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# Hydrophobic Vitamin $B_{12}$. III. Incorporation of Hydrophobic Vitamin $B_{12}$ Derivatives into Single-Compartment Vesicles and Their Alkylation in Various Molecular Aggregates[†]

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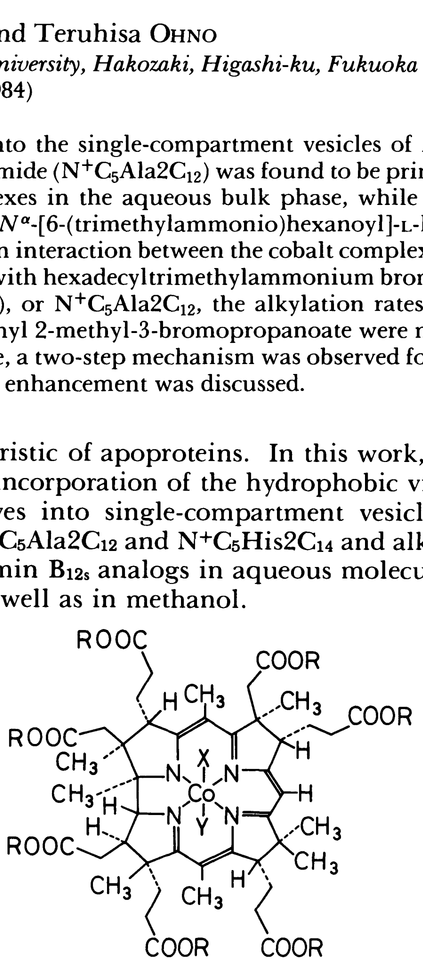
The incorporation of hydrophobic vitamin $B_{12}$ derivatives into the single-compartment vesicles of *N*,*N*-didodecyl-$N^{\alpha}$-[6-(tyimethylammonio)hexanoyl]-L-alaninamide bromide ($N^+C_5Ala2C_{12}$) was found to be primarily concerned with the extent of insolubility of the cobalt complexes in the aqueous bulk phase, while such incorporation into the single-walled vesicles of *N*,*N*-ditetradecyl-$N^{\alpha}$-[6-(trimethylammonio)hexanoyl]-L-histidinamide bromide ($N^+C_5His2C_{14}$) was enhanced by the coordination interaction between the cobalt complex and the imidazolyl group of the lipid. In molecular aggregates formed with hexadecyltrimethylammonium bromide, $\alpha$-[4-(1,1,3,3-tetramethylbutyl)phenyl]-$\omega$-hydroxypoly(oxyethylene), or $N^+C_5Ala2C_{12}$, the alkylation rates of a hydrophobic vitamin $B_{12}$ with 1-bromo-2-methylpropane and methyl 2-methyl-3-bromopropanoate were much enhanced relative to those in methanol. In the $N^+C_5Ala2C_{12}$ vesicle, a two-step mechanism was observed for the alkylation with the latter reagent. The possible origin of such rate enhancement was discussed.

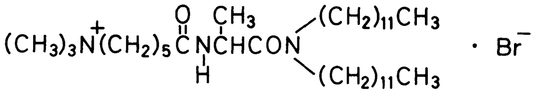
It is of great interest to evaluate the microenvironmental effects on reactions of vitamin $B_{12}$ in order to simulate the functions of apoproteins concerned in biological systems. Such enzymatic microenvironments for vitamin $B_{12}$ seem to be concerned with the extents of integrated mode of hydrogen-bonding, hydrophobic, and other weak interactions effective in the active sites of enzymes. On the other hand, the physicochemical properties of hydrophobic vitamin $B_{12}$ are subjected to change by the nature of reaction media as reported previously.[1,2] On the basis of the above viewpoints, the association behavior between micelles and the base-on and base-off methylcobalamin,[3] the interactions of vitamin $B_{12a}$ with cysteine and *N*-alkanoylcysteines,[4] the formation of glycine, imidazole, and azide adducts of vitamin $B_{12a}$,[5] and the methyl transfer reaction between methylcobalamin and mercuric acetate[6] have been investigated in aqueous and/or reversed micellar phases. The extents of these interactions and reactions have been discussed in connection with microenvironmental properties of the reaction sites concerned.

We have reported previously that amphiphiles involving an amino acid residue interposed between a polar head group and an aliphatic double-chain segment constitute stable single-compartment vesicles in aqueous media due to formation of the hydrogen-belt domain in the intramembrane region.[7] In this regard, these stable single-compartment vesicles have been utilized as media for various reactions to mimic the functions of apoproteins.[8] Futhermore, we have investigated the binding behavior of bilayer vesicles, formed with $N^+C_5Ala2C_{12}$, toward a hydrophobic cobalt complex, dicyano(8,12-diethyl-1,2,3,7,13,17,18,19-octamethyltetradehydrocorrinato)cobalt(III), and clarified that the bilayer vesicles may provide two binding sites which are largely different from each other in microenvironmental polarity.[9] Such previous studies prompted us to carry out various reactions of hydrophobic vitamin $B_{12}$ derivatives in single-compartment vesicles of the peptide amphiphiles in order to obtain a clue to understanding microenvironmental effects characteristic of apoproteins. In this work, we investigated incorporation of the hydrophobic vitamin $B_{12}$ derivatives into single-compartment vesicles formed with $N^+C_5Ala2C_{12}$ and $N^+C_5His2C_{14}$ and alkylation of the vitamin $B_{12s}$ analogs in aqueous molecular aggregates as well as in methanol.



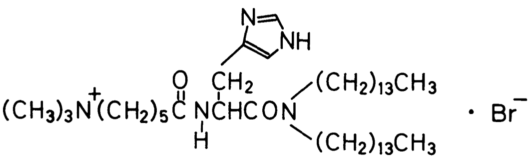
X=Y=CN

R=$CH_3$ : $(CN)_2Cob(III)C_1$ester
R=$C_2H_5$ : $(CN)_2Cob(III)C_2$ester
R=n-$C_3H_7$ : $(CN)_2Cob(III)C_3$ester
R=n-$C_4H_9$ : $(CN)_2Cob(III)C_4$ester



$$(CH_3)_3\overset{+}{N}(CH_2)_5\overset{\overset{O}{\|}}{C}\underset{H}{N}\overset{CH_3}{C}HCON\begin{smallmatrix}(CH_2)_{11}CH_3\\(CH_2)_{11}CH_3\end{smallmatrix}\cdot Br^-$$

$N^+C_5Ala\,2\,C_{12}$



$$(CH_3)_3\overset{+}{N}(CH_2)_5\overset{\overset{O}{\|}}{C}\underset{H}{N}\overset{\overset{\text{imidazolyl (N, NH)}}{|}}{\overset{CH_2}{C}}HCON\begin{smallmatrix}(CH_2)_{13}CH_3\\(CH_2)_{13}CH_3\end{smallmatrix}\cdot Br^-$$

$N^+C_5His\,2\,C_{14}$

## Experimental

*General Analyses and Measurements.* Elemental analyses were performed at the Microanalysis Center of Kyushu University. IR spectra were taken on a JASCO DS-

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403G grating spectrophotometer, while electronic absorption spectra were recorded on a Hitachi 340 or a Hitachi 220A spectrophotometer. pH-Measurements were carried out with a Beckman expandomatic SS-2 pH meter equipped with a Metrohm EA-125 combined electrode after calibration with a combination of appropriate standard aqueous buffers. A JEOL JEM-200B electron microscope, installed at the Research Laboratory for High Voltage Electron Microscopy of Kyushu University, was used for morphological measurements of the aggregates.[7b] Circular dichroism (CD) spectra were recorded on a JASCO J-500C spectropolarimeter.

*Materials.* Cyanocobalamin was purchased from Nakarai Chemicals and used for its modifications without further purification. Hexadecyltrimethylammonium bromide (CTAB) was purchased from Nakarai Chemicals and recrystallized from ethanol. Sodium dodecyl sulfate (SDS) of Nakarai Chemicals and $\alpha$-[4-(1,1,3,3-tetramethylbutyl)phenyl]-$\omega$-hydroxypoly(oxyethylene) ($n$=9—10) (Triton X-100) of Rohm and Haas were used without further purification. 1-Bromo-2-methylpropane was obtained commercially from Nakarai Chemicals as extra pure grade and fractionally distilled just before use. Methyl 2-methyl-3-bromopropanoate was synthesized from the corresponding carboxylic acid: bp 73.0 °C/$2.53\times10^3$ Pa; IR (neat) 1735 $\mathrm{cm^{-1}}$ (ester C=O); $^1$H-NMR ($\mathrm{CDCl_3}$, TMS) $\delta$=1.28 (3H, d, $\mathrm{CH_3}$), 2.84 (1H, m, CH), 3.47 (2H, dd, $\mathrm{CH_2}$) and 3.66 (3H, s, $\mathrm{COOCH_3}$). These alkyl bromides were confirmed to be sufficiently pure by GLC. *N,N*-Didodecyl-$N^\alpha$-[6-(trimethylammonio)hexanoyl]-L-alaninamide bromide ($\mathrm{N^+C_5Ala2C_{12}}$) was synthesized according to the method described previously.[7b] *N,N*-Ditetradecyl-$N^\alpha$-[6-(trimethylammonio)hexanoyl]-L-histidinamide bromide ($\mathrm{N^+C_5His2C_{14}}$) was prepared in a manner similar to that reported previously by utilizing the benzyl group in place of the tosyl as an imidazole-protecting moiety.[8c]

Preparation of heptamethyl dicyanocobyrinate, $\mathrm{(CN)_2Cob(III)C_1ester}$, and heptamethyl cobyrinate perchlorate, $\mathrm{[Cob(II)C_1ester]ClO_4}$, has been described previously.[1] Heptaethyl dicyanocobyrinate, $\mathrm{(CN)_2Cob(III)C_2ester}$, heptapropyl dicyanocobyrinate, $\mathrm{(CN)_2Cob(III)C_3ester}$, and heptabutyl dicyanocobyrinate, $\mathrm{(CN)_2Cob(III)C_4ester}$, were prepared from cyanocobalamin according to the method of Werthemann *et al.*,[10] adopted for the preparation of $\mathrm{(CN)_2Cob(III)C_1ester}$, with some modification as typically described below.

*Heptapropyl Dicyanocobyrinate, $\mathrm{(CN)_2Cob(III)C_3ester}$.* Cyanocobalamin (500 mg, 0.37 mmol) in 333 ml of dry 1-propanol was mixed with 33 ml of cold conc. sulfuric acid and 167 ml of dry 1-propanol. The solution was deoxygenated by bubbling nitrogen gas through it for 1 h, and heated to 60—62 °C for 250 h in the dark under nitrogen atmosphere.[11] The solution was concentrated to *ca.* 100 ml, diluted with 250 ml of cold water, and quickly neutralized with sodium carbonate. After addition of 2.5 g of potassium cyanide, the reaction mixture was extracted with carbon tetrachloride (70 ml×3) and dichloromethane (70 ml×3). The dichloromethane extract, which contained partially esterified corrins, was used for the above esterification reaction. The carbon tetrachloride extract was evaporated to dryness, and the residue was purified by TLC on silica gel (Kiesel gel 60H) with dichloromethane–methanol (10:1 v/v). The TLC analysis showed the presence of 5 components: A purple one with the highest $R_f$ was collected and dissolved in carbon tetrachloride. This solution was treated with aqueous potassium cyanide and evaporated to dryness to afford a dark purple solid: yield 190 mg (40%); $\lambda_{\max}$ ($\mathrm{C_6H_6}$) 316, 372, 426, 520 sh, 550, and 590 nm; IR (neat) 2130 (C≡N) and 1730 $\mathrm{cm^{-1}}$ (ester C=O). Found: C, 63.38; H, 7.98; N, 6.32. Calcd for $\mathrm{C_{68}H_{101}CoN_6O_{14}}$: C, 63.53; H, 7.92; N, 6.54.

*Heptaethyl Dicyanocobyrinate.* Found: C, 61.31; H, 7.43; N, 6.83. Calcd for $\mathrm{C_{61}H_{87}CoN_6O_{14}}$: C, 61.71; H, 7.39; N, 7.08.

*Heptabutyl Dicyanocobyrinate.* Found: C, 65.19; H, 8.40; N, 5.62. Calcd for $\mathrm{C_{75}H_{115}CoN_6O_{14}}$: C, 65.10; H, 8.38; N, 6.07.

*Heptapropyl Cobyrinate Perchlorate, $\mathrm{[Cob(II)C_3ester]ClO_4}$.* This complex was prepared after the method employed for the preparation of $\mathrm{[Cob(II)C_1ester]ClO_4}$.[1] Found: C, 59.17; H, 7.70; N, 4.42. Calcd for $\mathrm{C_{66}H_{101}ClCoN_4O_{18}}$: C, 59.47; H, 7.64; N, 4.20.

*Equilibrium Measurements for Incorporation of $\mathrm{(CN)_2Cob(III)C_n ester}$ into Single-compartment Vesicles.* A dichloromethane solution (1.1 ml) containing $\mathrm{N^+C_5Ala2C_{12}}$ ($1.0\times10^{-5}$ mol) and $\mathrm{(CN)_2Cob(III)C_n ester}$ ($n$=1,2,3,4; $1.0\times10^{-7}$ mol) was evaporated *in vacuo* to remove the solvent completely, and an aqueous potassium cyanide solution (1 ml, $5.0\times10^{-4}$ mol $\mathrm{dm^{-3}}$) 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 a hydrophobic vitamin $\mathrm{B_{12}}$ into single-compartment vesicles was examined by gel-filtration chromatography on a column of Sephadex G-50 with an aqueous potassium cyanide solution ($5.0\times10^{-4}$ mol $\mathrm{dm^{-3}}$) as an eluant; the bound complex was eluted first in the column void volume. An amount of the incorporated complex was determined by electronic spectroscopy.

*Kinetic Measurements.* A 3-ml sample of an aqueous buffer ($5.00\times10^{-3}$ mol $\mathrm{dm^{-3}}$ phosphate–$4.75\times10^{-2}$ mol $\mathrm{dm^{-3}}$ borate, pH 9.18) containing CTAB ($5.0\times10^{-3}$ mol $\mathrm{dm^{-3}}$) was placed in a spectrophotometric cell thermostated at 21.5±0.1 °C. The sample was deoxygenated by bubbling argon gas through it for 10 min. To this solution were added 10 μl of a methanol solution of $\mathrm{[Cob(II)C_3ester]ClO_4}$ (final concentration, *ca.* $2\times10^{-5}$ mol $\mathrm{dm^{-3}}$) and 10 μl of the aqueous buffer containing sodium tetrahydroborate (final concentration, *ca.* 1—$2\times10^{-4}$ mol $\mathrm{dm^{-3}}$) under argon atmosphere. The reduction of the complex to the cobalt(I) state took place instantaneously. A methanol solution of an alkyl bromide was injected into the medium containing the cobalt(I) complex under argon atmosphere to initiate the reaction. The mixture was quickly shaken, and the decrease in absorbance at 392 nm, characteristic of $\mathrm{Cob(I)C_3ester}$, was monitored. In all kinetic runs, an alkyl bromide was used in excess to assure the pseudo-first-order conditions and clear isosbestic points were observed along the progress of conversion of the cobalt(I) complex to the corresponding alkylated complex. All the kinetic runs under the pseudo-first-order conditions provided linear correlations for ln ($\mathrm{OD}_t-\mathrm{OD}_\infty$) *vs.* time, and the slopes (pseudo-first-order rate constants) were divided by the initial concentrations of alkyl bromides to give the corresponding second-order rate constants. The resulting alkylated complexes were confirmed by means of identification of their photolysis products obtained under aerobic conditions. The kinetic measurements in aqueous single-compartment vesicles were carried out as follows: an amphiphile ($2.0\times10^{-3}$ mol $\mathrm{dm^{-3}}$) dispersed in the aqueous buffer was sonicated for 1 min with a probe-type sonicator at 30 W; $\mathrm{[Cob(II)C_3ester]ClO_4}$, sodium tetrahydroborate, and an alkyl halide were added to the resulting clear vesicle solution in a manner as stated above.

## Results and Discussion

*Incorporation of Hydrophobic Vitamin $\mathrm{B_{12}}$ into Single-compartment Vesicles.* As shown in Table 1, the hydrophobic vitamin $\mathrm{B_{12}}$ derivatives, $\mathrm{(CN)_2Cob(III)C_n ester}$, are readily incorporated into the $\mathrm{N^+C_5Ala2C_{12}}$ vesicle when the $n$-value of $\mathrm{C}_n$ is equal to 2 or larger.

Table 1. Incorporation of hydrophobic vitamin $\mathrm{B_{12}}$ derivatives into single-compartment vesicles[a]

| Vesicle[b] | Complex[c] | Incorporated amount,[d] mol |
|---|---|---|
| $\mathrm{N^+C_5Ala2C_{12}}$ | $\mathrm{(CN)_2Cob(III)C_1ester}$ | 0 |
| $\mathrm{N^+C_5Ala2C_{12}}$ | $\mathrm{(CN)_2Cob(III)C_2ester}$ | $2.4\times10^{-7}$ |
| $\mathrm{N^+C_5Ala2C_{12}}$ | $\mathrm{(CN)_2Cob(III)C_3ester}$ | $5.0\times10^{-7}$ |
| $\mathrm{N^+C_5Ala2C_{12}}$ | $\mathrm{(CN)_2Cob(III)C_4ester}$ | $5.0\times10^{-7}$ |
| $\mathrm{N^+C_5His2C_{14}}$ | $\mathrm{[Cob(II)C_1ester]ClO_4}$[e] | $4.5\times10^{-7}$ |

a) Incorporation was examined by gel-filtration chromatography on a column ($\phi$0.9$\times$21 cm) of Sephadex G-50 with aqueous KCN solution ($5\times10^{-4}$ mol $\mathrm{dm^{-3}}$) as an eluant. b) Quantity used, $1.0\times10^{-5}$ mol. c) Qunatity used, $5.0\times10^{-7}$ mol. d) Incorporated quantity was determined by electronic spectroscopy. e) Aqueous $\mathrm{NaClO_4}$ solution ($5\times10^{-4}$ mol $\mathrm{dm^{-3}}$) was used as an eluant.

$\mathrm{(CN)_2Cob(III)C_1ester}$ is slightly soluble in water (*ca.* $10^{-4}$ mol $\mathrm{dm^{-3}}$), but $\mathrm{(CN)_2Cob(III)C_3ester}$ and $\mathrm{(CN)_2Cob(III)C_4ester}$ are almost insoluble. Thus, the partitioning trend of $\mathrm{(CN)_2Cob(III)C_{\mathit{n}}ester}$ to the membrane phase is subjected to change by the hydrophobicity of the ester groups placed in the peripheral positions of the corrinoid skeleton. On the other hand, when a membrane-forming amphiphile involves a functional group capable of coordinating to the cobalt complex, such a coordination effect acts to enhance the incorporation of $\mathrm{[Cob(II)C_1ester]ClO_4}$ having some limited solubility in water (*ca.* $10^{-3}$ mol $\mathrm{dm^{-3}}$); this cobalt complex is largely incorporated into the $\mathrm{N^+C_5His2C_{14}}$ vesicle (Table 1). The stability constant ($K$) for the coordination of the imidazolyl moiety of $\mathrm{N^+C_5His2C_{14}}$ to the nuclear cobalt of $\mathrm{[Cob(II)C_1ester]ClO_4}$ at one of its axial sites was measured in dichloromethane at 26.5 °C by the method described previously:[1] $\log K=5.5$. Consequently, the incorporation of $\mathrm{[Cob(II)C_1ester]ClO_4}$ into the vesicles of $\mathrm{N^+C_5His2C_{14}}$ originates in the axial coordination of the amphiphile to the complex. The experimental method for the equilibrium measurements (see Experimental) was not applied to the evaluation of an extent of incorporation of $\mathrm{[Cob(II)C_1ester]\,ClO_4}$ into the $\mathrm{N^+C_5His2C_{14}}$ vesicle since the complex was converted into the corresponding cobalt(III) complex during the sonication (see Fig. 1). Alternatively, a methanol solution of $\mathrm{[Cob(II)C_1ester]ClO_4}$ was added to the single-compartment vesicles of $\mathrm{N^+C_5His2C_{14}}$, and the cobalt(II) complex was confirmed to be bound to the vesicle in the base-on form in the light of our previous study[1] (Fig. 1). This clearly indicates that the imidazolyl moiety of $\mathrm{N^+C_5His2C_{14}}$ in the vesicular state coordinates to the nuclear cobalt of $\mathrm{Cob(II)C_1ester}$. The microenvironmental polarity for $\mathrm{(CN)_2Cob(III)C_3ester}$ was evaluated when the complex was incorporated into the micelles or the vesicle in reference to the $\alpha$- and $\gamma$-bands of the complex measured in various organic media (Table 2 and Fig. 2). The CTAB and Triton X-100 micelles provide a microenvironment comparable to acetonitrile, while the $\mathrm{N^+C_5Ala2C_{12}}$ vesicle provides one equivalent to dichloromethane.

The structure of molecular aggregates composed of $\mathrm{N^+C_5Ala2C_{12}}$, when $\mathrm{(CN)_2Cob(III)C_3ester}$ was incorporated into them, was measured directly by electron

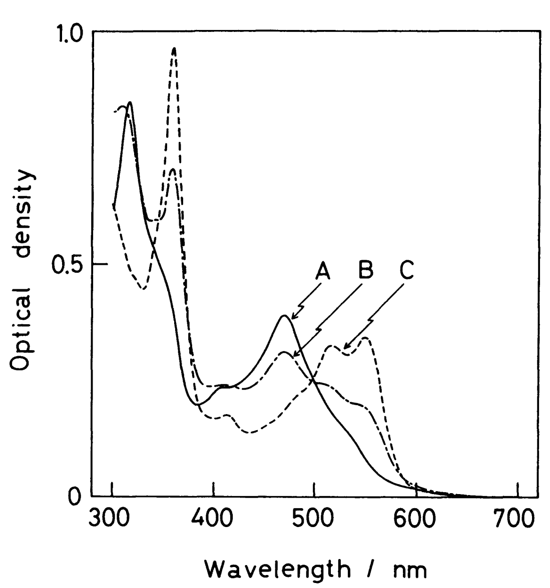

Fig. 1. Electronic spectra of $\mathrm{[Cob(II)C_1ester]ClO_4}$ in aqueous phosphate ($5.00\times10^{-3}$ mol $\mathrm{dm^{-3}}$)-borate ($4.75\times10^{-2}$ mol $\mathrm{dm^{-3}}$) buffer (pH 9.18) at 19.6±0.1 °C: A, $\mathrm{[Cob(II)C_1ester]ClO_4}$, $3.16\times10^{-5}$ mol $\mathrm{dm^{-3}}$: B, $\mathrm{[Cob(II)C_1ester]ClO_4}$, $3.16\times10^{-5}$ mol $\mathrm{dm^{-3}}$; $\mathrm{N^+C_5His2C_{14}}$, $2.0\times10^{-3}$ mol $\mathrm{dm^{-3}}$ (sonicated for 1 min at 30 W): C, sample B was sonicated for 1 min at 30 W.

Table 2. Characteristic absorption bands of $\mathrm{(CN)_2Cob(III)C_3ester}$ in various media

| Medium | Solvent parameters | | $\alpha$-Band,[c] nm | $\gamma$-Band,[c] nm |
|---|---|---|---|---|
| | $Z$[a] | $E_\mathrm{T}(30)$[b] | | |
| Benzene | 54.0 | 34.5 | 590.4 | 372.0 |
| Dichloromethane | 64.2 | 41.1 | 588.8 | 371.0 |
| Acetonitrile | 71.3 | 46.0 | 587.2 | 370.2 |
| Ethanol | 79.6 | 51.9 | 584.8 | 368.8 |
| Methanol | 83.6 | 55.5 | 584.0 | 368.4 |
| CTAB micelle | 75[d] | 48[d] | 586.1 | 370.2 |
| Triton X-100 micelle | 71[d] | 46[d] | 587.2 | 370.5 |
| $\mathrm{N^+C_5Ala2C_{12}}$ vesicle | 64[d] | 41[d] | 588.8 | 372.0 |

a) E. M. Kosower,"An Introduction to Physical Organic Chemistry," John Wiley and Sons, New York (1968), Part 2. b) C. Reichardt, "Solvent Effects in Organic Chemistry, " Verlag Chemie, Weinheim (1979). c) Electronic absorption bands characteristic of the corrinoid complex are named $\alpha$-, $\beta$-, and $\gamma$-bands by going from lower to higher energy regions. d) Numerical value was evaluated approximately in reference to the correlations given in Fig. 2.

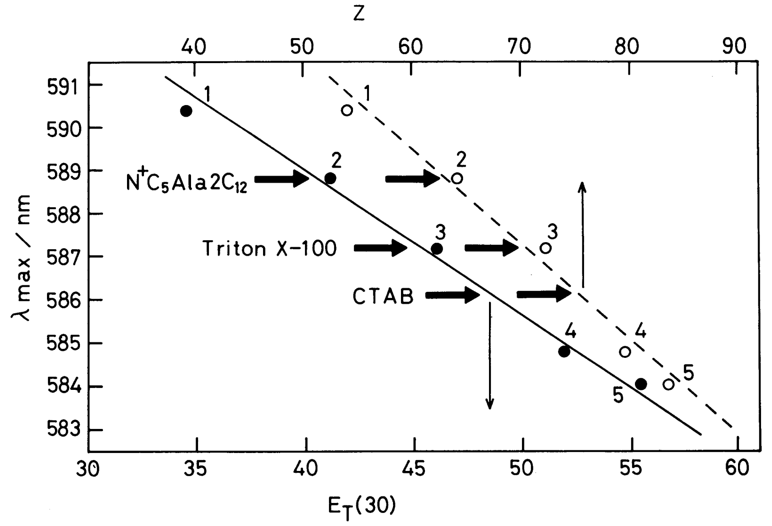

Fig. 2. Correlation of α-band of $(CN)_2Cob(III)C_3$ester with solvent polarity parameters, $Z$ and $E_T(30)$: 1, benzene; 2, dichloromethane; 3, acetonitrile; 4, ethanol; 5, methanol. α-Bands of $(CN)_2Cob(III)C_3$ester in molecular aggregates are shown by arrows.

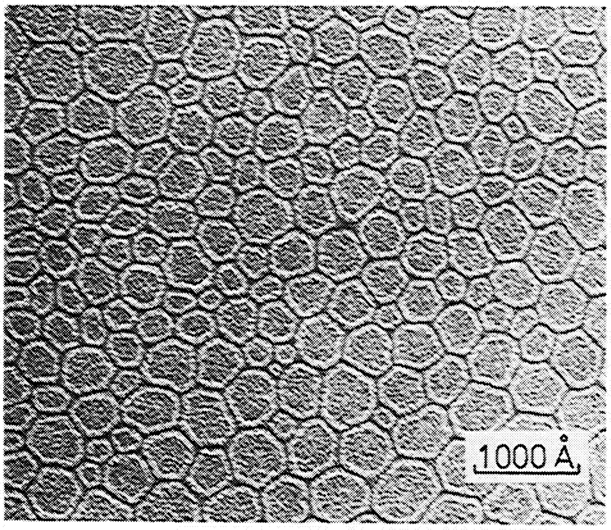

Fig. 3. Electron micrograph of single-compartment vesicles of $N^+C_5Ala2C_{12}$ entrapping $(CN)_2Cob(III)C_3$ester: $N^+C_5Ala2C_{12}$, $5.0\times10^{-3}$ mol dm$^{-3}$; $(CN)_2Cob(III)C_3$ester, $1.0\times10^{-4}$ mol dm$^{-3}$: negatively stained with uranyl acetate, sonicated for 1 min with a probe-type sonicator at 30 W, and allowed to stand for 10 min at 5 °C.

microscopy (Fig. 3). A large number of single-compartment vesicles ranging from 200 through 700 Å in diameter are observable, and the layer thickness is evaluated to be 40—50 Å, approximately twice the length of the hydrophobic double-chain segment. This means that the incorporation of a hydrophobic vitamin $B_{12}$, does not disturb the aggregate morphology of such peptite lipid.

The CD spectra of $(CN)_2Cob(III)C_1$ester and $[Cob(II)C_1ester]ClO_4$ are shown in Fig. 4 as typical examples. The spectral pattern of $(CN)_2Cob(III)C_1$ester is comparable to that of cyanocobalamin.[12] $[Cob(II)C_1ester]ClO_4$ was gradually oxidized upon addition of potassium cyanide under aerobic conditions and finally converted to $(CN)_2Cob(III)C_1$ester as confirmed by CD spectroscopy. In the light of these observations, $(CN)_2Cob(III)C_n$ester and $[Cob(II)C_n ester]ClO_4$ prepared in the present study retain the same enantiomeric configurations as the naturally occurring vitamin $B_{12}$'s even after the hydrophobic modifications.

*Alkylation of Hydrophobic Vitamin $B_{12}$ in Various Media.* The alkylation reactions of hydrophobic vitamin $B_{12}$ derivatives with alkyl halides in various media were followed by the spectrophotometric method. Figure 5 shows a typical spectral change observed along the progress of alkylation in the CTAB micelle under the conditions: in the aqueous phosphate-borate buffer at pH 9.18 and 21.5±0.1 °C under argon. Pseudo-first-order plots for these reactions gave satisfactory straight lines, and the corresponding second-order rate constants for the reaction of $Cob(I)C_3$ester with 1-bromo-2-methylpropane and methyl 2-methyl-3-bromopropanoate in various media are summarized in Table 3.

The same kinetic runs were also carried out in the anionic micelles of sodium dodecyl sulfate (SDS). However, $Cob(I)C_3$ester was not formed instantaneously, different from the above runs. The electrostatic repulsion between the anionic micellar surface and the tetrahydroborate ion does not seem to allow the effective permeation of the latter into the micellar interior and acts to lower the reactivity of the reductant toward $Cob(II)C_3$ester.

In order to characterize the reactivity of the hydrophobic vitamin $B_{12}$ derivatives toward alkyl halides relative to that of cobalamin in the cobalt(I) state, the second-order rate constants for the reactions of some hydrophobic vitamin $B_{12}$'s with 1-bromo-2-methylpropane were obtained in the same medium, methanol, as summarized in Table 4. The reactivity of the hydrophobic vitamin $B_{12}$'s in the alkylation decreases by 1/4- to 1/5-fold as compared with that of cobalamin. The reactivity of corrinoid complexes must be subjected to change by the nature of intramolecular cage, provided with the peripheral substituents, at the axial sites of the nuclear cobalt(I). Since the axial sites of the hydrophobic vitamin $B_{12}$'s containing ester groups are apolar as compared with those of cobalamin bearing the amide moieties as well as the nucleotide group, the nucleophilicity of the present hydrophobic complexes is lowered relative to that of the latter natural complex in the light of the solvent effect on the $Co^{II}/Co^{I}$ redox potential observed for Cob(II)$C_1$ester.[2] Furthermore, the transition state of alkylation becomes polar relative to the original state, so that the microenvironmental property of cobalamin provided at the cobalt(I) site would be in favor of such reaction. This environmental effect is reflected on the large stability of Cob(II)-

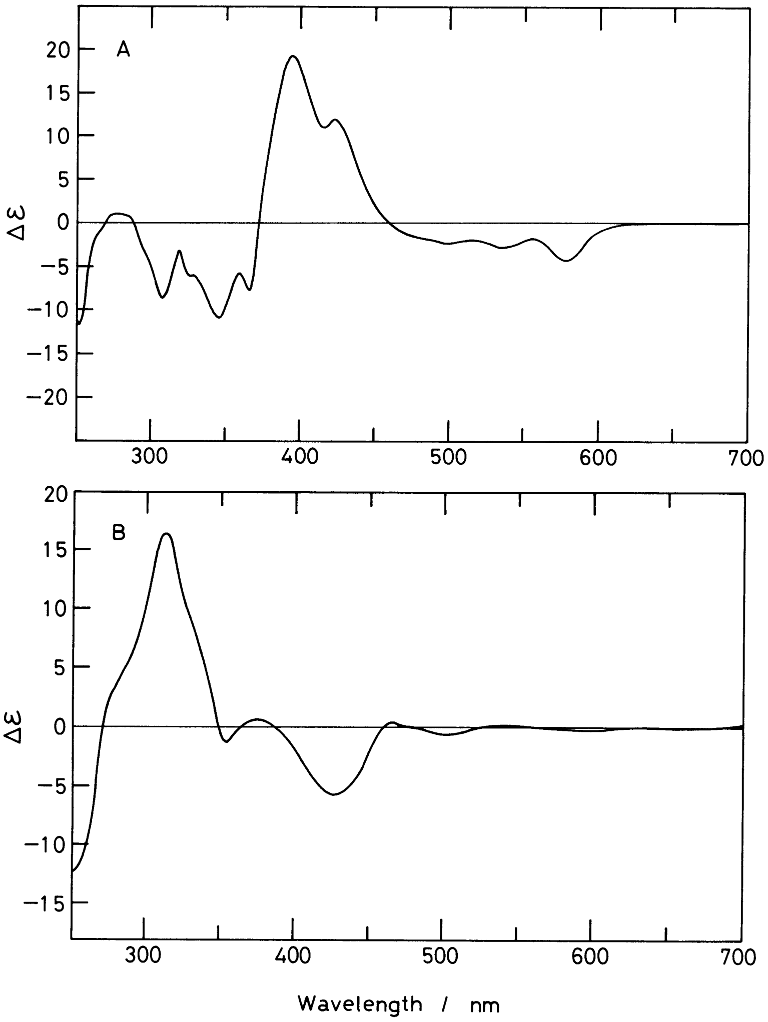

Fig. 4. CD spectra of $(CN)_2$Cob(III)$C_1$ester (A) and [Cob(II)$C_1$ester]-$ClO_4$ (B) in methanol at 20.0±0.1 °C.

Table 3. Second-order rate constants for the reactions of Cob(I)$C_3$ester with alkyl bromides in various media at 21.5±0.1 °C

| Medium[a,b] | $k_2$/mol$^{-1}$ dm$^3$ s$^{-1}$ | |
|---|---|---|
| | $(CH_3)_2CHCH_2Br$ | $(CH_3OOC)(CH_3)CHCH_2Br$ |
| Methanol | 0.21±0.03 | 0.87±0.05 |
| Triton X-100 micelle | 5.50±0.13 | 9.30±0.20 |
| CTAB micelle | 12.6±0.3 | 24.2±0.4 |
| $N^+C_5Ala2C_{12}$ vesicle | 10.8±0.2 | 39.0±0.8[c]<br>(two steps)[d] |

a) [Cob(I)$C_3$ester]=$2.0\times10^{-5}$ mol dm$^{-3}$. b) Amphiphile concentrations for molecular aggregates in phosphate ($5.00\times10^{-3}$ mol dm$^{-3}$)-borate ($4.75\times10^{-2}$ mol dm$^{-3}$) buffer: Triton X-100, $5.0\times10^{-3}$ mol dm$^{-3}$; CTAB, $5.0\times10^{-3}$ mol dm$^{-3}$; $N^+C_5Ala2C_{12}$, $2.0\times10^{-3}$ mol dm$^{-3}$. c) Rate constant for the first step of two-step kinetics. d) Pseudo-first-order rate constants ($k_{obsd}$) for the two steps were measured by changing the concentration of the alkyl bromide (RX): first step, $k_{obsd}=(2.46\pm0.15)\times10^{-2}$ s$^{-1}$ with [RX]=$6.31\times10^{-4}$ mol dm$^{-3}$; second step, $k_{obsd}=(2.41\pm0.16)\times10^{-2}$ s$^{-1}$ with [RX]=$5.32\times10^{-3}$ mol dm$^{-3}$ ($k_{obsd}$ for the first step under this condition was too large to evaluate accurately and the stopped-flow technique was not applicable due to the foam-forming property of $N^+C_5Ala2C_{12}$).

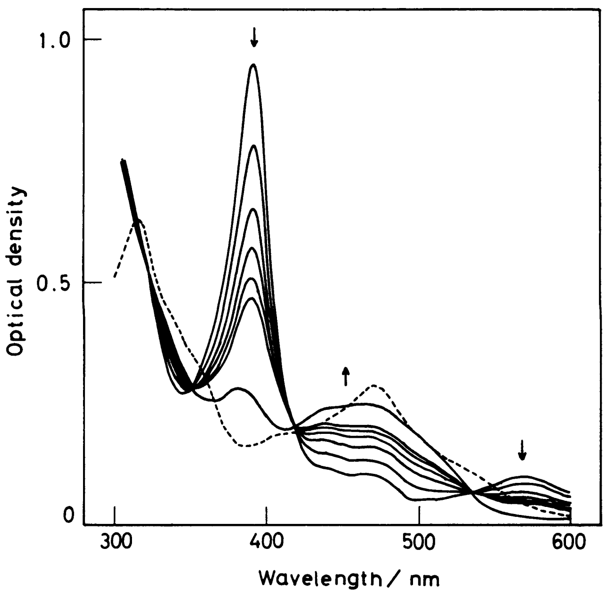

Fig. 5. Spectral change for the reaction of Cob(I)C$_3$ester ($2.1\times10^{-5}$ mol dm$^{-3}$) with 1-bromo-2-methylpropane ($2.1\times10^{-4}$ mol dm$^{-3}$) in the presence of CTAB micelles ($5.0\times10^{-3}$ mol dm$^{-3}$) in aqueous phosphate-borate buffer (pH 9.18) at 21.5±0.1 °C. Trends of spectral change with time are shown by arrows. Broken line represents the spectrum for [Cob(II)C$_3$ester]ClO$_4$ before reduced with NaBH$_4$.

TABLE 4. SECOND-ORDER RATE CONSTANTS FOR THE REACTIONS OF CORRINOID Co$^{I}$ COMPLEXES WITH 1-BROMO-2-METHYLPROPANE[a)] IN METHANOL AT 21.5±0.1 °C

| Corrinoid complex[b)] | $k_2$/mol$^{-1}$ dm$^3$ s$^{-1}$ |
|---|---|
| Cob(I)alamin | 1.12±0.12 |
| Cob(I)C$_1$ester | 0.27±0.08 |
| Cob(I)C$_3$ester | 0.21±0.04 |

a) Concentration range for the alkyl bromide: $1.0\times10^{-3}$—$2.1\times10^{-3}$ mol dm$^{-3}$. b) Aquacobalamin, [Cob(II)C$_1$ester]ClO$_4$, and [Cob(II)C$_3$ester]ClO$_4$ were used as starting materials and reduced with sodium tetrahydroborate (*ca.* $6\times10^{-4}$ mol dm$^{-3}$) to the corresponding cobalt(I) complexes under argon atmosphere.

C$_n$ester under aerobic conditions, while Cob(II)alamin is readily oxidized under the identical conditions. Even though the dioxygen adduct of Cob(II)C$_n$ester is formed to a limited extent, the oxidation of the hydrophobic vitamin B$_{12r}$ does not take place readily. This is plausibly due to the following effect. In order that the electron transfer from Cob(II)C$_n$ester to dioxygen takes place, the coordinated oxygen molecule needs to be much polarized in the transition state. The apolar microenvironment provided at the cobalt site of Cob(II)-C$_n$ester would not be favorable for the formation of such polarized structure.

The second-order rate constants for the reactions in the micellar and vesicular phases are 10—60 times greater than the corresponding values in methanol (Table 3). When methyl 2-methyl-3-bromopropanoate having an electron-withdrawing ester group is used as a substrate, the second-order rate constants are somewhat larger than the corresponding values for the reactions with 1-bromo-2-methylpropane. On the other hand, the rate enhancements in the molecular aggregates, relative to the reactivity in methanol, are much larger for the reaction with 1-bromo-2-methylpropane than those for the reaction with methyl 2-methyl-3-bromopropanoate. This would be due to the difference in hydrophobicity between the two substrates. The former substrate is to be more favorably incorporated into the hydrophobic interior of the molecular aggregates.

When 1-bromo-2-methylpropane is used as substrate, the second-order rate constant measured in the N$^+$C$_5$Ala2C$_{12}$ vesicle is comparable to or even slightly less than that in the CTAB micelle, while the rate constant in the nonionic Triton X-100 micelle is smaller than both of the above values. This must originate in the difference in polarizing power, acting on the transition state, between the cationic and nonionic molecular aggregates; *i.e.*, the transition state is more effectively stabilized in the cationic aggregates.

In the single-compartment vesicles of N$^+$C$_5$Ala2C$_{12}$, the reaction behavior between Cob(I)C$_3$ester and methyl 2-methyl-3-bromopropanoate was somewhat different from that in the micelles or in methanol. The reaction proceeded through a two-step mechanism; a faster step was followed by a slow one. The substrate-binding property of single-walled vesicles formed with *N*,*N*-ditetradecyl-*N*$^{\alpha}$-[6-(trimethylammonio)hexanoyl]-L-alaninamide bromide has been investigated previously from both static and dynamic viewpoints, hexyl *p*-nitrophenyl ether being used as a pseudo-substrate. The substrate-binding equilibrium was achieved through a two-step mechanism: the substrate molecules located in the bulk phase are incorporated readily into the surface area as the first step and such incorporated guest molecules penetrate rather slowly from the surface area into the hydrophobic interior of vesicular assembly.[7c)] This feature is consistent with the presence of a potential barrier between the surface and interior hydrophobic regions of the vesicular system for translocation of such a substrate. This barrier must be identical with the so-called hydrogen-belt domain located in the intramembrane region which has been identified by the use of dicyano(8,12-diethyl-1,2,3,7,13,17,18,19-octamethyltetradehydrocorrinato)cobalt(III) as a probe.[9)] In the light of these previous studies, the two kinetic steps observed for the alkylation with methyl 2-methyl-3-bromopropanoate may be defined as follows: (i) The first-step reaction takes place quite readily in the relatively polar domain near the vesicular surface; (ii) the second-step reaction occurs in the hydrophobic domain after permeation of the substrate into it. Under the experimental conditions for measurements of the first step ([RX]=$6.31\times10^{-4}$ mol dm$^{-3}$, RX being methyl 2-methyl-3-bromopropanoate), the absorbance at 392 nm characteristic of Cob(I)C$_3$ester decayed to about a 40% level of the initial one by following the pseudo-first-order kinetics; a further decay was extremely slow for accurate evaluation of the rate. Under the experimental conditions for measurements of the second step ([RX]=$5.32\times10^{-3}$ mol dm$^{-3}$), the 392 nm absorbance instantaneously decreased to about a 40% level of the initial one, followed by a slow decay in the pseudo-first-order kinetics. This result clearly indicates that the molar ratio of Cob(I)$C_3$ester distributing between the relatively polar and apolar domains is approximately 6:4. The second-order rate constant for the reaction of Cob(I)$C_3$ester with 1-bromo-2-methylpropane in the $N^+C_5Ala2C_{12}$ vesicle is more or less comparable to that measured in the CTAB micelle, while the rate constant for the reaction of the same complex with methyl 2-methyl-3-bromopropanoate in the former vesicle is significantly larger than that in the CTAB micelle. The rate enhancement would originate in the effective entrapment of the latter substrate into the hydrogen-belt domain of the vesicle due to the hydrogen-bonding interaction of the methoxycarbonyl group with the domain.

The net volume occupied by the CTAB micelle is determined on the basis of the partial molar volume of the surfactant monomer in the micellar state; $\bar{V}=$ 0.32—0.37 $dm^3\,mol^{-1}$.[13] The intrinsic volume occupied by the $N^+C_5Ala2C_{12}$ vesicle is assumed to be approximately equal to that by egg phosphatidylcholine; $\bar{V}=0.75$ $dm^3\,mol^{-1}$.[14] Thus, the molecular aggregates under the present experimental conditions hold about 1/600 of the total solution volume. Since Cob(I)$C_3$ester, being insoluble in water, is completely encapsulated in the aggregates, the micro-concentration effect exercised by the molecular aggregates on Cob(I)$C_3$ester is undoubtedly responsible for the rate enhancement. Since the extents of incorporation of the alkyl bromides were not determined by the gel-filtration chromatography applied to the hydrophobic vitamin $B_{12}$'s for their chemical instability, a conventional extraction method was employed here to evaluate them;[15] at least 25% of the total amount of the substrates was entrapped into the aggregates. In conclusion, multiple hydrophobic materials can be incorporated into the hydrophobic intramembrane region, the extents being dependent on their hydrophobic properties. The entrapped substances may undergo reactions in the significantly small compartments of molecular aggregates, and the apparent rate enhancement is attained at least by the micro-concentration effect.

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15) Diethyl ether ($E_T(30)=34.6$) was used as an extractant in the present work for technical reasons. $N^+C_5Ala2C_{12}$ in the vesicular state is hardly extracted with diethyl ether, while the present alkyl bromides present in the bulk aqueous phase were readily extracted. The extracted bromides were analyzed quantitatively by GLC. The fraction of the entrapped bromides was evaluated under consideration that those bromides bound to the vesicle are not readily extracted with diethyl ether.