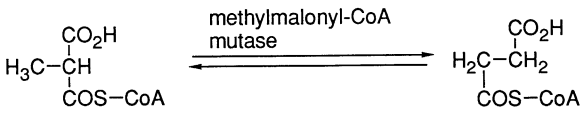
An artificial enzyme was constructed with a combination of the single-walled vesicle of *N*,*N*-dihexadecyl-N^{α}-[6-(trimethylammonio)hexanoyl]-L-alaninamide bromide ($N^{+}C_5$Ala2C_{16}) and a simple vitamin B_{12} model; (11-hydroxyimino-4,10-dipropyl-5,9-diazatrideca-4,9-dien-3-one oximato)cobalt, [Co(C_2C_3)(DO)(DOH)pn], or [*N*-(2-hydroxyiminocyclohexylidene)-*N'*-(cyclohexyliden-2-one oximato)-1,3-diaminopropane]cobalt, [Co(ch)(DO)(DOH)pn]. Carbon-skeleton rearrangement reactions of alkyl ligands bound to the cobalt complex were markedly favored in the single-walled vesicle, relative to the reactions in methanol and benzene, under anaerobic photolysis conditions at ordinary temperatures. The cobalt complex incorporated into the intramembrane domain is subjected to significant repression of its molecular motion and to desolvation as confirmed by fluorescence and fluorescence polarization measurements. The 1,2-migration of cyano and carboxylic ester groups involved in the alkyl ligands is plausibly enhanced by such effects.

It is well known that metal ions play important roles as essential trace elements in biological systems. Metallocoenzymes involved in holoenzymes exhibit efficient catalyses only under specific micro-environments provided by the corresponding apoproteins. Vitamin B_{12}-dependent enzymes catalyze various isomerization reactions as formally given by Eq. 1.[1] The catalysis includes four carbon-skeleton rearrangements of reversible nature as follows;[2,3] methylmalonyl-SCoA⇌succinyl-SCoA, isobutyryl-SCoA⇌butyryl-SCoA, β-methylaspartate⇌glutamate, and methylitaconate⇌α-methyleneglutarate, as shown by Eqs. 2—5, respectively.


(1)



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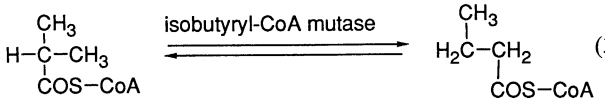

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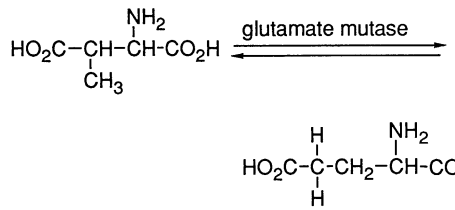

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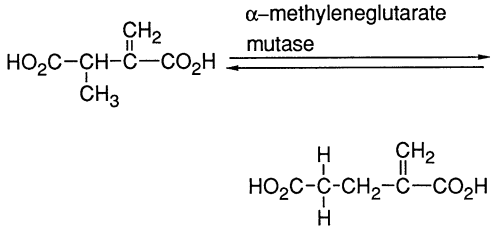
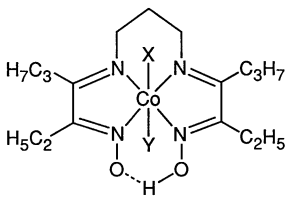


(5)

The naturally occurring apoproteins, which provide relevant reaction sites for vitamin B_{12}, are considered to play crucial roles in the molecular rearrangements.[4,5] In this regard, we have been interested in the catalytic activity of vitamin B_{12} in hydrophobic micro-environments to simulate the catalytic functions of the holoenzymes concerned. We have succeeded in constructing holoenzyme models,[6-8] as composed of a synthetic bilayer membrane and a hydrophobic vitamin B_{12} which has ester groups in place of the peripheral amide moieties of the naturally occurring vitamin B_{12}.[9-16] This finding prompted us to develop simple model complexes which are capable of catalyzing the carbon-skeleton rearrangements in similar bilayer membranes. We adopted Costa-type complexes with some modifications of the original one, [Co^{III}\{(C_2C_3)-

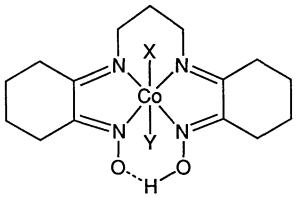


[Co^{III}\{(C_2C_3)(DO)(DOH)pn\}I_2] (**1**): X = Y = I

[Co^{III}\{(C_2C_3)(DO)(DOH)pn\}\{$CH_2C(CO_2C_2H_5)_2CH_3$\}Br] (**2**):
X = $CH_2C(CO_2C_2H_5)_2CH_3$, Y = Br

[Co^{III}\{(C_2C_3)(DO)(DOH)pn\}\{$CH_2C(CO_2C_2H_5)(CN)CH_3$\}Br] (**3**):
X = $CH_2C(CO_2C_2H_5)(CN)CH_3$, Y = Br

† Contribution No. 941 from this Department. Preliminary communication; see Ref. 31.

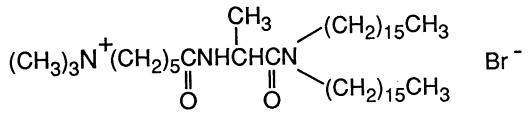


$[Co^{III}\{(ch)(DO)(DOH)pn\}Br_2]$ (**4**): X = Y = Br

$[Co^{III}\{(ch)(DO)(DOH)pn\}I_2]$ (**5**): X = Y = I

$[Co^{III}\{(ch)(DO)(DOH)pn\}\{CH_2C(CO_2C_2H_5)_2CH_3\}Br]$ (**6**): X = $CH_2C(CO_2C_2H_5)_2CH_3$, Y = Br

(DO)(DOH)pn}(XY)] and $\{Co^{III}\{(ch)(DO)(DOH)pn\}$-(XY)] (refer to **1**—**6**), since these complexes show redox behavior analogous to that of vitamin B_{12} with respect to the nuclear cobalt.[17-19] A single-walled bilayer membrane composed of a synthetic peptide lipid is quite effective as an apoprotein model.[20-23] On these grounds, we report here the carbon-skeleton rearrangements of alkyl ligands coordinated to the vitamin B_{12} model complexes in the $N^+C_5Ala2C_{16}$ vesicle.

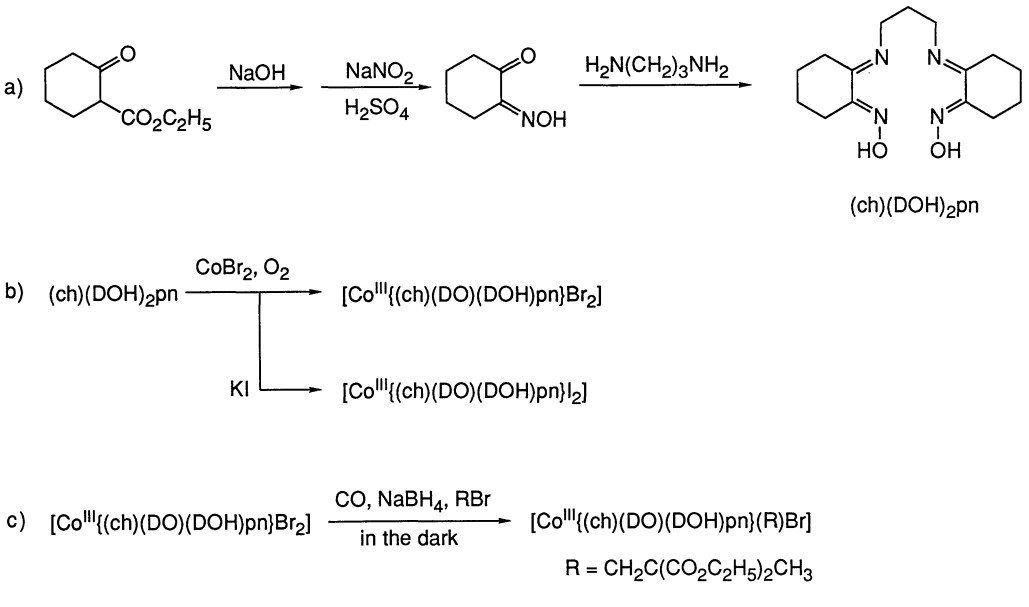


$N^+C_5Ala2C_{16}$

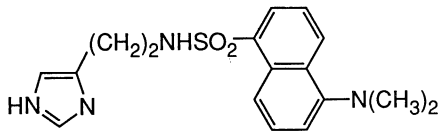
Experimental

General Analyses and Measurements. Elemental analyses were performed at the Microanalysis Center of Kyushu University. IR spectra were taken on a JASCO IR-810 infrared spectrophotometer, while electronic absorption spectra were recorded on a Hitachi 220A or a Hitachi 340 spectrophotometer. ^1H NMR spectra were taken on a Hitachi R-24B, a Bruker AC-250P, and a Bruker AMX-500 spectrometer installed at the Advanced Instrumental Analysis Center of Kyushu University. Assignments of NMR signals for the cobalt complexes were performed by means of the 2D-NMR technique (H–H COSY). Fluorescence spectra were obtained with a Hitachi 650-60 spectrophotometer, and fluorescence polarization measurements were performed with a Union Giken FS-501A fluorescence polarization spectrophotometer equipped with a Union Giken System-77 computer; emission at 530 nm was monitored upon excitation at 340 nm with a slit width of 3.5 nm for both excitation and emission sides. Fluorescence polarization (P) was calculated according to a previous method.[24,25] GLC analyses were carried out on a Shimadzu GC-9A apparatus equipped with a Shimadzu C-R3A-FFC chromatopac for data processing.

Materials. Diiodo(11-hydroxyimino-4,10-dipropyl-5,9-diazatrideca-4,9-dien-3-one oximato)cobalt(III), $[Co^{III}\{(C_2C_3)(DO)(DOH)pn\}I_2]$ (**1**), and its alkylated complexes (**2** and **3**), were prepared by methods identical with those reported previously.[26] *N*,*N*-Bis(2-hydroxyiminocyclohexylidene)propane-1,3-diamine, $(ch)(DOH)_2pn$, and its cobalt complexes with various axial ligands (**4**, **5**, and **6**) were prepared by referring to reported procedures[18,27-29] along with some modifications as shown in Scheme 1. An artificial lipid, *N*,*N*-dihexadecyl-N^{α}-[6-(trimethylammonio)hexanoyl]-L-alaninamide bromide ($N^+C_5Ala2C_{16}$), was prepared after a reported procedure.[20,21] Preparation of a fluorescent probe, *N*-[5-(dimethylamino)-1-naphthylsulfonyl]-2-(4-imidazolyl)-ethylamine (dansylhistamine), was described elsewhere.[14] Alkyl



Scheme 1.



dansylhistamine

halides, diethyl 2-bromomethyl-2-methylmalonate and ethyl 3-bromo-2-cyano-2-methylpropanoate, and authentic samples of the corresponding products such as diethyl 2,2-dimethylmalonate (**A**), diethyl 2-methylsuccinate (**B**), ethyl 2-cyano-2-methylpropanoate (**C**), ethyl 3-cyanobutyrate (**D**), and ethyl 3-cyano-2-methylpropanoate (**E**) were prepared according to literature methods[13] and confirmed to be sufficiently pure by ^{1}H NMR and GLC.

***N*,*N*′-Bis(2-hydroxyiminocyclohexylidene)propane-1,3-diamine, (ch)(DOH)$_2$pn.** This ligand was synthesized by the condensation of 1,2-cyclohexanedione monooxime (15.8 g, 0.12 mol), which was prepared from ethyl 2-oxocyclohexanecarboxylate and sodium nitrite,[30] with 1,3-diaminopropane (5.20 g, 0.07 mol): Yield 12.5 g (71.0%), mp 50—51 °C; IR (KBr) 3300 (OH), 1640 (C=N), 970 (N–O) cm^{-1}; 60 MHz ^{1}H NMR ($CDCl_3$, TMS) δ=1.75 (8H, m, ONCCH$_2$C<u>H$_2$</u>C<u>H$_2$</u>-CH$_2$), 2.10 (2H, m, NCH$_2$C<u>H$_2$</u>CH$_2$N), 2.55 (4H, t, J=6.1 Hz, ONCCH$_2$CH$_2$CH$_2$C<u>H$_2$</u>), 2.92 (4H, t, J=6.1 Hz, ONCC<u>H$_2$</u>-CH$_2$CH$_2$CH$_2$), 4.91 (4H, t, J=4.5 Hz, NC<u>H$_2$</u>CH$_2$C<u>H$_2$</u>N), 7.20 (2H, br s, OH). Found: C, 59.51; H, 8.24; N, 19.28%. Calcd for $C_{15}H_{24}N_4O_2$: C, 59.62; H, 8.27; N, 19.17%.

Dibromo[*N*-(2-hydroxyiminocyclohexylidene)-*N*′-(cyclohexliden-2-one oximato)-1,3-diaminopropane]cobalt(III), [Co^{III}{(ch)(DO)(DOH)pn}Br$_2$] (4). An acetone solution (5 mL) of (ch)(DOH)$_2$pn (1.46 g, 5.0×10^{-3} mol) was added dropwise to an aqueous solution (150 ml) of cobalt dibromide hexahydrate (2.0 g, 6.0×10^{-3} mol) while air was bubbled through the latter aqueous solution. After the reaction mixture was stirred for 4 h, precipitates were recovered by filtration and washed with water. The resulting cobalt complex was recrystallized from acetone–water (1:1 v/v) to afford green needles: yield 1.66 g (65%), mp 251—252 °C; IR (KBr) 720 (Co–N) cm^{-1}; 500 MHz ^{1}H NMR ($CDCl_3$, TMS) δ=1.86 (4H, m, ONCCH$_2$C<u>H$_2$</u>CH$_2$CH$_2$), 1.90 (4H, m, ONCCH$_2$CH$_2$C<u>H$_2$</u>CH$_2$), 2.59 (2H, m, NCH$_2$C<u>H$_2$</u>CH$_2$N), 3.01 (4H, t, J=6.16 Hz, ONCCH$_2$CH$_2$CH$_2$C<u>H$_2$</u>), 3.21 (4H, t, J=6.46 Hz, ONCC<u>H$_2$</u>CH$_2$CH$_2$CH$_2$), 4.07 (4H, t, J=4.52 Hz, NC<u>H$_2$</u>CH$_2$C<u>H$_2$</u>N), 19.0 (1H, s, OH). Found: C, 35.36; H, 4.54; N, 10.92%. Calcd for $C_{15}H_{23}Br_2CoN_4O_2$: C, 35.32; H, 4.54; N, 10.99%.

Diiodo[*N*-(2-hydroxyiminocyclohexylidene)-*N*′-(cyclohexyliden-2-one oximato)-1,3-diaminopropane]cobalt(III), [Co^{III}{(ch)(DO)(DOH)pn}I$_2$] (5). This complex was prepared in the presence of a large excess of potassium iodide by the method employed for the preparation of **4.** The crude product was purified by column chromatography on silica gel (Wakogel C-100) with benzene–acetone (4:1 v/v) as eluant to give dark green powder: Yield 61%, mp 262—263 °C; λ_{max} (CH_2Cl_2) 340 (ϵ 1.15×10^4), 462 nm (6.27×10^3); IR (KBr) 720 (Co–N) cm^{-1}; 500 MHz ^{1}H NMR ($CDCl_3$, TMS) δ=1.83 (4H, m, ONCCH$_2$C<u>H$_2$</u>CH$_2$CH$_2$), 1.85 (4H, m, ONCCH$_2$CH$_2$-C<u>H$_2$</u>CH$_2$), 2.63 (2H, br s, NCH$_2$C<u>H$_2$</u>CH$_2$N), 2.94 (4H, t, J=6.09 Hz, ONCCH$_2$CH$_2$CH$_2$C<u>H$_2$</u>), 3.18 (4H, t, J=6.45 Hz, ONCC<u>H$_2$</u>CH$_2$CH$_2$CH$_2$), 4.10 (4H, br s, NC<u>H$_2$</u>CH$_2$C<u>H$_2$</u>N), 19.0 (1H, s, OH). Found: C, 29.94; H, 3.93; N, 9.50%. Calcd for $C_{15}H_{23}I_2CoN_4O_2$: C, 29.82; H, 3.85; N, 9.28%.

Bromo{2,2-bis(ethoxycarbonyl)propyl}[*N*-(2-hydroxyiminocyclohexylidene)-*N*′-(cyclohexliden-2-one oximato)-1,3-diaminopropane]cobalt(III), [Co^{III}{(ch)(DO)(DOH)pn}-{CH$_2$C(CO$_2$C$_2$H$_5$)$_2$CH$_3$}Br] (6). A methanol solution (300 mL) containg **4** (400 mg, 7.8×10^{-4} mol) and diethyl 2-bromomethyl-2-methylmalonate (800 mg, 3.0×10^{-3} mol) was stirred while carbon monoxide was introduced into it until saturation was attained. After being stirred for 1 h, the solution turned pale yellow, indicating formation of the Co^{II} complex. Sodium tetrahydroborate in methanol was added dropwise to the resulting solution until the solution turned deep blue under carbon monoxide atmosphere. The solution changed its color to reddish orange, after it was refluxed for 4 h in the dark. The solution was evaporated to dryness, a solid material insoluble in dichloromethane (15 mL) was removed by filtration, and then the dichloromethane solution was concentrated to ca. 3 mL. Hexane (50 mL) was added to the resulting solution, and precipitates were recovered by filtration and purified by gel-filtration chromatography on a column of Sephadex LH-20 with methanol as eluant to give reddish brown powder: Yield 262 mg (55%); IR (KBr) 1725 (C=O) cm^{-1}; λ_{max} (CH_3OH) 475 nm. Found: C, 46.03; H, 6.23; N, 8.91%. Calcd for $C_{24}H_{38}BrCoN_4O_6\cdot1/2H_2O$: C, 46.02; H, 6.28; N, 8.94%.

Equilibrium Measurements for Incorporation of Vitamin B$_{12}$ Model Complexes into Single-Compartment Vesicles. A dichloromethane solution (1 mL) containing N$^+$C$_5$Ala2C$_{16}$ (1.0×10^{-5} mol) and a cobalt complex (2.0×10^{-7} mol), [Co^{III}-{(C$_2$C$_3$)(DO)(DOH)pn}I$_2$] or [Co^{III}{(ch)(DO)(DOH)pn}I$_2$], was evaporated in vacuo to remove the solvent completely, and an aqueous potassium iodide solution (1 mL, 5.0×10^{-4} mol dm^{-3}) was added to the residue. The resulting mixture was sonicated for 30 s with a probe-type sonicator at 30 W to give a clear solution. An extent of incorporation of the cobalt complex into the single-walled vesicle was examined by gel-filtration chromatography on a column of Sephadex G-50 with an aqueous potassium iodide solution (5.0×10^{-4} mol dm^{-3}) as eluant. The bound complex was eluted first in the column void volume, and the free complex was followed as the second fraction with a retention time close to the bed volume. An amount of the incorporated complex was determined by electronic spectroscopy. As for complexes with the cobalt–carbon bond, [Co^{III}{(C$_2$C$_3$)(DO)(DOH)pn}{CH$_2$C(CO$_2$C$_2$H$_5$)$_2$-CH$_3$}Br] and [Co^{III}{(ch)(DO)(DOH)pn}{CH$_2$C(CO$_2$C$_2$H$_5$)$_2$-CH$_3$}Br], a methanol solution (10 μL) of each complex was injected into the vesicular solution which was prepared in a manner as stated above, so that any cleavage of the cobalt–carbon bond caused by sonication was avoided.

Photolysis and Product Analyses. N$^+$C$_5$Ala2C$_{16}$ (5.0×10^{-5} mol) was dispersed in aqueous potassium bromide (10 mL, 5.0×10^{-4} mol dm^{-3}) by Vortex mixing, and the dispersion sample was then sonicated for 2 min with a probe-type sonicator at 30 W to give a clear solution of single-walled vesicles. A methanol solution (10 μL) of the alkylated complex (5.0×10^{-7} mol), [Co^{III}{(C$_2$C$_3$)(DO)(DOH)pn}RBr] or [Co^{III}{(ch)(DO)(DOH)pn}RBr], was added to the vesicular solution. Then, the resulting solution was irradiated with a 500 W tungsten lamp at a distance of 30 cm and at an appropriate temperature. After the alkylated complex was

completely decomposed as confirmed by electronic spectroscopy, the products were extracted with dichloromethane (10 mL×3) and analyzed by GLC. Reaction products were identified by coinjection of the corresponding authentic samples into columns of Silicone DC-550 (Shimadzu Co.) and Silicone SE-30 (Gasukuro Kogyo Inc.) as described previously in detail.[8] A capillary column of Polyethylene Glycol-20M (Gasukuro Kogyo) was used for identification of isomers having similar structures. Each product separated by preparative GLC on Silicone DC-550 was identified by means of ^{1}H NMR spectroscopy. As for reactions in methanol and benzene, a reaction mixture was evaporated to dryness in vacuo before dichloromethane was added. Total yields listed in Tables 1—3 are less than 100% owing to losses during extraction and evaporation treatments in vacuo. However, we confirmed that no other by-productus were obtained.

Results and Discussion

Incorporation of Vitamin B_{12} Model Complexes into Single-Compartment Vesicles. Complexes **1**, **2**, **5**, and **6** were incorporated into the $N^+C_5Ala2C_{16}$ vesicle to the extents of 82—90, 55—60, 16—20, and 54—56% of their total amounts in molar fraction, respectively.[31] The amounts of the complexes incorporated into the vesicle were little affected by temperature change in the range of 5—50 °C. The extents of solubility of these complexes in water were found as follows: **1**, 1.1×10^{-4} mol dm^{-3}; **2**, 2.0×10^{-4} mol dm^{-3}; **5**, 3.1×10^{-4} mol dm^{-3}; **6**, 8.1×10^{-4} mol dm^{-3}. We have already found that the extent of incorporation of hydrophobic vitamin B_{12} derivatives into the $N^+C_5Ala2C_{16}$ vesicle is primarily controlled by the hydrophobicity of the complexes.[8] In other words, the incorporation of those complexes into the vesicle becomes increasingly favored as those become less soluble in aqueous media. However, the solubility parameter alone can not provide a reasonable explanation for the incorporation behavior of the present complexes. The incorporation behavior seems to depend on various factors, such as size, dipolar property, and hydrophobicity of the complex, which give out different modes of interactions with the membrane.

Table 1. Product Analyses for Photolysis of **2** in Various Media[a]

Medium[b]	Temp[c]/°C	Yield[d]/%		B/A[e]
		A	B	
CH_3OH	20.0	74—77	8—11	0.13
C_6H_6	20.0	76—80	5—9	0.09
$N^+C_5Ala2C_{16}$ vesicle	5.0	41—44	46—50	1.13
	20.0	48—51	39—42	0.82
	50.0	54—59	31—33	0.57

a) A solution containing **2** (5.0×10^{-5} mol dm^{-3}) was irradiated with a 500 W tungsten lamp at a distance of 30 cm for 4 h. b) $N^+C_5Ala2C_{16}$ (5.0×10^{-3} mol dm^{-3}) in aqueous potassium bromide (5.0×10^{-4} mol dm^{-3}). c) Accuracy, ±0.1 °C. d) Products were analyzed by GLC. e) Ratio of the rearrangement product.

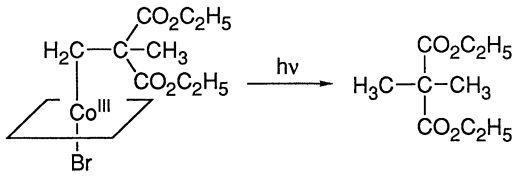
Product Analyses for Photolysis of Alkylated Complexes in Various Media. The cobalt–carbon bond involved in alkylated complexes **2** and **6** was cleaved in the vesicular solution upon irradiation with visible light as reported previously.[31] Likewise, **3** underwent the photolytic cleavage. After the alkylated complex was completely decomposed as confirmed by electronic spectroscopy, the products were analyzed by GLC. Product analyses for the photolysis of alkylated complexes **2** and **3** in various media are summarized in Tables 1 and 2. We identified the products, which were obtained by the photolysis reaction of complex **2**, as diethyl 2,2-dimethylmalonate (**A**, the simple hydrogenated product without rearrangement) and diethyl 2-methylsuccinate (**B**, the rearrangement product) (refer to Eq. 6). As for complex **3**, ethyl 2-cyano-2-methylpropanoate (**C**, the simple hydrogenated product), ethyl 3-cyanobutyrate (**D**, the ester-migrated product), and ethyl 3-cyano-2-methylpropanoate (**E**, the cyano-migrated product) were confirmed to be obtained (refer to Eq. 7).

The following findings are based on the product analyses. (i) The major products are the unrearranged ones in benzene and methanol, but the rearrangement products are largely obtained in the vesicular solution. Consequently, the carbon-skeleton rearrangement is

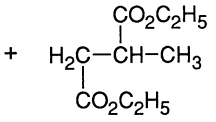
Table 2. Product Analyses for Photolysis of **3** in Various Media[a]

Medium[b]	Temp[c]/°C	Yield[d]/%			(D+E)/C[e]
		C	D	E	
CH_3OH	5.0	80—85	0	0	0
	20.0	80—85	0	0	0
	50.0	80—85	0	0	0
C_6H_6	5.0	80—85	0	0	0
	20.0	80—85	0	0	0
	50.0	80—85	0	0	0
$N^+C_5Ala2C_{16}$ vesicle	5.0	42—45	21—24	14—16	0.86
	20.0	47—50	19—22	12—15	0.70
	50.0	52—56	16—20	10—14	0.56

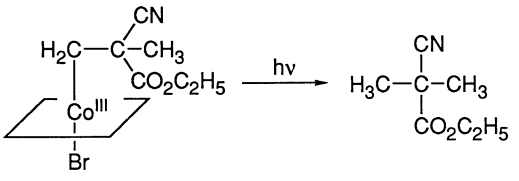
a) A solution containing **3** (5.0×10^{-5} mol dm^{-3}) was irradiated with a 500 W tungsten lamp at a distance of 30 cm for 4 h. b) $N^+C_5Ala2C_{16}$ (5.0×10^{-3} mol dm^{-3}) in aqueous potassium bromide (5.0×10^{-4} mol dm^{-3}). c) Accuracy, ±0.1 °C. d) Products were analyzed by GLC. e) Ratio of the rearrangement products.



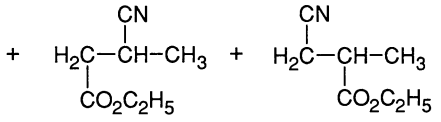
2 or 6 A



B (6)



3 C



D E (7)

much enhanced in the vesicle relative to the reaction in homogeneous solutions. (ii) The product ratio **B**/**A** for complex **2** in the vesicular solution is quite large as compared with the one for the corresponding hydrophobic vitamin B_{12} (**B**/**A**=0.12).[8] The photolysis must cause the homolytic cleavage of the cobalt–carbon bond in the initial stage.[32] The radical mechanism was assigned to the carbon-skeleton rearrangement of hydrophobic vitamin B_{12} derivatives which do not hold an anionic ligand trans to the substituted alkyl moiety. However, the ester group migrates more readily via formation of an anionic intermediate relative to the reaction via formation of a radical intermediate even in the vesicle.[33] Therefore, the bromide ion coordinated to the cobalt atom at the axial site seems to take a role in inducing the heterolytic cleavage of the cobalt–carbon bond involved in the model complex so that an anionic intermediate is produced, as caused by one-electron transfer immediately after the homolytic cobalt–carbon cleavage. (iii) The product ratios of the rearranged ones, **B**/**A** and (**D**+**E**)/**C**, increase in the vesicle along with temperature decrease as shown in Tables 1 and 2. Since the single-walled $N^+C_5Ala2C_{16}$ vesicle was found to show a broad phase transition from the gel to the liquid-crystalline state at 20±5 °C by differential scanning calorimetry,[21] the synthetic bilayer membrane favors formation of the rearrangement product in the gel state. A similar result was obtained for complex **6** bearing cyclohexane moieties in the ligand portion, as is apparent from the data in Table 3.

Table 3. Product Analyses for Photolysis of **6** in Various Media[a]

Medium[b]	Temp[c]/°C	Yield[d]/%		**B**/**A**[e]
		A	**B**	
CH_3OH	20.0	75—79	7—9	0.10
C_6H_6	20.0	81—87	0—3	0.02
$N^+C_5Ala2C_{16}$ vesicle	5.0	49—54	31—35	0.64
	20.0	59—63	21—25	0.38
	50.0	76—80	5—9	0.09

a) A solution containing **6** (5.0×10^{-5} mol dm^{-3}) was irradiated with a 500 W tungsten lamp at a distance of 30 cm for 4 h. b) $N^+C_5Ala2C_{16}$ (5.0×10^{-3} mol dm^{-3}) in aqueous potassium bromide (5.0×10^{-4} mol dm^{-3}). c) Accuracy, ±0.1 °C. d) Products were analyzed by GLC. e) Ratio of the rearrangement product.

Microenvironment around Vitamin B_{12} Model Complexes in Single-Compartment Vesicle. In order to understand microenvironmental properties experienced by the vitamin B_{12} model complexes in the vesicle, the cobalt complex coordinated at the axial site with dansylhistamine as a fluorescent probe was adopted. The axial ligation constants (K) for [Co^{III}\{(C_2C_3)(DO)(DOH)pn\}Br_2] and [Co^{III}\{(ch)(DO)(DOH)pn\}I_2] with dansylhistamine were determined by electronic spectroscopy after a reported procedure:[9] log K=5.2 and 5.0 in methanol, and 5.3 and 5.2 in benzene, respectively. These data indicate that the metal complexes are largely coordinated with dansylhistamine under the present experimental conditions. The microscopic polarity experienced by the dansyl moiety bound to the vitamin B_{12} model complex is reflected in its fluorescence maximum.[34] In order to obtain the reference data, the fluorescence maxima of dansylhistamine coordinated to the vitamin B_{12} model complexes were measured in various mixtures of water and dioxane as shown in Fig. 1. The fluorescence maximum is shifted to lower wavelength as the solvent polarity decreases. It is clear that the single-walled vesicle of $N^+C_5Ala2C_{16}$ provides a microenvironment for the dansyl moiety that is equivalent to a medium polarity between those of methanol and ethanol. These values are comparable to that obtained for a hydrophobic vitamin B_{12}.[8] The result leads us to conclude that an axial ligand bound to the complex is significantly desolvated in the vesicle.

The microscopic viscosity experienced by the dansyl moiety bound to the vitamin B_{12} model was examined by means of fluorescence polarization (P) measurements. P values for the dansylhistamine-coordinated vitamin B_{12} models in methanol and benzene are 0.004—0.005 and 0.009—0.02, respectively, in the temperature

range 5—50 °C. On the other hand, the identical complexes gave large *P* values in the vesicular solution as shown in Fig. 2. This apparently indicates that the molecular motion of the guest molecules in the vesicle is markedly suppressed.[35] Thus, the microenvironmental effect provided by the vesicle is quite different from those given by simple organic solvents which solubilize the vitamin B_{12} models homogeneously.

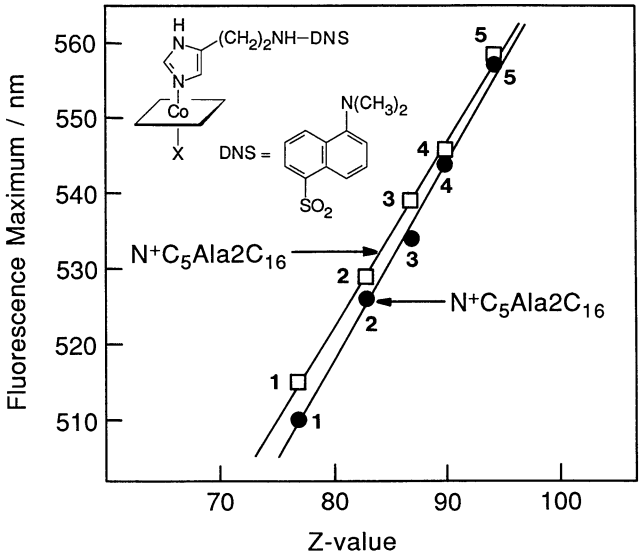


Fig. 1. Medium effect on fluorescence of dansylhistamine (1.0×10^{-5} mol dm^{-3}) coordinated to [Co^{III}{(ch)(DO)(DOH)pn}I] (□, X=I; 1.0×10^{-5} mol dm^{-3}) and [Co^{III}{(C_2C_3)(DO)pn}Br] (●, X=Br; 1.0×10^{-5} mol dm^{-3}), both complexes being incorporated into the $N^+C_5Ala2C_{16}$ (5.0×10^{-3} mol dm^{-3}) vesicle in phosphate–borate buffer (0.05 mol dm^{-3}; pH 9.2) at 20.0±0.1°C. Reference data obtained in water-dioxane at the following ratios (v/v): 1, 1:9; 2, 3:7; 3, 1:1; 4, 7:3; 5, 1:0. The Z-value refers to Kosower's parameter.

The present study demonstrates that a vitamin B_{12}-dependent artificial holoenzyme can be prepared by utilizing a synthetic bilayer membrane and a simple vitamin B_{12} model complex. It is clear that the carbon-skeleton rearrangement of an alkyl ligand coordinated to the simple vitamin B_{12} model takes place much favorably in the vesicular phase. This 1,2-migration must arise from both repression of molecular motion and desolvation effects operating on the alkylated complex in the vesicle, in a manner as observed by utilizing a hydrophobic vitamin B_{12}.[8]

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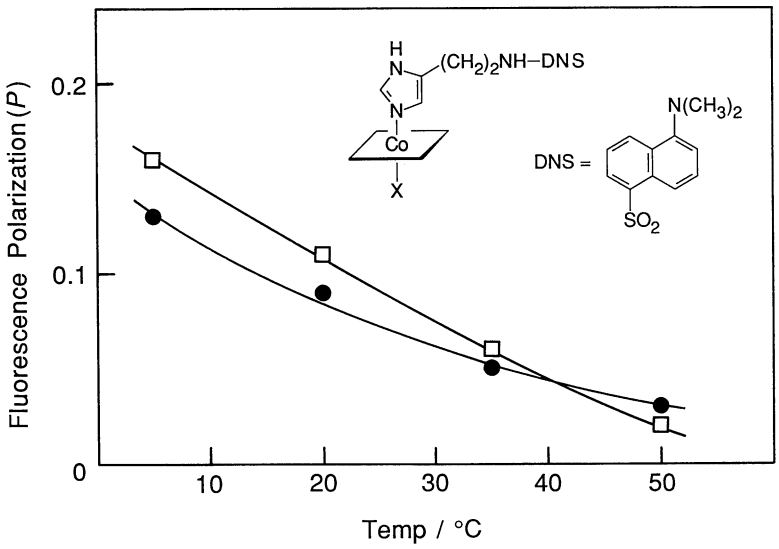

Fig. 2. Temperature effect on fluorescence polarization (*P*) of dansylhistamine (1.0×10^{-5} mol dm^{-3}) coordinated to [Co^{III}{(ch)(DO)(DOH)pn}I] (□, X=I; 1.0×10^{-5} mol dm^{-3}) and [Co^{III}{(C_2C_3)(DO)(DOH)pn}Br] (●, X=Br; 1.0×10^{-5} mol dm^{-3}), both complexes being incorporated into the $N^+C_5Ala2C_{16}$ (5.0×10^{-3} mol dm^{-3}) vesicle in phosphate–borate buffer (0.05 mol dm^{-3}; pH 9.2).

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