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Photolysis of Hydrophobic Vitamin B_{12} Derivatives Covalently Bound to Lipid in Aqueous Media

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An alkyl ligand coordinated to hydrophobic vitamin B_{12} derivatives covalently bound to *N,N*-dihexadecyl-*N*$^{\alpha}$-[6-(trimethylammonio)hexanoyl]-L-aspartamide bromide underwent a novel bromination reaction along with its rearrangement in the single-walled vesicle of *N,N*-dihexadecyl-*N*$^{\alpha}$-[6-(trimethylammonio)hexanoyl]-L-alaninamide bromide under photolysis conditions.

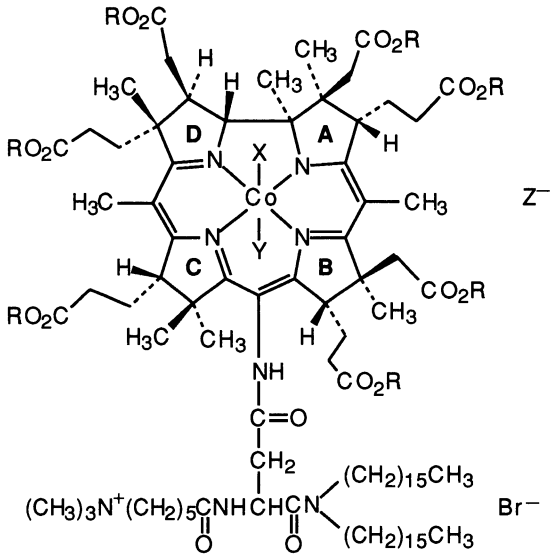
In order to place hydrophobic vitamin B_{12} derivatives more tightly in the hydrophobic microenvironment provided by synthetic bilayer membranes for efficient performance of enzyme-mimetic reactions,[1] we have designed novel hydrophobic vitamin B_{12} derivatives (**1** and **2**) covalently bound to *N,N*-dihexadecyl-*N*$^{\alpha}$-[6-(trimethylammonio)hexanoyl]-L-aspartamide bromide ($N^+C_5Asp2C_{16}$) through formation of an amide linkage between the 10-amino moiety of the hydrophobic vitamin B_{12} species and the β-carboxy group of the aspartate residue involved in the lipid molecule.

Complex **2** with peripheral propyl ester groups was synthesized after a method reported previously for preparation of complex **1**.[2] Heptapropyl (10-amino)dicyanocobyrinate (107 mg, 0.089 mmol) underwent reaction with *N,N*-dihexadecyl-*N*$^{\alpha}$-[6-(trimethylammonio)hexanoyl]-L-aspartamide bromide (73 mg, 0.089 mmol) in dry dichloromethane (3 mL) in the presence of *N,N'*-dicyclohexylcarbodiimide (25 mg, 0.12 mmol) to afford a dark purple solid: yield 106 mg (56.7%); $\lambda_{max}(CH_3OH)$ 284, 308, 318, 370, 422, 554, and 592 nm. Found: C, 64.55; H, 9.05; N, 6.62%. Calcd for $C_{113}H_{190}BrCoN_{10}O_{17}$: C, 64.64; H, 9.12; N, 6.67%.

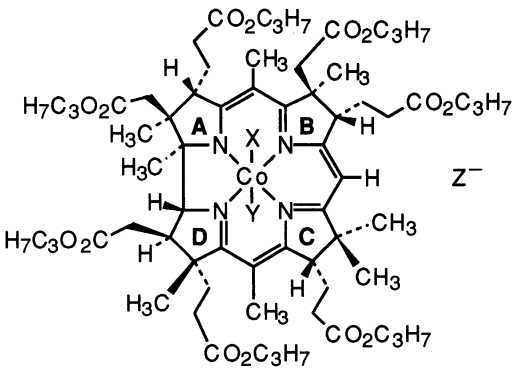
Alkylated hydrophobic vitamin B_{12} derivatives (**5** and **8**) were prepared via formation of the corresponding divalent cobalt complexes (**3** and **7**) according to the methods reported previously.[1-3] Complex **6** was prepared in a similar manner to give a brown solid: yield 59%; $\lambda_{max}(CH_2Cl_2)$ 268, 315, 410, and 469 nm. Found: C, 59.94; H, 8.54; N, 4.88%. Calcd for $C_{120}H_{207}BrClCoN_8O_{26}\cdot 5/2H_2O$: C, 60.12; H, 8.91; N, 4.67%. In order to remove an excess amount of the bromide ion in alkylated complexes **5**, **6**, and **8**, these compounds were dissolved in dichloromethane and shaken with aqueous sodium perchlorate (10 %) at the final synthetic step of these alkylated complexes. After the organic and aqueous layers were separated, the organic layer was dried over sodium sulfate and evaporated to dryness at room temperature.

Peptide amphiphile $N^+C_5Ala2C_{16}$ was prepared previously.[4] The following compounds were obtained from commercial sources (Aldrich Chemical Co., Inc., Milwaukee, WI, U.S.A.) as guaranteed reagents and used without further purification: α-phenyl-*N*-(*t*-butyl)nitrone (PBN) as a spin-trapping reagent, tetrabutylammonium bromide, and tributyltinhydride (*n*-Bu_3SnH). Diethyl 2,2-dimethylmalonate (**A**), diethyl 2-methylsuccinate (**B**), diethyl 2-bromomethyl-2-methylmalonate (**C**) were prepared as authentic samples for

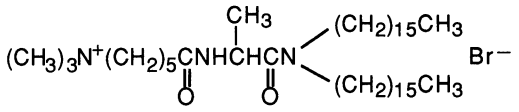
the corresponding reaction products after the procedures reported previously[1] and confirmed to be sufficiently pure by ^{1}H NMR and GLC.



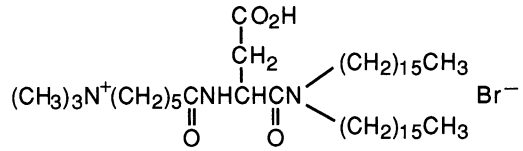
X=Y=CN, Z=None, R=CH_3 : **1**
X=Y=CN, Z=None, R=C_3H_7 : **2**
X=Y=None, Z=ClO_4 , R=CH_3 : **3**
X=Y=None, Z=ClO_4 , R=C_3H_7 : **4**
X=$CH_2C(CH_3)(CO_2C_2H_5)_2$, Y=$H_2O$, Z=$ClO_4$, R=$CH_3$: **5**
X=$CH_2C(CH_3)(CO_2C_2H_5)_2$, Y=$H_2O$, Z=$ClO_4$, R=$C_3H_7$: **6**
X=CN, Y=H_2O, Z=ClO_4, R=C_3H_7 : **9**



X=Y=None, Z=ClO_4 : **7**
X=$CH_2C(CH_3)(CO_2C_2H_5)_2$, Y=$H_2O$, Z=$ClO_4$: **8**

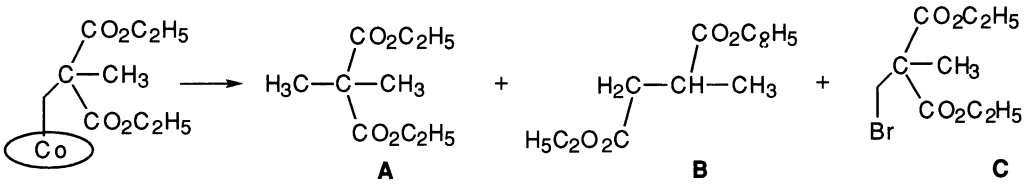


$N^+C_5Ala2C_{16}$



$N^+C_5Asp2C_{16}$

Photolysis reactions of the alkylated complexes and product analyses were performed by following procedures similar to those described previously.[1] Total yields listed in Table 1 are less than 100% owing to losses during extraction and evaporation treatments. It needs to be noted that no other by-products were obtained as confirmed by a GLC technique. Although the ester-migration efficiency in the photolysis of the alkylated hydrophobic vitamin B_{12} derivatives covalently bound to the lipid moiety (**5** and **6**) is less than that observed in the photolysis of the hydrophobic vitamin B_{12} derivative without a covalent linkage with a lipid (**8**), the bromination to afford **C** becomes predominant in the former reaction system.



A **B** **C**

In order to investigate a mechanism involved in the bromination reaction, ESR and GC-MS measurements were carried out in addition to a GLC method. A plausible reaction mechanism for production of the brominated product (**C**) is considered to be radical coupling between an alkyl radical species liberated

from the alkylated hydrophobic vitamin B_{12} and a bromine radical generated from bromide ions upon the photolysis treatment. In order to confirm this state of affairs, a spin-trapping technique was adopted here.

Table 1. Product Analyses for Photolysis of Alkylated Hydrophobic Vitamin B_{12} Derivatives in Various Media at 20.0 °C[a)]

		Yield[c)] / %		
Medium[b)]	Complex	A	B	C
$N^+C_5Ala2C_{16}$ vesicle	**5**	26.0	0.88	65.5
$N^+C_5Ala2C_{16}$ vesicle	**6**	11.3	0.85	67.1
$N^+C_5Ala2C_{16}$ vesicle	**8**	69.8	6.7	14.5
CH_3OH	**8**	88	0	0
C_6H_6	**8**	82	1.3	0

a) A solution containing a complex (1.0×10^{-4} mol dm^{-3}) was irradiated with a 500 W tungsten lamp at a distance of 30 cm under argon atmosphere for 1 h. b) $N^+C_5Ala2C_{16}$ (5.0×10^{-3} mol dm^{-3}) in phosphate buffer (20 mL, 1.0×10^{-3} mol dm^{-3}, μ 0.01 with KCl, pH 7.0). c) Products were analyzed by GLC.

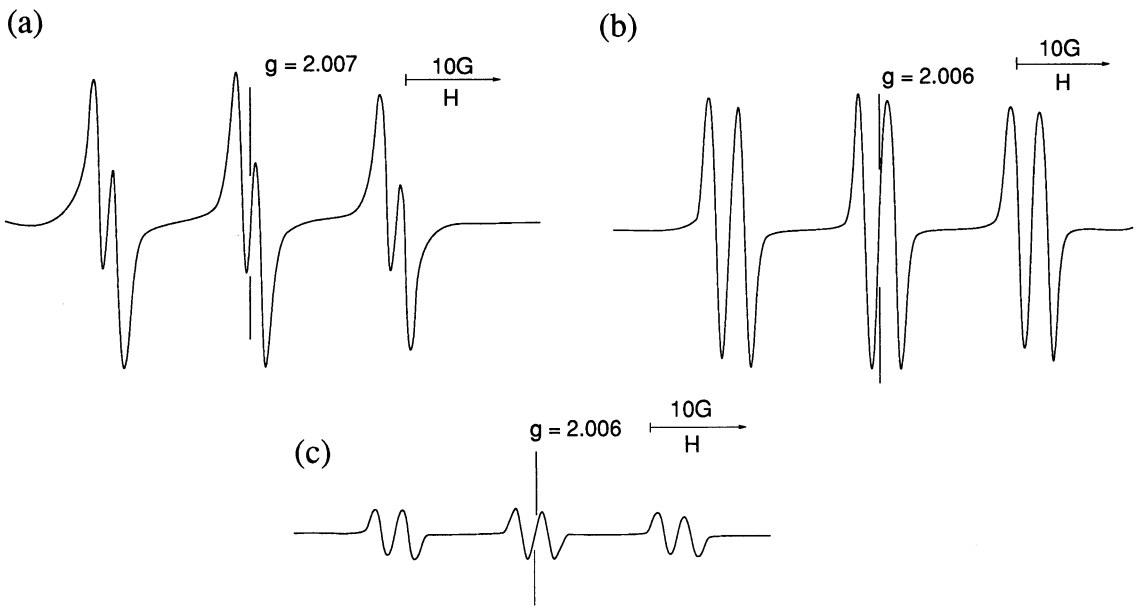
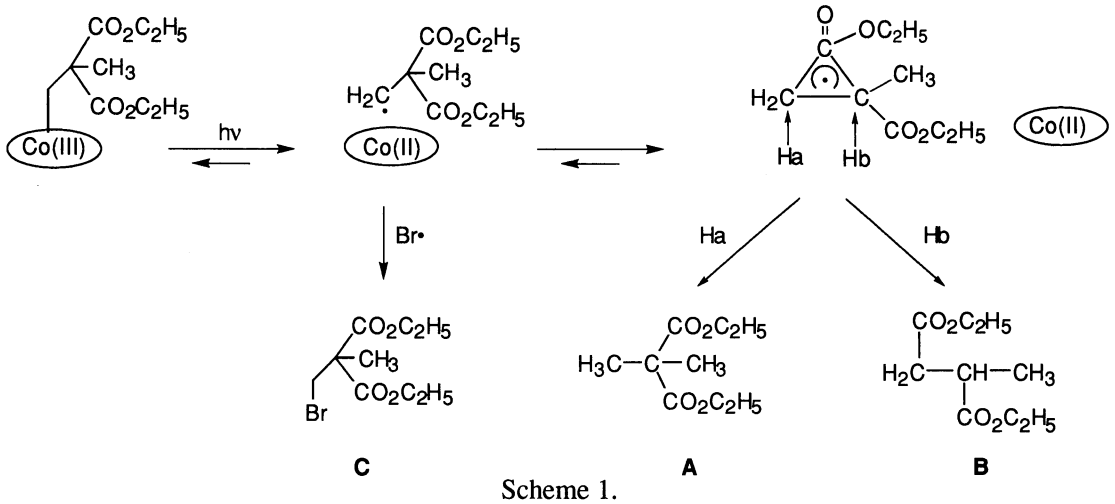


Fig. 1. ESR spectra of spin adducts in the presence of PBN (0.1 mol dm^{-3}) at room temperature when benzene solutions were irradiated with a 500 W tungsten lamp at a distance of 30 cm: (a), tetrabutylammonium bromide (1.0×10^{-3} mol dm^{-3}); (b), complex **5** (1.0×10^{-3} mol dm^{-3}); (c), complex **5** (1.0×10^{-3} mol dm^{-3}) and tetrabutylammonium bromide (1.0×10^{-3} mol dm^{-3}).

Photolysis of tetrabutylammonium bromide (TBAB) was examined under anaerobic condition in the presence of PBN. ESR signals of the PBN spin adduct (A_N = 14.4 G, A_H = 2.0 G; 10^4 G = 1 T) were clearly observed in benzene (Fig. 1, a), indicating formation of the bromine radical by photolysis of bromide ions. It must be noted that such radical formation is markedly depressed in aqueous media. When complex **5** was irradiated with visible light, ESR signals attributable to the PBN spin adduct (A_N = 14.7 G, A_H = 2.9 G; 10^4 G = 1 T) were also clearly observed in benzene (Fig. 1, b). This apparently indicates that the alkyl radical species is

generated by the photolytic cobalt–carbon bond cleavage. Furthermore, ESR signals of the PBN spin adduct (A_N = 15.0 G, A_H = 2.9 G; 10^4 G = 1 T) were clearly observed in benzene when a mixture of complex **5** and TBAB was irradiated (Fig. 1, c). The signal intensities are much weaker than those of the other spectra in Fig. 1. The results indicate that the radical coupling reaction between the alkyl radical and the bromine radical takes place more quickly than the reaction between the radical species and PBN to generate the corresponding PBN spin adducts.

Since the photolysis must take place in an apolar membrane domain, the radical species seem to be generated in a manner as observed in benzene. Under such circumstances, the photolysis to afford **A**, **B**, and **C** can be elucidated on the basis of reaction steps shown in Scheme 1. The alkylated hydrophobic vitamin B_{12} undergoes the cobalt–carbon cleavage to afford the alkyl radical and the Co(II) species. The alkyl radical abstracts a hydrogen atom from its vicinity[5] to give **A** and **B** and undergoes coupling with the bromine radical to generate the brominated product (**C**). Yields of the brominated product are much larger with complexes **5** and **6** relative to that with complex **8** in the bilayer membrane. This seems to be due to the fact that the hydrophobic vitamin B_{12} derivative covalently bound to the lipid molecule is subjected to tight association with the bilayer membrane to provide an efficient apolar microenvironment around the cobalt complex.



Scheme 1.

References

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