Hydrophobic Vitamin B_{12}. Part 9.† An Artificial Holoenzyme Composed of Hydrophobic Vitamin B_{12} and Synthetic Bilayer Membrane for Carbon-skeleton Rearrangements

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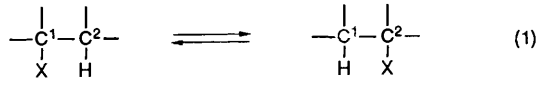
The incorporation of hydrophobic vitamin B_{12} derivatives, which have ester groups in place of the peripheral amide moieties of the naturally occurring vitamin B_{12}, into single-compartment vesicles composed of synthetic lipids having an alanyl residue in the single-chain segment, is primarily controlled by the hydrophobicity of the peripheral ester groups. Such incorporation into vesicles of another synthetic lipid, having a histidyl residue in place of an alanyl residue, was much enhanced when coordination was allowed to take place between the nuclear cobalt of the hydrophobic vitamin B_{12} and the imidazolyl moiety of the lipid. Microenvironmental properties around heptapropyl cobyrinate derivatives placed in single-compartment vesicles of the former lipid were examined by electronic, fluorescence and fluorescence polarization measurements as well as by differential scanning calorimetry. The hydrophobic vitamin B_{12} was incorporated into the intramembrane domain composed of assembly of the single-chain segment of each lipid molecule, and its molecular motion was markedly suppressed under such microenvironmental conditions. Carbon-skeleton rearrangement reactions of alkyl ligands bound to heptapropyl cobyrinate were markedly favoured in the single-compartment vesicle, relative to the reactions in methanol and benzene, under anaerobic photolysis conditions at ordinary temperatures. The 1,2-migration of electron-withdrawing groups, such as acetyl, cyano, carboxylic ester and thioester, apparently arises both from suppression of molecular motion and desolvation effects operating on the alkylated hydrophobic vitamin B_{12} in the vesicle. Finally, the catalytic mediator, constituted with heptapropyl cobyrinate perchlorate and the single-compartment vesicle in aqueous media, was coupled with a substrate-activation system, composed of atmospheric oxygen and vanadium(III) ions, to establish a real artifical holoenzyme. 2-Acetyl-2-ethoxycarbonylpropane, 2-cyano-2-ethoxycarbonylpropane and 1-acetyl-1-ethoxycarbonylethane were converted catalytically into the corresponding rearrangement products under aerobic photolysis conditions at 20 °C. A plausible reaction mechanism for the catalytic reaction is discussed.

Coenzyme B_{12}-dependent enzymes which catalyse isomerization reactions, leading to the intramolecular exchange of a functional group (X) and a hydrogen atom between neighbouring carbon atoms [eqn. (1)], have received much attention



(1)

because of the novel nature of these reactions from the viewpoints of organic and organometallic chemistry. Various cobalt complexes have been synthesized as vitamin B_{12} model complexes, and their physicochemical properties and catalytic performance have been investigated in attempts to clarify mechanisms involved in vitamin B_{12}-dependent enzymatic reactions.[1] Recently, these complexes have been extensively used not only as vitamin B_{12} models but also as novel catalysts for organic syntheses by taking advantage of the high nucleophilicity of their univalent cobalt species.[2–6] However, most of those complexes cannot be qualified as favourable ones for functional simulation of the vitamin B_{12}-dependent enzymes. For example, cobaloxime [bis(dimethylglyoximato)-cobalt], which is readily prepared and frequently utilized, is capable of forming the cobalt–carbon bond with various ligands in the same manner as observed for vitamin B_{12}.[7] Nevertheless, this cobalt complex is not considered to be a good model since its redox behaviour is quite different from that of vitamin B_{12}.[8] We have previously prepared the cobalt complex of 1,19-dimethyl-AD-didehydrocorrin, which has additional double bonds at peripheral sites and an extra angular methyl group compared with the parent corrinoid.[9] This complex was found to be analogous to the corrinoid as far as electronic properties are concerned, but failed to simulate the reactions mediated by vitamin B_{12} because of the marked steric repulsion effect exerted by the angular methyl groups.

In order to design the most favourable model complexes, we focused on the following aspects: (i) the redox behaviour of the central cobalt, which is primarily controlled by the basicity of an equatorial ligand, must be identical with that for the naturally occurring vitamin B_{12}; (ii) the electronic properties must be equivalent to those of the natural B_{12}, which are provided by the corrinoid skeleton with eight double bonds and direct bonding between A and D rings; and (iii) the steric effects, which are generated in vitamin B_{12} by the methyl moiety and the hydrogen atom at the C(1) and C(19) positions in the corrin ring, respectively, and by four propionamides and three acetamides protruding at the α- and β-peripheral sites, respectively, must be retained by model complexes. We have been dealing with hydrophobic vitamin B_{12} derivatives,[10–12] which have ester groups in place of the peripheral amide moieties of the naturally occurring vitamin B_{12}, in order to simulate various functions of vitamin B_{12} as exerted in the hydrophobic active sites of the various enzymes concerned. These cobalt complexes certainly satisfy all the above requirements.

Carbon-skeleton rearrangement reactions, mediated by

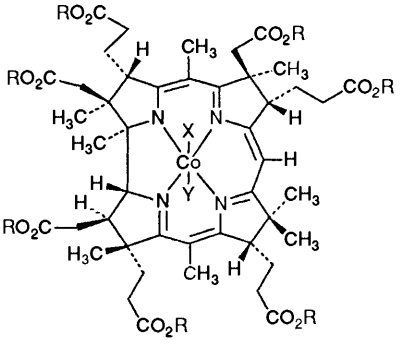
† Part 8, ref. 35.

methylmalonyl–CoA mutase, glutamate mutase and α-methyleneglutarate mutase, are referred to reversible interconversions represented as follows: methylmalonyl–SCoA ⇌ succinyl–SCoA, β-methylaspartate ⇌ glutamate and methylitaconate ⇌ α-methyleneglutarate. These enzymatic reactions do not require additional cofactors, unlike other isomerization reactions, and radical intermediates have not been detected by EPR spectroscopy.[13] This raised questions as to whether the reactions proceed *via* radical or anionic mechanisms, and whether or not the nuclear cobalt of coenzyme B_{12} participates in the rearrangement process. Thus, clarification of the reaction mechanisms involved has become a challenging area of biomimetic research.[14] Even though the real reaction mechanisms involved in the carbon-skeleton rearrangements mediated by the vitamin B_{12}-dependent enzymes are not clarified at present, radical mechanisms are considered to be the most plausible ones for other rearrangement reactions on the basis of various evidence.[15–18] However, no relevant apoprotein models, which are capable of generating substrate radicals in the dark, have been developed up to now. To compromise on these circumstances, we have been adopting photolysis conditions to generate substrate radicals.

As for the role of the cobalt species, Halpern *et al.* proposed a *reversible free radical carrier* mechanism; coenzyme B_{12} is referred simply to a source of the 5′-deoxyadenosyl free radical that acts to generate a substrate radical by abstracting a hydrogen atom from the substrate to initiate the reaction, and behaves as a reversible free radical carrier.[19,20] They excluded possible participation of the cobalt species in the rearrangement process. In connection with the biological reaction, a free radical rearrangement reaction, promoting 1,2-migration of a thioester group in a substrate radical generated by reaction of the corresponding bromide with n-Bu_3SnH and 2,2′-azobisisobutyronitrile, was investigated in methanol at relatively high temperatures (60–114 °C) as a model reaction for methylmalonyl–CoA mutase; the rearrangement product was obtained in a relatively small yield (1–9%).[21,22] On the other hand, thermal decomposition of the dimethylthiomalonate complex of vitamin B_{12} was carried out in aqueous media (pH 8–9) in the dark at room temperature, and the rearrangement product was obtained in a good yield (50–70%).[23–25] In addition, there is some experimental evidence indicating that a thioester group in ligands coordinated to a cobalt complex more readily migrates to the adjacent carbon compared with the same group involved in the corresponding free radicals.[26–28] Therefore, it is not reasonable to exclude the possible participation of the cobalt species in the course of 1,2-migration reactions, even though free radicals are spontaneously converted into the corresponding isomerization products to a certain extent. Since the participation mode of the cobalt complex has not yet been clarified for the rearrangement reaction occurring in biological systems, this is also a challenging subject of investigation.

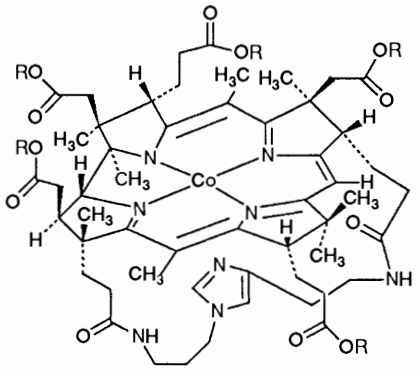
The naturally occurring apoproteins, which provide relevant reaction sites for vitamin B_{12}, are considered to play crucial roles that lead to desolvation and close association of reacting species.[17,29] In this regard, we have been interested in the catalytic activity of vitamin B_{12} in hydrophobic microenvironments, in order to simulate the catalytic functions of the holoenzymes concerned. Various macrocyclic compounds have been studied extensively in view of their potential for functional simulation of enzymes.[30] It was necessary for our study to develop macrocycles which provide a three-dimensionally extended cavity so that a bulky vitamin B_{12} model complex could be incorporated into it. From this viewpoint, we have prepared an octopus cyclophane having eight hydrocarbon chains, which behaves as an effective cationic host over a wide pH range in aqueous media.[31] An artificial holoenzyme, composed of the octopus cyclophane and a hydrophobic vitamin B_{12} derivative [Cob(II)7C_3ester]ClO_4,* was found to be an effective catalyst for carbon-skeleton rearrangement reactions such as simulation of the methylmalonyl–CoA mutase and glutamate mutase reactions, and for ring-expansion reactions which are not observed in biological systems.[28,32–35]

In this work, we adopted a single-compartment bilayer membrane composed of a synthetic peptide lipid as an effective apoprotein model. Synthetic peptide lipids, *e.g.*, N^+C_5Ala-$2C_{14}$, N^+C_5His2C_{14} and $(SO_3^-)C_5$Ala2C_{14}, were designed under the concept that intravesicular hydrogen-bonding interactions between amino acid residues may give sufficient morphological stability to single-walled bilayer membranes.[36–39] Thus, an amino acid residue was utilized as a structural component of a synthetic lipid in the following manner. The α-carboxylato and α-amino groups of an amino acid residue are connected with a hydrophobic double-chain segment by a tertiary amide linkage and with a connector unit to a polar head moiety by a secondary amide linkage, respectively. The connector unit is introduced on the grounds that a microenvironment around the amino acid residue in the lipid molecule is sufficiently separated from the bulk aqueous phase so as to become relatively hydrophobic in the aggregated state and the intravesicular hydrogen-bonding interaction becomes favoured under such conditions. In fact, the single-compartment bilayer membrane stays in solution for a prolonged period of time without meaningful morphological change. Thus, the interior domain can be used as a steady reaction site for a hydrophobic vitamin B_{12}. The single-compartment vesicle must be used to avoid any kinetic complication, which is anticipated if multi-walled vesicles are used. The extent of incorporation of various hydrophobic vitamin B_{12} derivatives into single-compartment vesicles was examined in the first place in order to find out which hydrophobic vitamin B_{12}'s can be anchored completely in the vesicular domain so that a steady catalytic mediator is readily set up in combination with them. Secondly, microenvironmental properties around the hydrophobic vitamin B_{12} in the single-compartment vesicle were investigated to characterize kinetic roles of the vesicle in the catalytic performance. Then we examined carbon-skeleton rearrangement reactions of alkyl ligands bound to the hydrophobic vitamin B_{12}, Cob7C_3ester, in the hydrophobic domain of bilayer membrane formed with N^+C_5Ala2C_{16} under anaerobic photolysis conditions at ordinary temperatures. Finally, a true artificial

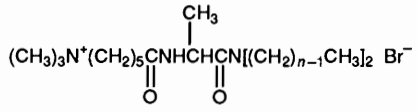


X = Y = CN, R = CH_3 : $(CN)_2$Cob(III)7C_1ester
X = Y = CN, R = C_2H_5 : $(CN)_2$Cob(III)7C_2ester
X = Y = CN, R = n-C_3H_7 : $(CN)_2$Cob(III)7C_3ester
X = Y = CN, R = n-C_4H_9 : $(CN)_2$Cob(III)7C_4ester
X = Y = None, R = CH_3 : [Cob(II)7C_1ester]ClO_4
X = Y = None, R = n-C_3H_7 : [Cob(II)7C_3ester]ClO_4
X = alkyl(L), Y = H_2O
R = n-C_3H_7 : [L-Cob(III)7C_3ester]ClO_4

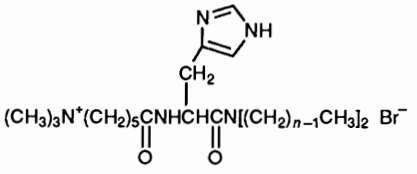
* The cobalt complex of corrin is conventionally known as cob.



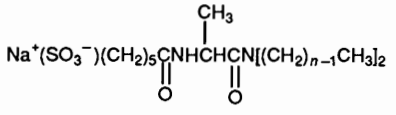
X = Y = CN, R = n-C_3H_7 : $(CN)_2$Cob(III)(Im : cap)5C_3ester
X = Y = CN, R = n-C_4H_9 : $(CN)_2$Cob(III)(Im : cap)5C_4ester
X = None, Y = Intramolecular imidazole,
R = CH_3 : [Cob(II)(Im : cap)5C_1ester]ClO_4
X = Y = None, R = CH_3 : [Cob(II)(Im^+-H : cap)5C_1ester]$(ClO_4)_2$
(X and Y : Axial ligands)



n = 12 : $N^+C_5Ala2C_{12}$
n = 14 : $N^+C_5Ala2C_{14}$
n = 16 : $N^+C_5Ala2C_{16}$



n = 14 : $N^+C_5His2C_{14}$



n = 14 : $(SO_3^-)C_5Ala2C_{14}$

holoenzyme system, which is capable of exhibiting turnover of the hydrophobic vitamin B_{12}, was established in combination with a substrate-activation process provided by vanadium trichloride and atmospheric oxygen under irradiation with visible light.

Experimental

General Analyses and Measurements.—Elemental analyses were performed at the Microanalysis Centre of Kyushu University. A Beckman Φ71 pH meter equipped with a Beckman 39505 combined electrode was used for pH measurements after calibration with a combination of appropriate standard aqueous buffers. IR spectra were taken on a JASCO IR-810 spectrophotometer, while electronic absorption spectra were recorded on a Hitachi 220A or a Hitachi 340 spectrophotometer. Fluorescence spectra were obtained with a Hitachi 650-60 spectrofluorometer, and fluorescence polarization measurements were performed with a Union Giken FS-501A fluorescence polarization spectrophotometer equipped with a Sord microcomputer M200 Mark II; emission at λ = 480 nm was monitored upon excitation at λ = 325 nm with a slit width of 3.5 nm for both excitation and emission sides. Fluorescence polarization (P) was calculated according to a method used previously.[40,41] EPR spectra were recorded on a JEOL JES-FE1G X-band spectrometer equipped with an Advantest TR-5213 microwave counter and an Echo Electronics EFM-200 NMR field meter, while ^{1}H NMR spectra were taken on a Hitachi R-24B, a JEOL JNM-GSX400 and a Bruker AMX-500 spectrometer installed at the Centre of Advanced Instrumental Analysis, Kyushu University. Mass spectroscopic analyses were performed with a JEOL JMS-01SG-2 spectrometer. Phase transition parameters [T_m, temperature at a peak maximum of differential scanning calorimetry (DSC) thermogram; ΔH, enthalpy change evaluated from a peak area] for bilayer membrane systems were measured with a Daini Seikosha SSC-560U differential scanning calorimeter. GLC analyses were carried out on a Shimadzu GC-4C or a Shimadzu GC-9A apparatus equipped with a Shimadzu C-R3A-FFC chromatopac for data processing.

Materials.—Peptide amphiphiles having the L-alanyl residue, such as *N,N*-didodecyl-*N*$^{\alpha}$-[6-(trimethylammonio)hexanoyl]-L-alaninamide bromide ($N^+C_5Ala2C_{12}$), *N,N*-ditetradecyl-*N*$^{\alpha}$-[6-(trimethylammonio)hexanoyl]-L-alaninamide bromide ($N^+C_5Ala2C_{14}$), *N,N*-dihexadecyl-*N*$^{\alpha}$-[6-(trimethylammonio)-hexanoyl]-L-alaninamide bromide ($N^+C_5Ala2C_{16}$) and sodium *N,N*-ditetradecyl-*N*$^{\alpha}$-(6-sulphohexanoyl)-L-alaninamide [$(SO_3^-)C_5Ala2C_{14}$], were prepared according to the methods reported previously.[36–38] A peptide lipid having the histidyl residue, *N,N*-ditetradecyl-*N*$^{\alpha}$-[6-(trimethylammonio)hexanoyl]-L-histidinamide bromide ($N^+C_5His2C_{14}$), was also prepared after the reported method.[39] Preparation of 2-(imidazol-4-yl)-*N*-[5-(dimethylamino)-1-naphthylsulphonyl]-ethylamine (dansylhistamine) as a fluorescent probe has been reported previously.[28] 2-Acetyl-2-ethoxycarbonylpropane **1a**, 2-acetyl-1-ethoxycarbonylpropane **1b**, 1-acetyl-2-ethoxycarbonylpropane **1c**, 2-cyano-2-ethoxycarbonylpropane **2a**, 2-cyano-1-ethoxycarbonylpropane **2b**, 1-cyano-2-ethoxycarbonylpropane **2c**, 2,2-bis(ethoxycarbonyl)propane **3a**, 1,2-bis-(ethoxycarbonyl)propane **3b**, 2-(ethylthio)carbonylpropane **4a**, 2-(ethylthio)carbonylprop-1-ene **4b**, 1-(ethylthio)carbonyl-propane **4c**, 1-acetyl-1-ethoxycarbonylethane **6a** and 1-acetyl-2-ethoxycarbonylethane **6b** were prepared as substrates and authentic samples for the corresponding reaction products after the procedures reported previously.[42] Diethyl β-methyl-DL-aspartate **5a** was prepared by esterification of β-methyl-DL-aspartic acid in a manner similar to that adopted for esterification of various α-amino acids,[43] and isolated as the *hydrochloride salt* (Found: C, 44.75; H, 7.55; N, 5.9. $C_9H_{18}ClNO_4 \cdot \frac{1}{8}H_2O$ requires C, 44.7; H, 7.6; N, 5.8%): δ_H(60 MHz; $CDCl_3$) 1.28 (6 H, dt, $CO_2CH_2CH_3$), 1.49 (3 H, d, $CHCH_3$), 3.39 (1 H, m, CH_3CH), 4.14 (1 H, d, CHN^+H_3), 4.20 (4 H, dq, $CO_2CH_2CH_3$) and 8.53 (3 H, br s, N^+H_3). Diethyl glutamate **5b** was purchased from Fluka Chemie AG (Switzerland), and used without further purification.

Hydrophobic Vitamin B_{12} *Derivatives.*—$(CN)_2$Cob(III)7C_1-ester, $(CN)_2$Cob(III)7C_2ester, $(CN)_2$Cob(III)7C_4ester, [Cob(II)-7C_1ester]ClO_4 and [Cob(II)7C_3ester]ClO_4, were prepared from cyanocobalamin with reference to the methods reported previously.[10–12] Capped hydrophobic vitamin B_{12} derivatives, [Cob(II)(Im : cap)5C_1ester]ClO_4 and [Cob(II)(Im^+–H : cap)-5C_1ester]$ClO_4)_2$, were also synthesized after the reported procedure.[44]

(*i*) $(CN)_2$Cob(III)7C_3*ester.** This complex was prepared from $(CN)_2$Cob(III)7C_1ester by transesterification in a good yield, as compared with the previous method.[12] Propan-1-ol (60 cm^3) saturated with hydrogen chloride was added to $(CN)_2$Cob(III)-7C_1ester (500 mg, 4.6 × 10^{-4} mol) dissolved in propan-1-ol (300

* This synthesis was carried out by Mr. Xi-Ming Song of this laboratory.

cm^3). After being stirred at 30–40 °C for 40 h under a nitrogen atmosphere, the solution was evaporated to dryness. The residue was dissolved in dichloromethane (200 cm^3) and shaken with aqueous potassium cyanide (2.5 g/50 cm^3). After the layers were separated, the organic layer was dried (Na_2SO_4) and evaporated to dryness. The product was purified by TLC on silica gel (Kiesel gel 60 H) with dichloromethane–methanol (10:1 v/v); the major purple fraction was collected and dissolved in carbon tetrachloride. The solution was then shaken with aqueous potassium cyanide, the layers separated, and the organic layer dried (Na_2SO_4) and evaporated to dryness to afford a *dark purple solid*; yield 464 mg (83%) (Found: C, 62.05; H, 7.7; N, 6.6. $C_{68}H_{101}CoN_6O_{14}$·1.5H_2O requires C, 62.2; H, 8.0; N, 6.4%); ν_{max}(KBr)/cm^{-1} 2950 (C–H str.), 2130 (C≡N str.) and 1730 (ester C=O str.); λ_{max}(C_6H_6)/nm 281 (ε/dm^3 mol^{-1} cm^{-1} 1.2 × 10^4), 316 (9.5 × 10^3), 375 (2.8 × 10^4), 425 (3.1 × 10^3), 520 (4.7 × 10^3), 553 (8.7 × 10^3) and 591 (1.2 × 10^4).

(*ii*) $(CN)_2$Cob(III)(Im:cap)5C_3*ester*. [$(CN)_2$Cob(III)(Im^+–H:cap)5C_1ester]Cl[44] (50 mg, 4.0 × 10^{-5} mol) was added to propan-1-ol (100 cm^3) saturated with hydrogen chloride, and the solution was stirred for 50 h at room temperature under a nitrogen atmosphere. After the precipitated species had been removed by filtration, the filtrate was evaporated to dryness under reduced pressure. The product was purified by gel-filtration chromatography on a column of Sephadex LH-20 with methanol as eluent. The major red fraction was evaporated to dryness and the species which was soluble in benzene but insoluble in carbon tetrachloride was separated as the desired product. The benzene layer was shaken with aqueous potassium cyanide, the layers separated, and the organic layer was dried (Na_2SO_4). The product was reprecipitated from benzene upon addition of hexane to afford a *purple powder*; yield 21 mg (38%) (Found: C, 63.35; H, 7.4; N, 10.6. $C_{70}H_{101}CoN_{10}O_{12}$ requires C, 63.05; H, 7.65; N, 10.5%); ν_{max}(KBr)/cm^{-1} 2940 (C–H str.), 2130 (C≡N str.), 1735 (ester C=O str.) and 1640 (amide C=O str.); λ_{max}(C_6H_6)/nm 316 (ε/dm^3 mol^{-1} cm^{-1} 6.1 × 10^3), 366 (1.2 × 10^4), 504 (3.5 × 10^3), 546 (3.8 × 10^3) and 585 (3.8 × 10^3).

(*iii*) $(CN)_2$Cob(III)(Im:cap)5C_4*ester*. This complex was prepared from [$(CN)_2$Cob(III)(Im^+–H:cap)5C_1ester]Cl by transesterification and purified by gel-filtration chromatography in a manner similar to that described above. The product, which was soluble in benzene and carbon tetrachloride, was treated with aqueous potassium cyanide in a manner as described above and reprecipitated from benzene upon addition of hexane to afford a *purple powder*; yield 20 mg (35%) (Found: C, 64.65; H, 7.95; N, 9.95. $C_{75}H_{111}CoN_{10}O_{12}$ requires C, 64.15; H, 7.95; N, 10.0%); ν_{max}(KBr)/cm^{-1} 2940 (C–H str.), 2130 (C≡N str.), 1735 (ester C=O str.) and 1640 (amide C=O str.); λ_{max}(C_6H_6)/nm 316 (ε/dm^3 mol^{-1} cm^{-1} 6.1 × 10^3), 366 (1.2 × 10^4), 504 (3.5 × 10^3), 546 (3.8 × 10^3) and 585 (3.8 × 10^3).

Alkylated Hydrophobic Vitamin B_{12}*'s*, L-Cob(III)7C_3*ester*.*— Various alkylated hydrophobic vitamin B_{12}'s were prepared by two different methods: (*a*) [Cob(II)7C_3ester]ClO_4 was reduced with sodium tetrahydroborate to the cobalt(I) species, which subsequently underwent reaction with an alkyl halide by oxidative addition;[45,46] (*b*) the bivalent cobalt complex underwent reaction with a substituted alkane in the presence of vanadium trichloride under aerobic conditions.[14i,47–50] Preparation of the adduct of the hydrophobic vitamin B_{12} and methylaspartate is described below as a representative example.

(*i*) [$CH(NH_2)(CO_2C_2H_5)CH(CO_2C_2H_5)CH_2$-Cob(III)7$C_3$-*ester*]$ClO_4$ **5**. The following experiment was carried out in the dark. A methanol solution (100 cm^3) containing [Cob(II)7C_3-ester]ClO_4 (300 mg, 2.2 × 10^{-4} mol) and diethyl β-methyl-DL-aspartate (a mixture of *erythro* and *threo* diastereoisomers; 100 mg, 4.2 × 10^{-4} mol) was mixed with 60 cm^3 of aqueous sodium carbonate buffer (0.02 mol dm^{-3}; pH 11.2), and then 25 cm^3 of 15% (w/w) aqueous perchloric acid containing vanadium trichloride (50 mg, 3.2 × 10^{-4} mol) was added to the solution. The resulting mixture was stirred vigorously for 5 min at room temperature, air was introduced into it for 2 min, and stirring was continued further for 1 h. The product was extracted with dichloromethane and isolated as its perchlorate salt by gel-filtration chromatography on a column of Sephadex LH-20 with methanol as eluent; yield 151 mg (46%) (Found: C, 57.55; H, 7.45; N, 4.4. $C_{75}H_{117}ClCoN_5O_{22}$·1.5$H_2O$ requires C, 57.65; H, 7.75; N, 4.5%); ν_{max}(KBr)/cm^{-1} 2945 (C–H str.), 1725 (ester C=O str.), 1100 and 620 (ClO_4^- str.); λ_{max}(CH_2Cl_2)/nm 271 (ε/dm^3 mol^{-1} cm^{-1} 2.4 × 10^4), 311 (2.2 × 10^4) and 458 (1.0 × 10^4).

(*ii*) [$(COCH_3)(CO_2C_2H_5)(CH_3)CCH_2$-Cob(III)7$C_3$*ester*]-$ClO_4$ **1**. ν_{max}(KBr)/cm^{-1} 2950 (C–H str.), 1730 (ester C=O str.), 1100 and 620 (ClO_4^- str.); λ_{max}(CH_2Cl_2)/nm 273 (ε/dm^3 mol^{-1} cm^{-1} 2.2 × 10^4), 309 (2.4 × 10^4), 376 (8.7 × 10^3) and 456 (1.0 × 10^4).

(*iii*) [$(CN)(CO_2C_2H_5)(CH_3)CCH_2$-Cob(III)7$C_3$*ester*]$ClO_4$ **2**. ν_{max}(KBr)/cm^{-1} 2940 (C–H str.), 1730 (ester C=O str.), 1100 and 620 (ClO_4^- str.); λ_{max}(CH_2Cl_2)/nm 269 (ε/dm^3 mol^{-1} cm^{-1} 2.2 × 10^4), 310 (2.4 × 10^4), 378 (8.4 × 10^3) and 458 (1.0 × 10^4) (Found: C, 59.25; H, 7.8; N, 4.95. $C_{73}H_{113}ClCoN_5O_{21}$ requires C, 58.8; H, 7.65; N, 4.7%).

(*iv*) [$(CO_2C_2H_5)_2(CH_3)CCH_2$-Cob(III)7$C_3$*ester*]$ClO_4$ **3**. ν_{max}(KBr)/cm^{-1} 2950 (C–H str.), 1730 (ester C=O str.), 1090 and 620 (ClO_4^- str.); λ_{max}(CH_2Cl_2)/nm 272 (ε/dm^3 mol^{-1} cm^{-1} 2.2 × 10^4), 311 (2.4 × 10^4), 377 (8.8 × 10^3) and 457 (1.1 × 10^4) (Found: C, 59.0; H, 7.5; N, 3.95. $C_{75}H_{118}ClCoN_4O_{23}$ requires C, 58.55; H, 7.75; N, 3.65%).

(*v*) [$(COSC_2H_5)(CH_3)CHCH_2$-Cob(III)7C_3*ester*]ClO_4 **4**. ν_{max}(KBr)/cm^{-1} 2945 (C–H str.), 1725 (ester C=O str.), 1100 and 630 (ClO_4^- str.); λ_{max}(CH_2Cl_2)/nm 270 (ε/dm^3 mol^{-1} cm^{-1} 2.3 × 10^4), 309 (2.2 × 10^4), 375 (8.5 × 10^3) and 455 (1.1 × 10^4).

Complexes **1** and **4** were quite labile and could not be subjected to critical elemental analyses, so that these were used immediately after preparation.

Equilibrium Measurements for Incorporation of Hydrophobic Vitamin B_{12} *Derivatives into Single-compartment Vesicles*.—A dichloromethane solution (1 cm^3) containing a peptide amphiphile (an appropriate quantity) and a hydrophobic vitamin B_{12} derivative with axial ligation of the cyano groups (1.0 × 10^{-7} mol) was evaporated *in vacuo* to remove the solvent completely, and an aqueous potassium cyanide solution (1 cm^3, 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. The vesicular solution was applied on a column of Sephadex G-50 and eluted with aqueous potassium cyanide (5.0 × 10^{-4} mol dm^{-3}). In the case of [Cob(II)7$C_n$ester]ClO_4 (n = 1 and 3), aqueous sodium perchlorate (5.0 × 10^{-4} mol dm^{-3}) was used for elution. The bound complex was eluted first in the column void volume, and its amount was determined from absorbances at the α- and γ-bands (λ = *ca.* 590 and *ca.* 370 nm, respectively).

Photolysis of Alkylated Hydrophobic Vitamin B_{12}*'s in Single-compartment Vesicles*.—N^+C_5Ala2C_{16} (77.3 mg, 1.0 × 10^{-4}

* We have carried out NMR (500 MHz) measurements on all the alkylated hydrophobic vitamin B_{12}'s reported here. Because of the highly complicated spectral data, we have refrained from making complete assignments at present. Detailed analyses of NMR spectra will, however, be reported in the near future.

mol) was dispersed in an aqueous buffer (20 cm^3, phosphate–borate 0.05 mol dm^{-3}; pH 9.2) by Vortex mixing (mechanical mixing), and the dispersion sample was sonicated for 2 min with a probe-type sonicator at 30 W to give a clear solution, which was then allowed to stand at 0 °C for 5 min. After the solution was deoxygenated with argon gas, a methanol solution (0.2 cm^3) of L-Cob(III)7C_3ester (1.0×10^{-6} mol) was added to it, resulting in the following final concentrations: L-Cob(III)7C_3-ester, 5.0×10^{-5} mol dm^{-3}; $N^+C_5Ala2C_{16}$, 5.0×10^{-3} mol dm^{-3}. The resulting solution was then 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, in *ca.* 1 h (as confirmed by electronic spectroscopy), the products were extracted with dichloromethane (3×20 cm^3). Any trace amount of water was separated from the dichloromethane extract with phase-separation filter paper (Whatman 1PS) which was washed with dichloromethane in advance. The dichloromethane solution was evaporated to dryness, and an appropriate amount of diethyl ether (500 mm^3) was added to the residue. The products were identified by means of GLC, with co-injection of authentic samples into columns of Silicone DC-550 (Shimadzu Co., Japan), Silicone SE-30 (Gasukuro Kogyo Inc., Japan), Thermon-3000 (Shimadzu) and dioctyl phthalate (Gasukuro Kogyo). A capillary column of Polyethylene Glycol-20M (Gasukuro Kogyo) was used for identification of isomers having similar structures. Quantitative analyses of the products were carried out by GLC on the basis of correlation lines established independently by using authentic samples. The following GLC conditions were employed. (i) **1a**, **1b** and **1c**: Silicone DC-550 (Φ5 mm × 3 m column), injection temp. 200 °C, column temp. 120 °C, carrier gas N_2 70 cm^3 min^{-1}; retention time t_R 4.1 min for **1a**, 7.4 min for **1b** and 6.9 min for **1c**. (ii) **2a**, **2b** and **2c**: Thermon-3000 (Φ3 mm × 3 m column), injection temp. 260 °C, column temp. 100 °C, carrier gas N_2 50 cm^3 min^{-1}; t_R 6.2 min for **2a**, 26.0 min for **2b** and 24.6 min for **2c**. Polyethylene Glycol-20M (Φ0.25 mm × 50 m capillary column), injection temp. 220 °C, column temp. 140 °C, carrier gas N_2 50 cm^3 min^{-1} (split ratio, 1/80); t_R 4.8 min for **2a**, 10.3 min for **2b** and 9.9 min for **2c**. (iii) **3a** and **3b**: Silicone DC-550 (Φ5 mm × 3 m column), injection temp. 200 °C, column temp. 160 °C, carrier gas N_2 60 cm^3 min^{-1}; t_R 2.5 min for **3a** and 4.1 min for **3b**. (iv) **4a**, **4b** and **4c**: dioctyl phthalate (Φ5 mm × 3 m column), injection temp. 140 °C, column temp. 120 °C, carrier gas N_2 70 cm^3 min^{-1}; t_R 12.5 min for **4a**, 19.7 min for **4b** and 17.8 min for **4c**. (v) **5a** and **5b**: Silicone SE-30 (Φ5 mm × 3 m column), injection temp. 250 °C, column temp. 190 °C, carrier gas N_2 30 cm^3 min^{-1}; t_R 4.0 min for **5a** and 6.8 min for **5b**. Silicone DC-550 (Φ5 mm × 3 m column), injection temp. 250 °C, column temp. 200 °C, carrier gas N_2 70 cm^3 min^{-1}; t_R 2.5 min for **5a** and 6.5 min (broad) for **5b**. (vi) **6a** and **6b**: Silicone DC-550 (Φ5 mm × 3 m column), injection temp. 200 °C, column temp. 120 °C, carrier gas N_2 50 cm^3 min^{-1}; t_R 3.1 min for **6a** and 5.2 min for **6b**. The rearrangement products separated by preparative GLC were also identified by means of ^1H NMR and mass spectroscopic measurements. **1b**: δ_H(500 MHz; $CDCl_3$) 1.15 (3 H, d, $CHCH_3$), 1.24 (3 H, t, CH_2CH_3), 2.21 (3 H, s, $COCH_3$), 2.28 (1 H, dd, $CHCH_2$), 2.74 (1 H, dd, $CHCH_2$), 3.02 (1 H, m, CH) and 4.11 (2 H, q, CH_2CH_3). **1c**: δ_H(500 MHz; $CDCl_3$) 1.16 (3 H, d, $CHCH_3$), 1.25 (3 H, t, CH_2CH_3), 2.23 (3 H, s, $COCH_3$), 2.29 (1 H, dd, $CHCH_2$), 2.76 (1 H, dd, $CHCH_2$), 3.02 (1 H, m, CH) and 4.12 (2 H, q, CH_2CH_3); M^+(EI ionization) 158. **2b**: δ_H(500 MHz; $CDCl_3$) 1.29 (3 H, t, CH_2CH_3), 1.39 (3 H, d, $CHCH_3$), 2.53 (1 H, dd, $CHCH_2$), 2.72 (1 H, dd, $CHCH_2$), 3.10 (1 H, m, $CHCH_2$) and 4.20 (2 H, q, CH_2CH_3); M^+ (EI ionization) 141. **2c**: δ_H(60 MHz; $CDCl_3$) 1.28 (3 H, t, CH_2CH_3), 1.32 (3 H, d, $CHCH_3$), 2.52 (2 H, br s, $CHCH_2$), 2.60 (1 H, m, $CHCH_2$) and 4.12 (2 H, q, CH_2CH_3). **3b**: δ_H(400 and 500 MHz; $CDCl_3$) 1.22 (3 H, d, $CHCH_3$), 1.25 (3 H, t, CH_2CH_3), 1.26 (3 H, t, CH_2CH_3), 2.39 (1 H, dd, CH_2), 2.73 (1 H, dd, CH_2), 2.90 (1 H, m, CH) and 4.14 (4 H, q, CH_2CH_3); M^+ (EI ionization) 188. **4c**: δ_H(60 MHz; $CDCl_3$) 0.95 (3 H, t, $CH_2CH_2CH_3$), 1.24 (3 H, t, SCH_2CH_3), 1.63 (2 H, m, $CH_2CH_2CH_3$), 2.58 (2 H, q, SCH_2CH_3) and 2.88 (2 H, t, $CH_2CH_2CH_3$). **5b**: δ_H(500 MHz; $CDCl_3$) 1.26 (3 H, t, CH_2CH_3), 1.28 (3 H, t, CH_2CH_3), 1.56 (2 H, br s, NH_2), 1.84 (1 H, m, $CHCH_2CH_2$), 2.08 (1 H, m, $CHCH_2CH_2$), 2.46 (2 H, t, $CHCH_2CH_2$), 3.46 (1 H, dd, $CHCH_2CH_2$), 4.14 (2 H, q, CH_2CH_3) and 4.18 (2 H, q, CH_2CH_3). **6b**: δ_H(60 MHz; $CDCl_3$) 1.25 (3 H, t, CH_2CH_3), 2.20 (3 H, s, $COCH_3$), 2.67 (4 H, m, CH_2CH_2) and 4.15 (2 H, q, CH_2CH_3).

Catalytic Reaction in Single-compartment Vesicles.—An aqueous dispersion (20 cm^3) of $N^+C_5Ala2C_{16}$ (49.2 mg, 6.35×10^{-5} mol) was subjected to sonication for 2 min with a probe-type sonicator (30 W) at room temperature to give a clear solution of single-compartment vesicles. A methanol solution (0.2 cm^3) containing [Cob(II)7C_3ester]ClO_4 (1.4 mg, 1.1×10^{-6} mol) and a substrate (6.4×10^{-5} mol) was then added to the vesicular solution at room temperature. Aqueous 10% (w/w) perchloric acid (1 cm^3) containing vanadium trichloride (334 mg, 2.12×10^{-3} mol; final concentration, 0.1 mol dm^{-3}) was added to the resulting solution at room temperature to give a final pH of 1.0. When the reaction was carried out at pH 7.0, aqueous 10% (w/w) sodium hydroxide (*ca.* 0.55 cm^3) was added to the reaction sample to attain the desired pH. The reaction mixture, maintained at 20.0 ± 0.1 °C, was irradiated with a 500 W tungsten lamp at a distance of 30 cm while air was bubbled through the mixture. Samples were taken out at appropriate time intervals for product analysis by GLC, in a manner as described above. No oxygenation products were detected by GLC under the present experimental conditions.

Results and Discussion

Incorporation of Hydrophobic Vitamin B_{12} Derivatives into Single-compartment Vesicles.—The extents of incorporation of the following vitamin B_{12} derivatives into single compartment vesicles, composed independently of $N^+C_5Ala2C_n$, $N^+C_5His2C_{14}$ and $(SO_3^-)C_5Ala2C_{14}$, were investigated as summarized in Table 1; $(CN)_2$Cob(III)7$C_n$ester (n = 1–4), [Cob(II)7$C_n$ester]ClO_4 (n = 1 and 3), $(CN)_2$Cob(III)(Im:cap)-5$C_n$ester (n = 3 and 4), [Cob(II)(Im:cap)5C_1ester]ClO_4 and [Cob(II)(Im^+–H:cap)5C_1ester]$(ClO_4)_2$. All the hydrophobic vitamin B_{12} derivatives with two cyano ligands, $(CN)_2$Cob(III)-$C_n$ester, were completely distributed to the organic layer when these complexes were mixed together with aqueous and dichloromethane phases. On the other hand, the incorporation behaviour of the $N^+C_5Ala2C_{12}$ vesicle, which provides an intramembrane hydrophobic domain toward the hydrophobic vitamin B_{12} was different from that of organic media. $(CN)_2$Cob(III)-7$C_n$ester was readily incorporated into the vesicle when the n-value of C_n was equal to 2 or larger, but $(CN)_2$Cob(III)-7C_1ester was not bound to the vesicle at all. The latter complex is slightly soluble in water (*ca.* 10^{-4} mol dm^{-3}), but $(CN)_2$Cob(III)7C_3ester and $(CN)_2$Cob(III)7C_4ester are almost insoluble. Thus, the extent of incorporation of $(CN)_2$Cob(III)7$C_n$ester into the vesicle is primarily controlled by the hydrophobicity of the ester groups placed at the peripheral sites of the corrinoid skeleton.

When a membrane-forming amphiphile involves a functional group capable of coordinating to the cobalt complex, the incorporation of [Cob(II)7C_1ester]ClO_4, having some limited solubility in water (*ca.* 10^{-3} mol dm^{-3}) was much enhanced by the coordination effect; this cobalt complex was largely incorporated into the $N^+C_5His2C_{14}$ vesicle (Table 1). The stability constant (K) for coordination of the imidazolyl moiety

Table 1 Incorporation of hydrophobic vitamin B_{12} derivatives into single-compartment vesicles at 20.0 ± 0.1 °C[a]

Complex[b]	Vesicle (quantity/10^{-5} mol)	Incorporated complex, %[c]
$(CN)_2$Cob(III)7C_1ester	N^+C_5Ala2C_{12} (1.0)	0[e]
$(CN)_2$Cob(III)7C_2ester	N^+C_5Ala2C_{12} (1.0)	48[e]
$(CN)_2$Cob(III)7C_3ester	N^+C_5Ala2C_{12} (1.0)	100[e]
$(CN)_2$Cob(III)7C_4ester	N^+C_5Ala2C_{12} (1.0)	100[e]
[Cob(II)7C_1ester]ClO_4[d]	N^+C_5Ala2C_{12} (1.0)	0
[Cob(II)7C_1ester]ClO_4[d]	N^+C_5His2C_{14} (1.0)	90[e]
[Cob(II)7C_3ester]ClO_4[d]	N^+C_5Ala2C_{12} (1.0)	100
[Cob(II)7C_3ester]ClO_4[d]	N^+C_5Ala2C_{14} (1.0)	100
[Cob(II)7C_3ester]ClO_4[d]	N^+C_5Ala2C_{16} (1.0)	100
[Cob(II)7C_3ester]ClO_4[d]	N^+C_5His2C_{14} (1.0)	100
$(CN)_2$Cob(III)(Im:cap)5C_3ester	N^+C_5Ala2C_{14} (0.5)	79
$(CN)_2$Cob(III)(Im:cap)5C_3ester	N^+C_5Ala2C_{14} (2.0)	100
$(CN)_2$Cob(III)(Im:cap)5C_3ester	$(SO_3^-)C_5$Ala2C_{14} (0.5)	81
$(CN)_2$Cob(III)(Im:cap)5C_3ester	$(SO_3^-)C_5$Ala2C_{14} (2.0)	100
$(CN)_2$Cob(III)(Im:cap)5C_4ester	N^+C_5Ala2C_{14} (0.5)	100
$(CN)_2$Cob(III)(Im:cap)5C_4ester	N^+C_5Ala2C_{14} (2.0)	100
$(CN)_2$Cob(III)(Im:cap)5C_4ester	$(SO_3^-)C_5$Ala2C_{14} (0.5)	100
$(CN)_2$Cob(III)(Im:cap)5C_4ester	$(SO_3^-)C_5$Ala2C_{14} (2.0)	100
[Cob(II)(Im:cap)5C_1ester]ClO_4[d]	N^+C_5His2C_{14} (0.5)	0
[Cob(II)(Im^+–H:cap)5C_1ester]$(ClO_4)_2$[d]	N^+C_5His2C_{14} (0.5)	91

[a] Incorporation was examined by gel-filtration chromatography on a column of Sephadex G-50 with aqueous potassium cyanide solution (5.0×10^{-4} mol dm^{-3}) as eluent. [b] Quantity used, 1.0×10^{-7} mol. [c] Incorporated quantity was determined by electronic spectroscopy. [d] Aqueous sodium perchlorate (5.0×10^{-4} mol dm^{-3}) was used as eluent. [e] Cited from ref. 12.

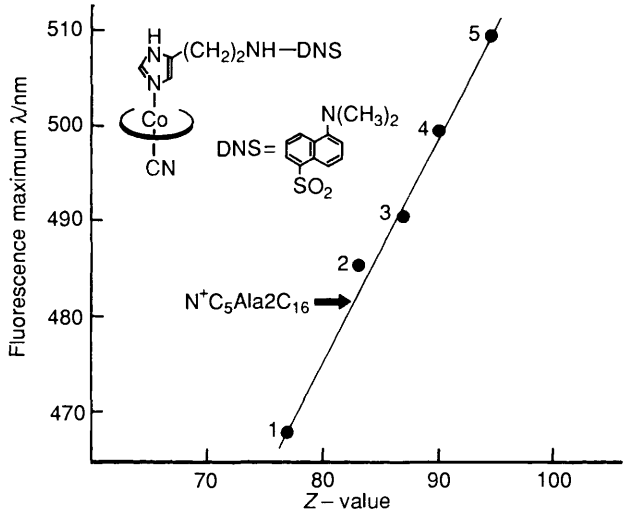
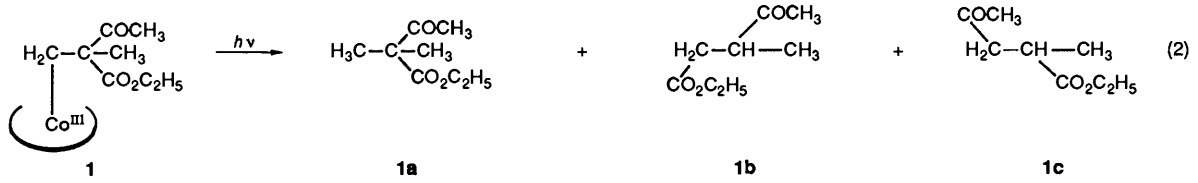


Fig. 1 Medium effect on fluorescence of dansylhistamine (1.0×10^{-5} mol dm^{-3}) coordinated to [(CN)Cob(III)7C_3ester]ClO_4 (1.0×10^{-5} mol dm^{-3}) incorporated into the N^+C_5Ala2C_{16} (5.0×10^{-3} mol dm^{-3}) vesicle in phosphate–borate buffer (0.05 mol dm^{-3}; pH 9.2) at 20.0 ± 0.1 °C. Reference 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 polarity parameter.

of N^+C_5His2C_{14} to the nuclear cobalt of [Cob(II)7C_1ester]ClO_4 at one of its axial sites was measured in dichloromethane at 26.5 °C as reported previously;[12] log*K* = 5.5. Consequently, the incorporation of [Cob(II)7C_1ester]ClO_4 into the vesicle of N^+C_5His2C_{14} is primarily caused by the axial coordination of the amphiphile to the complex. When a methanol solution of [Cob(II)7C_1ester]ClO_4 was added to the single-compartment vesicles of N^+C_5His2C_{14}, the cobalt(II) complex was confirmed to be bound to the vesicle in the 'base-on' form. The inclusion behaviour of the N^+C_5His2C_{14} vesicle is subjected to marked change by the axial coordination status of an intramolecular base of the hydrophobic vitamin B_{12}. A capped hydrophobic vitamin B_{12}, [Cob(II)(Im:cap)5C_1ester]ClO_4, in the 'base-on' state was not incorporated into the vesicle, while the corresponding protonated complex, [Cob(II)(Im^+–H:cap)5C_1ester]$(ClO_4)_2$, is in the 'base-off' state and was largely incorporated into the vesicle because of the coordination effect exercised by the amphiphile, even though both complexes are soluble in water and were not bound to the N^+C_5Ala2C_n (*n* = 12 and 14) vesicles.

Regardless of the charge mode of head groups, cationic or anionic, of amphiphiles, the incorporation behaviour of the vesicles was found to be identical with each other as shown with $(CN)_2$Cob(III)(Im:cap)5$C_n$ester (*n* = 3 and 4).

Microenvironmental Properties around Hydrophobic Vitamin B_{12} in Vesicle.—The aggregate morphology of peptide lipids remains the same even upon incorporation of a hydrophobic vitamin B_{12}, as clarified previously by means of electron microscopy.[12] The effects of an added hydrophobic vitamin B_{12} in aggregate morphology of peptide lipids was also examined by means of DSC. The phase transition of the single-compartment N^+C_5Ala2C_{16} vesicle from the gel to the liquid-crystalline state was observed as a broad endothermic peak: T_m, 20 °C (a broad peak ranged over 18–23 °C); ΔH, 18 kJ mol^{-1}. A similar DSC thermogram was observed when the same vesicle (5.0×10^{-3} mol dm^{-3}) included $(CN)_2$Cob(III)7C_3ester (1.0×10^{-4} mol dm^{-3}): T_m, 23 °C (a broad peak ranged over 19–25 °C); ΔH, 16 kJ mol^{-1}. As for multilayered vesicles of the same lipid, a sharp endothermic peak was observed under the identical conditions; the T_m and ΔH values were 25.5 °C and 32.6 kJ mol^{-1}, respectively, without the complex; these were 24.5 °C and 34.0 kJ mol^{-1}, respectively, in the presence of $(CN)_2$Cob(III)7C_3-ester. Since these phase transition parameters are primarily dependent on a packing mode of lipid molecules in their double hydrocarbon-chain domains,[37] the parameters clearly indicate that the hydrophobic vitamin B_{12} is incorporated into an apolar domain close to the amino acid residues and not into a nonpolar domain composed of the aliphatic double-chains. The microenvironmental polarity experienced by a hydrophobic vitamin B_{12} derivative, which is included in the vesicle, can be evaluated by referring to its characteristic absorption bands (α- and β-bands); the N^+C_5Ala2C_n (*n* = 14 and 16) vesicle provides a microenvironmental polarity equivalent to that of dichloromethane for $(CN)_2$Cob(III)7C_3ester.[12] Thus, this information is also in favour of the above conclusion.



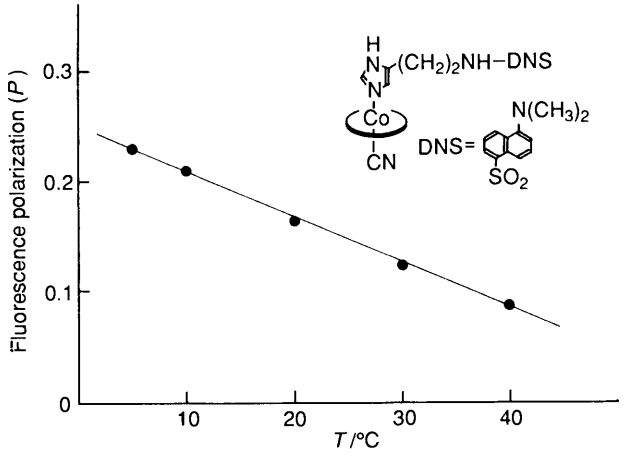


Fig. 2 Temperature effect on fluorescence polarization (P) of dansylhistamine (1.0×10^{-5} mol dm^{-3}) coordinated to [(CN)Cob(III)7C$_3$-ester]ClO$_4$ (1.0×10^{-5} mol dm^{-3}) incorporated into the N$^+$C$_5$Ala2C$_{16}$ (5.0×10^{-3} mol dm^{-3}) vesicle in phosphate–borate buffer (0.05 mol dm^{-3}; pH 9.2)

Table 2 Product analyses for photolysis of **1** in various media at 20.0 ± 0.1 °C[a]

Medium	Yield (%)		
	1a	**1b**	**1c**
Methanol[b]	70	Trace[d]	8.0
Benzene[b]	65	Trace[e]	10
N$^+$C$_5$Ala2C$_{16}$ vesicle[c]	25	Trace[e]	63

[a] A solution containing complex **1** (5.0×10^{-5} mol dm^{-3}) was irradiated with a 500 W tungsten lamp for 1 h at a distance of 30 cm. Products were analysed by GLC. [b] Cited from ref. 28. [c] Single-compartment vesicles of N$^+$C$_5$Ala2C$_{16}$ (5.0×10^{-3} mol dm^{-3}) in aqueous phosphate–borate buffer (0.05 mol dm^{-3}; pH 9.2). [d] Yield less than 0.1%. [e] Yield less than 1%.

In order to obtain further information about the microenvironment around the hydrophobic vitamin B_{12} in vesicles, [(CN)Cob(III)7C$_3$ester]ClO$_4$ coordinated at the residual axial site by dansylhistamine as a fluorescent probe was adopted. The microscopic polarity experienced by the dansyl moiety bound to the hydrophobic vitamin B_{12} is reflected in its fluorescence maximum.[51] First, the fluorescence maxima of dansylhistamine coordinated to [(CN)Cob(III)7C$_3$ester]ClO$_4$ 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 now clear that the single-compartment vesicle of N$^+$C$_5$Ala2C$_{16}$ provides a microenvironment for the dansyl moiety that is equivalent to a medium polarity between those of methanol and ethanol. Since the hydrophobic vitamin B_{12} itself in the vesicle is in a microenvironment equivalent in medium polarity to dichloromethane, the dansyl moiety seems to be placed at a site in the vesicle closer to the bulk aqueous phase. This result leads us to conclude that not only the hydrophobic vitamin B_{12} itself but also an axial ligand bound to the complex is significantly desolvated in the vesicle.

The dansylhistamine-coordinated hydrophobic vitamin B_{12}, incorporated into the vesicle, gave large fluorescence polarization (P) values at various temperatures, *i.e.* 0.1–0.23 in the temperature range 5–40 °C (Fig. 2). This apparently indicates that the molecular motion of the guest molecule in the vesicle is markedly suppressed, since P values in methanol and benzene are 0.005–0.006 and 0.02–0.04, respectively, in the same temperature range. Thus, the microenvironmental effect provided by the vesicle is quite different from those produced by simple organic solvents which solubilize the hydrophobic vitamin B_{12} derivative homogeneously.

Photochemical Carbon-skeleton Rearrangements of Alkyl Ligands Bound to Hydrophobic Vitamin B_{12}.—Hydrophobic vitamin B_{12} derivatives bearing various alkyl ligands at one axial site of the nuclear cobalt were incorporated into the N$^+$C$_5$Ala2C$_{16}$ vesicle in aqueous media and then irradiated with visible light under anaerobic conditions. Product analyses for the photolysis of the alkylated complex, (COCH$_3$)(CO$_2$-C$_2$H$_5$)(CH$_3$)CCH$_2$-Cob(III)7C$_3$ester (**1**), in the N$^+$C$_5$Ala2C$_{16}$ vesicle, methanol and benzene are summarized in Table 2 [refer to eqn. (2)]. The yield of acetyl-migration product **1c** was significantly increased in the vesicle as compared with those in methanol and benzene. The similar reaction behaviour with respect to the medium effect was observed for the 1,2-migration of electron-withdrawing groups such as cyano, carboxylic ester and thioester [refer to eqns. (3)–(6)], as summarized in Table 3. In the light of these product analyses, the apparent migratory aptitude of electron-withdrawing groups increases as follows: CN ≈ CO$_2$C$_2$H$_5$ < COCH$_3$. This order is in agreement with that obtained on the basis of MNDO SCF-MO calculations for free radicals; CN < CHO.[52] On the other hand, this aptitude is somewhat different from that observed for the electrochemical carbon-skeleton rearrangements, which proceed *via* formation of anionic intermediates.[42]

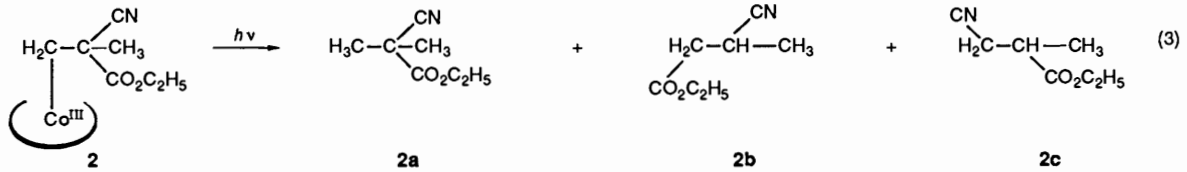
Correlations of the concentration of N$^+$C$_5$Ala2C$_{16}$ with product yields are shown in Fig. 3 for the photolysis of **1**. When the amphiphile concentration became less than *ca.* 30 times relative to that of the alkylated hydrophobic vitamin B_{12}, the yield of the acetyl-migrated product was progressively reduced. In the concentration range above 1.6×10^{-4} mol dm^{-3} while the concentration of the alkylated complex remained constant at 5.0×10^{-5} mol dm^{-3}, the product ratio reached a constant level; the reduction product **1a** *vs.* the rearrangement product **1c** being 1:3 in molar ratio. Similar behaviour was observed for the reaction of **2** (5.0×10^{-5} mol dm^{-3}), and the molar ratio of reduction product **2a** *vs.* rearrangement products, **2b** and **2c**, became constant (55:45) in a concentration range of the amphiphile above 1.6×10^{-4} mol dm^{-3}. These results indicate that all molecules of the alkylated vitamin B_{12}'s are completely incorporated into the vesicles above the threshold concentration of the amphiphile.

A bivalent copper salt, $CuCl_2$, is known to serve as a selective scavenger towards radicals that enter the bulk aqueous phase.[53,54] For this reason, $CuCl_2$ was added to the reaction solution containing the N$^+$C$_5$Ala2C$_{16}$ (5.0×10^{-3} mol dm^{-3})

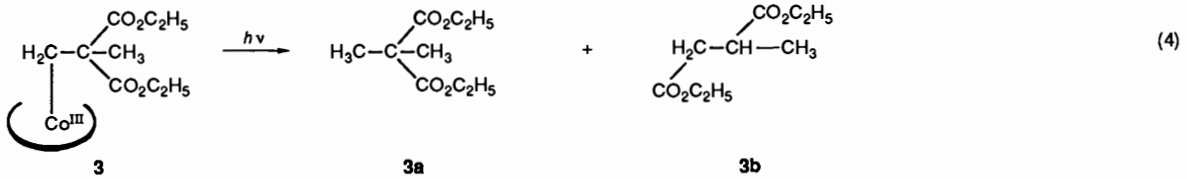
Table 3 Product analyses for photolyses of **2**, **3**, **4** and **5** in various media at 20.0 ± 0.1 °C[a]

Medium	Yield (%)			Yield (%)		Yield (%)			Yield (%)[e]	
	2a	**2b**	**2c**	**3a**	**3b**	**4a**	**4b**	**4c**	**5a**	**5b**
Methanol[b]	87	0	0	88	0	83	0	0	83	0
Benzene[b]	78	Trace[d]	Trace[d]	82	1.3	80	Trace[d]	Trace[d]	78	Trace[d]
Vesicle[c]	41	23	18	75	9.0	61	4	13	66	14

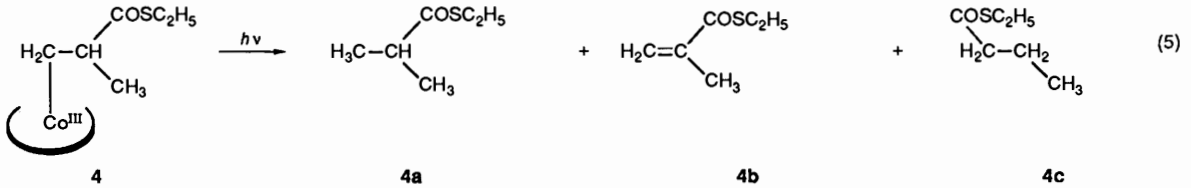
[a] A solution containing the alkylated complex (5.0×10^{-5} mol dm^{-3}) was irradiated with a 500 W tungsten lamp for 1 h at a distance of 30 cm. Products were analysed by GLC. [b] Cited from ref. 28. [c] Single-compartment vesicles of $N^+C_5Ala2C_{16}$ (5.0×10^{-3} mol dm^{-3}) in aqueous phosphate–borate buffer (0.05 mol dm^{-3}; pH 9.2). [d] Yield less than 1%. [e] Cited from ref. 46.



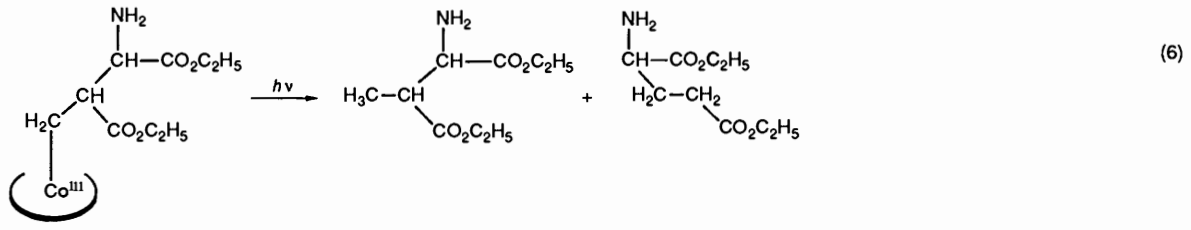
2 **2a** **2b** **2c**



3 **3a** **3b**



4 **4a** **4b** **4c**



5 **5a** **5b**

vesicle and the alkylated complex (5.0×10^{-5} mol dm^{-3}) in order to confirm that all the reaction took place in the vesicle. Regardless of the addition of the copper salt (5.0×10^{-3} mol dm^{-3}), the yields of all the products given in Table 2 remained unchanged. This undoubtedly indicates that the rearrangement reaction proceeded in the vesicle, but not in the bulk aqueous phase.

Reaction Mode of Carbon-skeleton Rearrangements in Single-compartment Vesicles.—The photolysis reaction was followed by spectroscopic methods in order to clarify the mechanism of the 1,2-migration of functional groups in the vesicle. An electronic spectral change observed for the photolysis of **1**, which was incorporated into the vesicle in an aqueous medium, is shown in Fig. 4. The alkylated complex was converted into the bivalent cobalt species upon irradiation with visible light under anaerobic conditions. Identical behaviour was observed for the photolysis in methanol and benzene.

The cleavage of the cobalt–carbon bond involved in **3** was examined under irradiation conditions by the spin-trapping technique with α-phenyl-*N*-(t-butyl)nitrone (PBN).[55] EPR signals attributable to the PBN spin adduct were clearly

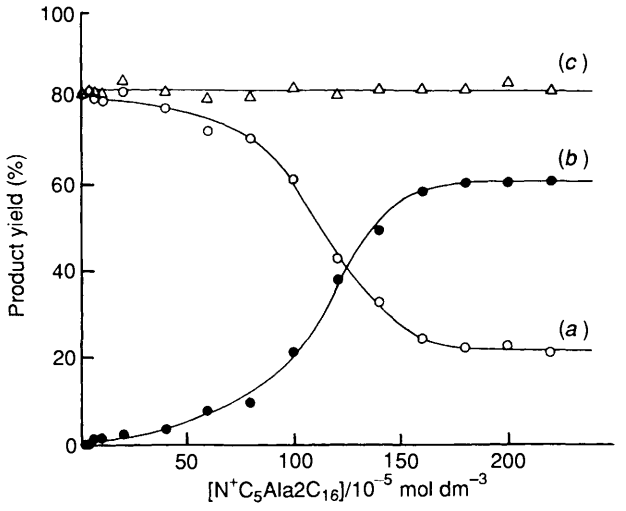


Fig. 3 Correlations between concentration of $N^+C_5Ala2C_{16}$ and product yield for photolysis of **1** (5.0×10^{-5} mol dm^{-3}) in phosphate–borate buffer (0.05 mol dm^{-3}; pH 9.2) at 20.0 ± 0.1 °C: (*a*) 2-acetyl-2-ethoxycarbonylpropane **1a**; (*b*) 1-acetyl-2-ethoxycarbonylpropane **1c**; (*c*) total yield

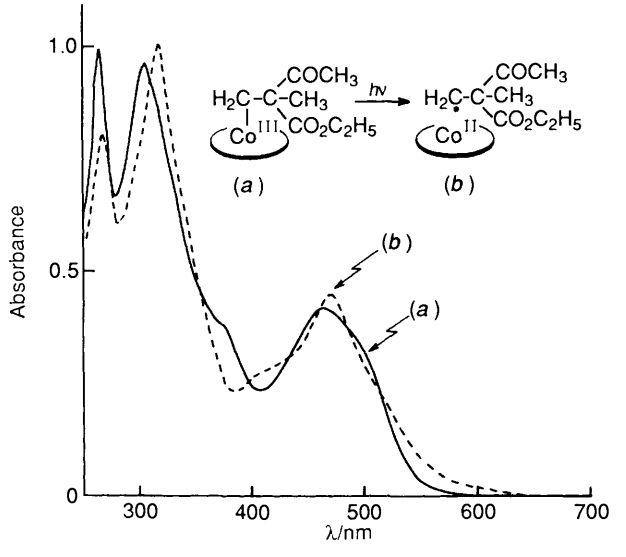


Fig. 4 Electronic spectra of cobalt species incorporated into the $N^+C_5Ala2C_{16}$ (5.0×10^{-3} mol dm^{-3}) vesicle in phosphate–borate buffer (0.05 mol dm^{-3}; pH 9.2) at 20.0 ± 0.1 °C: (*a*) **1** (4.0×10^{-5} mol dm^{-3}) before photolysis; (*b*), (*a*) irradiated with a 500 W tungsten lamp at a distance of 30 cm for 1 h under anaerobic conditions [formation of Cob(II)7C_3ester]

observed in methanol and benzene, as shown in our previous paper.[28] This clearly indicates that the radical species was produced by the photolytic cobalt–carbon cleavage. Although EPR signals were not detected at room temperature in the presence of the $N^+C_5Ala2C_{16}$ vesicle, typical signals attributable to the bivalent cobalt species were detected during the photolysis by EPR measurements at 77 K.[11] Since a very limited amount of PBN is incorporated into the vesicle in aqueous media, the spin adduct formation was hardly detected by EPR spectroscopy. In the light of the above results obtained by EPR and electronic spectroscopy, which were applied to the alkylated complex, the alkylated hydrophobic vitamin B_{12} must undergo homolysis of the cobalt–carbon bond in all the reaction media to afford the bivalent cobalt complex and the corresponding alkyl radical under anaerobic photolysis conditions.

In order to identify a hydrogen source, the anaerobic photolysis of [$C_6H_5CH_2$–Cob(III)7C_3ester]ClO_4 was carried out in deuteriated methanol (CD_3OD), and the product was analysed by NMR spectroscopy. Deuteriated toluene, $C_6H_5CH_2D$, was obtained as the major product; its isotopic purity was 80%D.* This apparently indicates that a hydrogen atom is mainly abstracted from a solvent and not from the corrinoid skeleton.

We have previously investigated the photolysis reactions of the alkylated complexes in benzene at various temperatures;[28] the yield of **1c** markedly increased in a temperature range below the melting point of benzene as compared with those observed above it. In the light of the previous results and the above information about the microenvironment around the hydrophobic vitamin B_{12} derivative in the vesicle, the hydrophobic cage effect provided by the vesicle acts to suppress the molecular motion of the alkylated hydrophobic vitamin B_{12}, so that the intermediate radical pair generated by photolysis is allowed to stay close to each other in the cage for a sufficient period of time to induce the 1,2-migration, as shown in Fig. 5.

Catalytic Reactions in Single-compartment Vesicles.—In the next stage of our investigation, we coupled an effective process for activation of substrates with the catalytic mediator, which consisted of [Cob(II)7C_3ester]ClO_4 and the $N^+C_5Ala2C_{16}$ vesicle in aqueous media. Schrauzer *et al.* developed a new method for syntheses of organo-cobalamins by utilizing both molecular oxygen and vanadium(III) ions as oxidizing and reducing reagents, respectively.[47–50] In reference to their method, a wide variety of non-activated organic compounds, including alkanes, can be used as substrates. We have previously succeeded in performing the glutamate mutase-mimic reaction,[14i] which converts diethyl β-methyl-DL-aspartate **5a** into diethyl glutamate **5b** along with turnover of the hydrophobic vitamin B_{12}, by adopting this activation process [refer to eqn. (7)].

An appropriate amount of [Cob(II)7C_3ester]ClO_4 and a large excess of vanadium trichloride were dissolved in an aqueous medium (pH 1.0) containing the $N^+C_5Ala2C_{16}$ vesicle and a sufficient excess of a substrate, **1a**, **2a** or **6a**, and the reaction solution was irradiated with visible light at 20 °C under aerobic conditions. The results, given in Figs. 6–8 [refer to eqns. (8)–(10)], apparently indicate that the reactions proceed catalytically, and the substrates are expected to be completely converted into the corresponding rearrangement products after a sufficient period of reaction time. On the other hand, much smaller amounts of the rearrangement products were detected without the hydrophobic vitamin B_{12} under otherwise identical conditions. The catalytic reactions were also carried out at pH 7.0 in order to understand the pH effect on the reaction (see Figs. 6–8). The reactions proceeded more efficiently at pH 7.0. Vanadium ions are present as V^{3+} and VO^{2+} species in acidic aqueous media, while the VO_3^{2-} ion is the predominant species in neutral and weakly acidic media.[56,57] Since the $N^+C_5Ala2C_{16}$ vesicle is of cationic nature, the VO_3^{2-} ion is much more readily concentrated on the vesicular surface due to an effective electrostatic interaction, relative to the other cationic vanadium species. This effect seems to be the primary reason for catalytic reactions taking place more efficiently at pH 7.0.

The reaction must proceed in the vesicle *via* the following sequence along with turnover of the catalyst species under aerobic photolysis conditions: the substrate is activated by vanadium(III) ions and molecular oxygen, and the resulting radical species undergoes coupling with [Cob(II)7C_3ester]$^+$ to

* Benzaldehyde was obtained as the major product under aerobic photolysis conditions. The isotopic experiment was carried out by Mr. Kenji Takasaki of this laboratory.

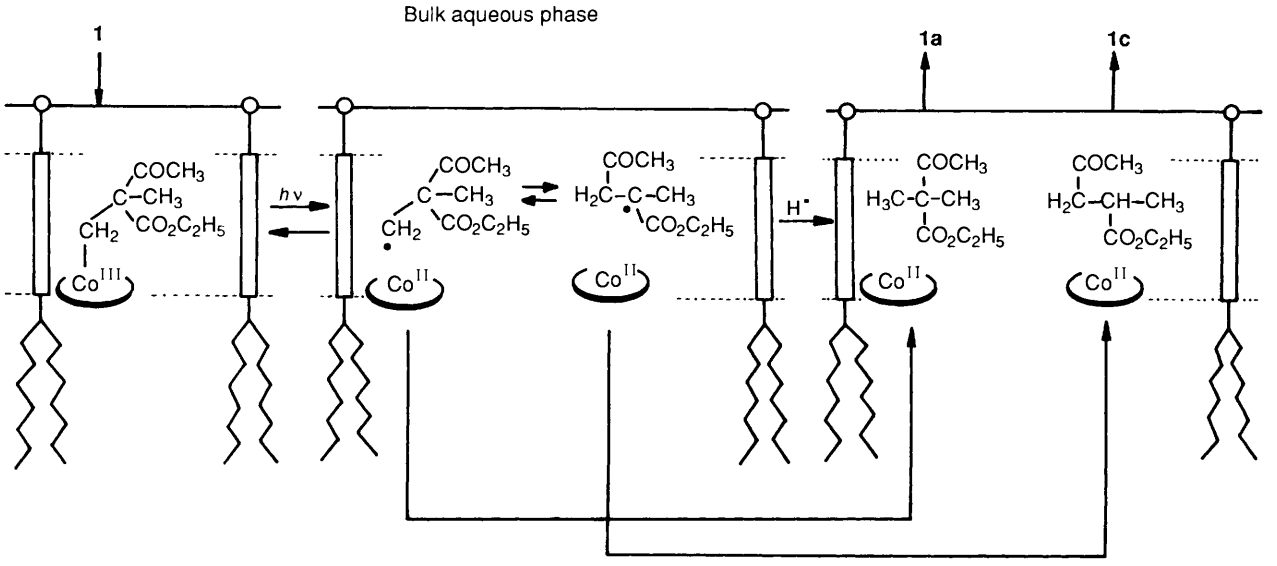
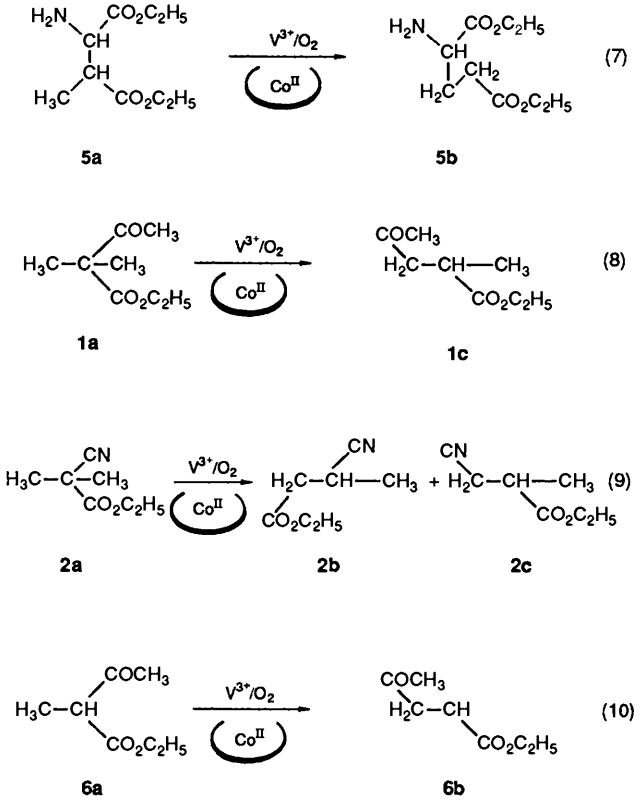


Fig. 5 Schematic representation of the photochemical carbon-skeleton rearrangement of an alkyl ligand bound to the hydrophobic vitamin B_{12} **1**



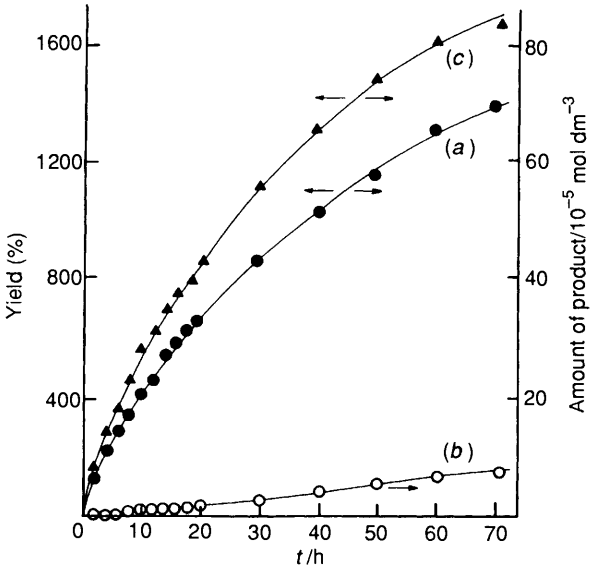


Fig. 6 Catalytic conversion of **1a** into **1c** in $N^+C_5Ala2C_{16}$ vesicular solution under aerobic irradiation conditions at 20.0 ± 0.1 °C; yield was based on an amount of the hydrophobic vitamin B_{12}. Compositions of the initial reaction mixture: $N^+C_5Ala2C_{16}$, 3.0×10^{-3} mol dm^{-3}; **1a**, 3.0×10^{-3} mol dm^{-3}; vanadium trichloride, 0.1 mol dm^{-3}. (*a*) [Cob(II)7C_3ester]ClO_4, 5.0×10^{-5} mol dm^{-3} (at pH 1.0); (*b*) without [Cob(II)7C_3ester]ClO_4 (at pH 1.0); (*c*) [Cob(II)7C_3ester]ClO_4, 5.0×10^{-5} mol dm^{-3} (at pH 7.0).

afford the corresponding alkylated complex; the alkylated complex is subjected to homolytic cleavage to give the original substrate and the rearrangement product(s); the original substrate thus obtained participates in the catalytic cycle. Vanadium trichloride acts not only as an activator for molecular oxygen to afford the substrate radical, but also as a reductant for the hydrophobic vitamin B_{12}, retaining the complex in the reactive Co^{II} state. The overall reaction cycle is shown in Fig. 9.

Conclusions

The single-compartment bilayer membrane of a synthetic peptide lipid was found to be effective as an apoprotein model for functional simulation of vitamin B_{12}-dependent enzymes. In the light of the present study, the following aspects became evident.

(i) Multiple hydrophobic molecules of different structural nature can be incorporated into the intramembrane domain composed of assembly of a single-chain segment of each lipid molecule. The extents of incorporation are primarily dependent on hydrophobic properties of guest molecules.

(ii) A combination of vanadium trichloride and atmospheric oxygen abstracts a hydrogen atom from the terminal methyl substituent of a substrate species to form the corresponding radical species, which then undergoes reaction with [Cob(II)7C_3-ester]$^+$ to form the alkylated complex.

(iii) The nuclear cobalt of the hydrophobic vitamin B_{12} promotes the rearrangement reaction in the single-compartment vesicle *via* formation of a tight pair with the substrate radical which is produced by homolytic cleavage of the cobalt–carbon bond upon photolysis of the alkylated complex. The 1,2-migration of the electron-withdrawing groups must arise from

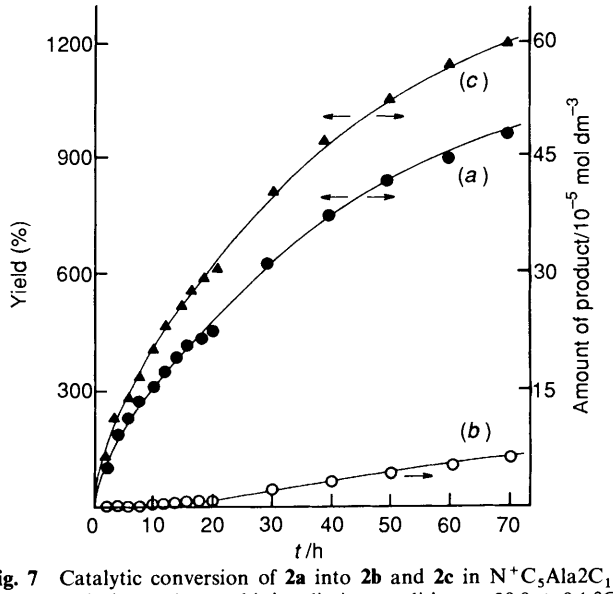


Fig. 7 Catalytic conversion of **2a** into **2b** and **2c** in $N^+C_5Ala2C_{16}$ vesicular solution under aerobic irradiation conditions at 20.0 ± 0.1 °C; yield was based on an amount of the hydrophobic vitamin B_{12}. Compositions of the initial reaction mixture: $N^+C_5Ala2C_{16}$, 3.0×10^{-3} mol dm^{-3}; **2a**, 3.0×10^{-3} mol dm^{-3}; vanadium trichloride, 0.1 mol dm^{-3}. The molar ratio of **2b** *vs.* **2c** (55:45) remained constant in the course of reaction. (*a*) [Cob(II)7C_3ester]ClO_4, 5.0×10^{-5} mol dm^{-3} (at pH 1.0); (*b*) without [Cob(II)7C_3ester]ClO_4 (at pH 1.0); (*c*) [Cob(II)7C_3ester]ClO_4, 5.0×10^{-5} mol dm^{-3} (at pH 7.0).

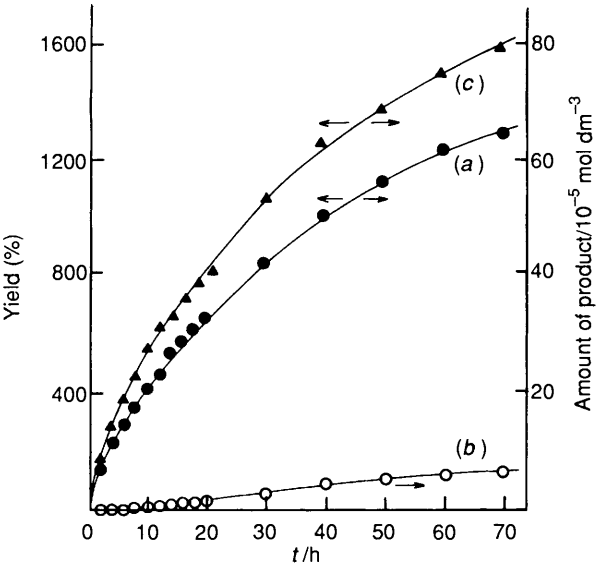


Fig. 8 Catalytic conversion of **6a** into **6b** in $N^+C_5Ala2C_{16}$ vesicular solution under aerobic irradiation conditions at 20.0 ± 0.1 °C; yield was based on an amount of the hydrophobic vitamin B_{12}. Compositions of the initial reaction mixture: $N^+C_5Ala2C_{16}$, 3.0×10^{-3} mol dm^{-3}; **6a**, 3.0×10^{-3} mol dm^{-3}; vanadium trichloride, 0.1 mol dm^{-3}. (*a*) [Cob(II)7C_3ester]ClO_4, 5.0×10^{-5} mol dm^{-3} (at pH 1.0); (*b*) without [Cob(II)7C_3ester]ClO_4 (at pH 1.0); (*c*) [Cob(II)7C_3ester]ClO_4, 5.0×10^{-5} mol dm^{-3} (at pH 7.0).

both suppression of molecular motion and desolvation effects operating on the alkylated cobalt complexes in the bilayer membrane.

(iv) The migratory aptitude of the present electron-withdrawing groups follows the sequence: CN ≈ $CO_2C_2H_5$ < $COCH_3$. Since the real reaction mechanism involved in the rearrangement of diethyl β-methyl-DL-aspartate is not known at present, a migratory tendency of the corresponding functional group cannot be defined.

The real catalytic cycle for carbon-skeleton rearrangements,

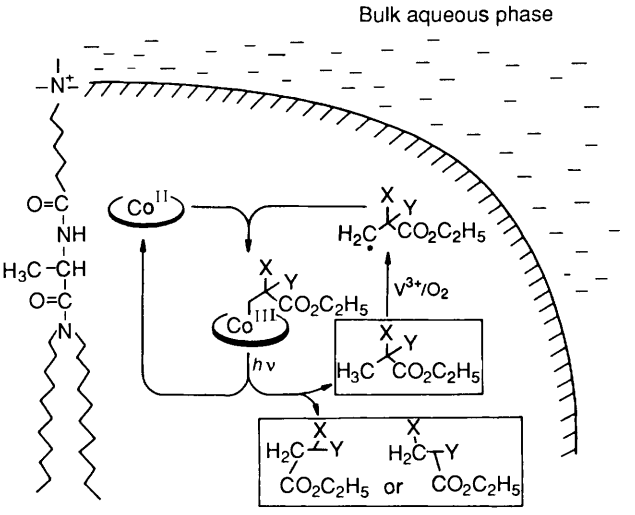


Fig. 9 Schematic representation of catalytic carbon-skeleton rearrangement reactions in the vesicle

as simulation of catalytic functions exerted by methylmalonyl–CoA mutase and glutamate mutase, is established by utilizing a novel substrate-activation process without using activated substrates such as halogenated ones. The present artificial holoenzyme system is expected to be applied to other non-enzymatic reactions which go through similar reaction mechanisms.

Acknowledgements

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