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Hydrogen Source in the Photochemical Cobalt-Carbon Cleavage of Hydrophobic Vitamin B_{12} Derivatives in Hydrophobic Microenvironments

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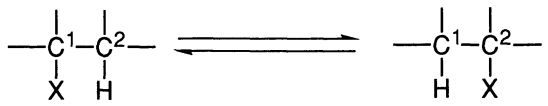
The photolysis of a hydrophobic vitamin B_{12} with a benzyl moiety at the *Coβ* site was carried out in a D_2O solution containing single-walled vesicles, and a major product was the non-deuterated toluene. A hydrogen atom was apparently abstracted from the hydrophobic vitamin B_{12} or a lipid molecule, and not from bulk water.

Coenzyme B_{12}-dependent enzymes catalyze isomerization reactions, which lead to the intramolecular exchange of a functional group (X) and a hydrogen atom between neighboring carbon atoms, as shown by Eq. 1. These reactions include carbon-skeleton rearrangement reactions mediated by methylmalonyl-CoA mutase and glutamate mutase (refer to Eqs. 2 and 3). Such metalloenzymes have attracted much attention because of novel nature of these reactions from the viewpoints of organic and organometallic chemistry.[1] The naturally occurring apoproteins, which provide relevant reaction sites for vitamin B_{12}, are considered to play crucial roles in the molecular rearrangements. In this regard, we have been interested in the catalytic activity of vitamin B_{12} in hydrophobic microenvironments, such as those provided by synthetic bilayer membranes and octopus cyclophanes, to simulate the catalytic functions of the holoenzymes concerned. We have constructed a vitamin B_{12} holoenzyme model by combination of a synthetic bilayer membrane composed of *N,N*-dihexadecyl-N^{α}-[6-(trimethylammonio)-hexanoyl]-L-alaninamide bromide ($N^{+}C_5Ala2C_{16}$) and a hydrophobic vitamin B_{12}, which has



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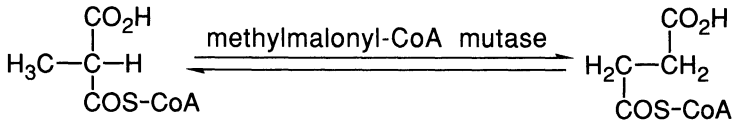

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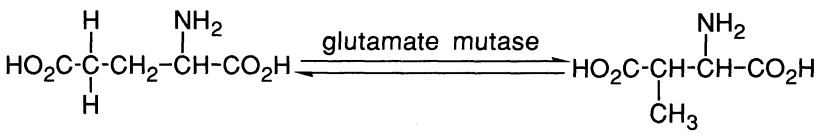


(3)

carboxylic ester groups in place of the peripheral amide moieties of the naturally occurring vitamin B_{12}[2] (Fig. 1). Simulation of catalytic functions, that are exercised by methylmalonyl-CoA mutase and glutamate mutase, were successfully performed by following the catalytic cycle given in Fig. 2.[3] However, it was not clear where a hydrogen atom comes from in this catalytic system for formation of the product species. In the present study, we clarified a hydrogen source for the product formation in the photochemical cobalt–carbon cleavage of hydrophobic vitamin B_{12} derivatives in hydrophobic microenvironments. The photolysis of a hydrophobic vitamin B_{12} with a benzyl moiety at the *Coβ* site was carried in various media, and the products were analyzed by 500 MHz ^{1}H-NMR spectroscopy.

We prepared heptamethyl *Coβ*-benzyl(cobyrinate) perchlorate (R=CH_3, X=$C_6H_5CH_2$, Y=H_2O in Fig. 1; [$(C_6H_5CH_2)(H_2O)$Cob(III)7C_1ester]ClO_4) as follows. Heptamethyl cobyrinate perchlorate[2a] (R=CH_3, X=Y=none in Fig. 1; [Cob(II)7C_1ester]ClO_4) (150 mg, 1.3 x 10^{-4} mol) was dissolved in methanol (100 mL), and the solution was deoxygenated by bubbling nitrogen gas through it at room temperature for 30 min. All the following treatments were performed at room temperature under subdued light. Sodium tetrahydroborate (500 mg, 1.3 x 10^{-2} mol) in water (25 mL) was added dropwise to the deoxygenated solution under nitrogen atmosphere, and benzyl bromide (220 mg, 1.3 x 10^{-3} mol) was added after the mixture turned dark green. After the reaction mixture changed its color to orange, it was stirred for 15 min. Then, 60% aqueous perchloric acid was added carefully to decompose an excess amount of sodium tetrahydroborate, and the mixture was extracted with dichloromethane. The extract was washed with distilled water, and the product was isolated by gel-filtration chromatography on a column of Sephadex LH-20 with methanol to afford brown powder: yield 65 mg (40%); λ_{max}(methanol) 262, 301, 340sh, and 424 nm. Found: C, 56.12; H, 6.87; N, 4.83%. Calcd for $C_{59}H_{82}ClCoN_4O_{19}\cdot 0.7H_2O$: C, 56.32; H, 6.68; N, 4.45%. Heptapropyl *Coβ*-benzyl(cobyrinate) perchlorate (R=C_3H_7, X=$C_6H_5CH_2$, Y=H_2O in Fig. 1; [$(C_6H_5CH_2)(H_2O)$Cob(III)7C_3ester]ClO_4) was prepared from heptapropyl cobyrinate perchlorate, (R=C_3H_7, X=Y=none in Fig. 1; [Cob(II)7C_3ester]ClO_4) by the similar procedure. Found: C, 60.16; H, 7.51; N, 4.20%. Calcd for $C_{73}H_{110}ClCoN_4O_{19}\cdot 0.5H_2O$: C, 60.42; H, 7.71; N, 3.86%.

The photolysis of hydrophobic vitamin B_{12} derivatives with a benzyl moiety at the *Coβ* site was carried out in various media. Firstly, [$(C_6H_5CH_2)(H_2O)$Cob(III)7C_1ester]ClO_4 (25 mg, 2.0 x 10^{-5} mol) was dissolved in methanol-d_4 (CD_3OD) and irradiated with a 500 W tungsten lamp at a distance of 30 cm for 4 h under anaerobic conditions. The electronic spectral change, analogous to that observed previously,[4] indicated that the cobalt–carbon bond was cleaved completely. Then, the solution containing the product was evaporated to dryness under reduced pressure. The product was analyzed by 500 MHz ^{1}H-NMR and GC-MS spectroscopy. The major product was toluene containing one deuterium atom (80%D). On the other hand, the major product was benzaldehyde, an oxygenated product, under aerobic photolysis conditions. The similar experiment with [$(C_6H_5CH_2)(H_2O)$Cob(III)7C_3ester]ClO_4 was carried out in benzene-d_6 (C_6D_6) under anaerobic conditions. The major product was bibenzyl as confirmed by 500 MHz ^{1}H-NMR analysis.

Then, we carried out the similar experiment in a deuterium oxide (D_2O) solution containing N^+C_5Ala2C_{16} vesicles as follows.[3c] [$(C_6H_5CH_2)(H_2O)$Cob(III)7C_3ester]ClO_4 (15 mg, 8.9 x 10^{-6} mol) was dissolved in a small amount of methanol (*ca.* 0.5 mL), and the solvent was removed by intro-

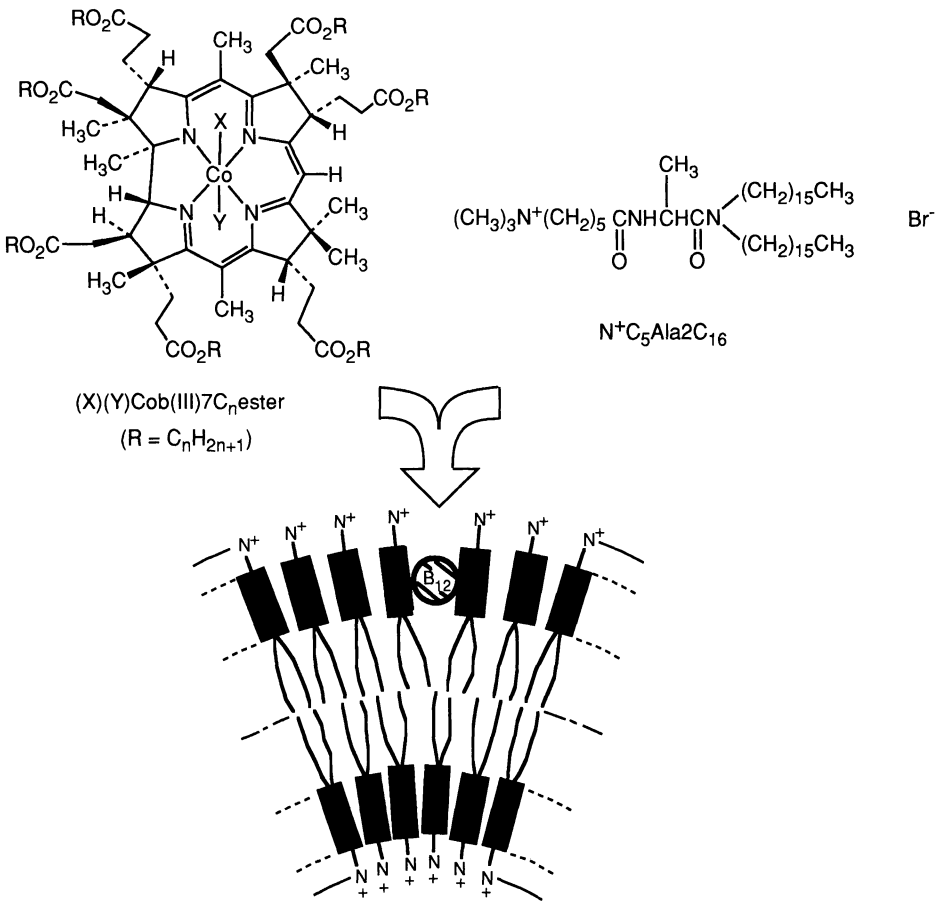


Fig. 1. Artificial vitamin B_{12}-dependent holoenzyme composed of a hydrophobic vitamin B_{12} and a synthetic bilayer membrane.

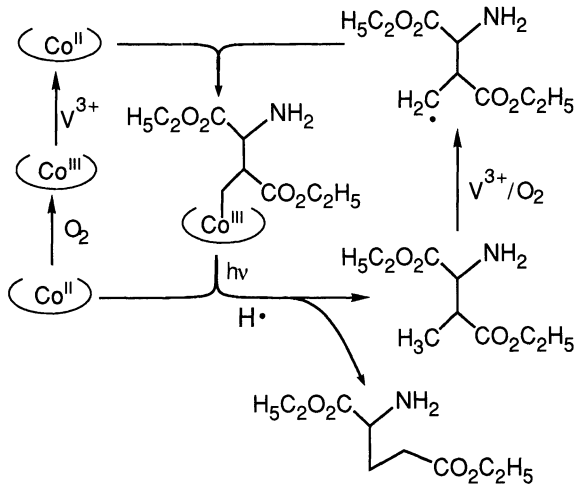
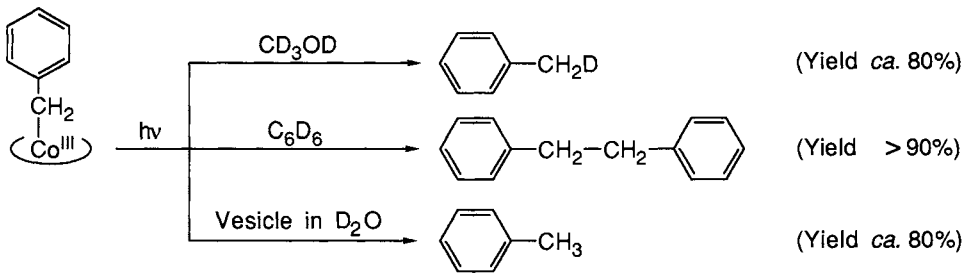


Fig. 2. Schematic representation for catalytic cycle of artificial glutamate mutase.[3b)]

ducing nitrogen gas into the methanol solution to obtain a thin film. The $N^+C_5Ala2C_{16}$ (155 mg, 2.0 x 10^{-4} mol) single-walled vesicle solution (20 mL) in D_2O, prepared after a previous procedure,[5] was added to the film with stirring, so that the cobalt complex was completely incorporated into the vesicle. After being deoxygenated by freeze-pump-and-thaw method, the resulting solution was irradiated with a 500 W tungsten lamp at a distance of 30 cm for 4 h. After the solution containing the product was evaporated to dryness under reduced pressure, the product was extracted from the evaporated fraction with tetrachlorocarbon (1 mL) and analyzed by 500 MHz ^{1}H-NMR spectroscopy. The major product was the non-deuterated toluene (deuterium content, below 5%). When $[(CH_3OCOCH_2CH_2)(H_2O)Cob(III)7C_3ester]ClO_4$ (R=C_3H_7, X=$CH_3OCOCH_2CH_2$, Y=H_2O in Fig. 1) was used as an alkylated complex, the major product was the non-deuterated methyl propionate.

The product analyses for the photochemical cleavage of $[(C_6H_5CH_2)(H_2O)Cob(III)-7C_nester]ClO_4$ (n=1 or 3) in various deuterated media under anaerobic conditions are summarized in Scheme 1. The following findings are based on the product analyses by GLC and 500 MHz ^{1}H-NMR spectroscopy. (i) The major product in CD_3OD was toluene, which was deuterated to an extent of 80%D. (ii) Bibenzyl was the major product in C_6D_6, and toluene was not detected by 500 MHz ^{1}H-NMR spectroscopy because of lack of a hydrogen source. (iii) The major product in the vesicular phase with D_2O as a bulk solvent was the non-deuterated toluene. This result indicates that a hydrogen atom is abstracted from the hydrophobic vitamin B_{12} or the lipid molecule in the vesicular phase, and not from bulk water. On this ground, it needs to be pointed out that the hydrophobic vitamin B_{12} is significantly desolvated in the vesicle.



Scheme 1.

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