Isomerization Catalysis by Hydrophobic Vitamin B_{12} Covalently Bound to a Lipid Species in a Bilayer Membrane

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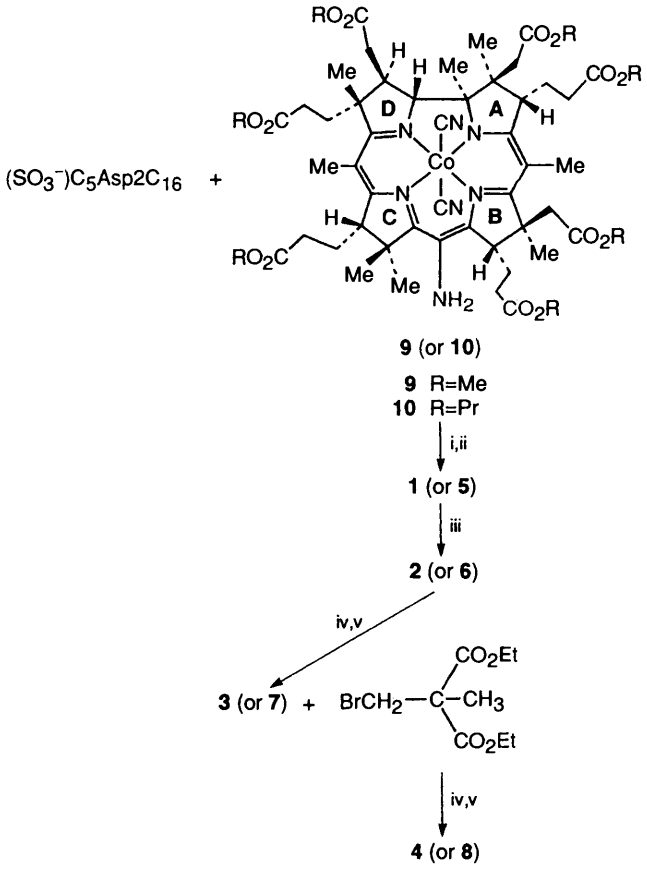
A novel artificial vitamin B_{12} holoenzyme composed of a hydrophobic vitamin B_{12} covalently bound to a lipid species and a bilayer matrix of sodium *N,N*-dihexadecyl-*N*$^{\alpha}$-(6-sulfohexanoyl)-L-alaninamide enhanced a methylmalonyl-CoA mutase model reaction.

Naturally occurring apoproteins are considered to provide hydrophobic microenvironments for vitamin B_{12}[1] to enhance the coenzyme activity in various isomerization reactions.[2] In this context, we have previously succeeded in constructing artificial vitamin B_{12} holoenzymes by combining non-covalently bound apoprotein models, such as bilayer membranes and octopus cyclophanes, with hydrophobic vitamin B_{12} derivatives, and found that those artificial enzymes were effective in various carbon-skeleton rearrangements.[3] We report here a reactivity of an artificial holoenzyme, composed of a hydrophobic vitamin B_{12} covalently bound to an anionic lipid species and a single-walled bilayer matrix, in a methylmalonyl-CoA mutase model reaction.

The novel hydrophobic vitamin B_{12} derivatives were prepared in a manner similar to that reported previously[4] by following the reaction steps shown in Scheme 1. All the products were characterized by ^{1}H NMR, IR and electronic spectroscopy as well as elemental analyses. Complex **1** was prepared by condensation of sodium *N,N*-dihexadecyl-*N*$^{\alpha}$-(6-sulfohexanoyl)-L-aspartamide, $(SO_3^-)C_5Asp2C_{16}$,† which was synthesized by a method similar to that reported for the preparation of $(SO_3^-)C_5Ala2C_{16}$,[5] with hydrophobic vitamin B_{12} **9**.‡§

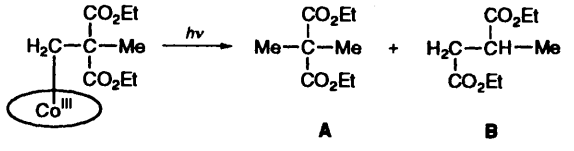
Complex **5** was prepared from $(SO_3^-)C_5Asp2C_{16}$ and **10**‡ to afford a purple solid.¶

Alkylated complexes **4** || and **8**** were prepared *via* formation of divalent cobalt complexes **3** and **7** according to a method similar to those described previously.[3]



Scheme 1 *Reagents:* i, DCC; ii, KCN; iii, $HClO_4$; iv, $NaBH_4$; v, $HClO_4$

One of the most interesting reactions catalysed by vitamin B_{12}-dependent enzymes is the carbon-skeleton rearrangement reaction as typically performed by methylmalonyl-CoA mutase. We examined a carbon-skeleton rearrangement of the 2,2-bis(ethoxycarbonyl)propyl moiety bound to the hydrophobic vitamin B_{12} derivatives (**3** and **7**), **4** and **8**, respectively, in the synthetic bilayer membrane matrix, formed with $(SO_3^-)C_5Ala2C_{16}$, under anaerobic photolysis conditions. After each of the alkylated complexes had been completely photolysed as confirmed by electronic spectroscopy, products were extracted with dichloromethane and analysed by GLC [refer to eqn. (1) and Table 1]. The analytical results indicate that the isomerization of the substrate bound to a hydrophobic vitamin B_{12} apparently takes place in a bilayer matrix of (SO_3^-)-



(1)

† (Found: C, 65.0; H, 10.45; N, 3.3. $C_{42}H_{81}N_2NaO_7S$ requires: C, 64.58; H, 10.45; N, 3.59%).

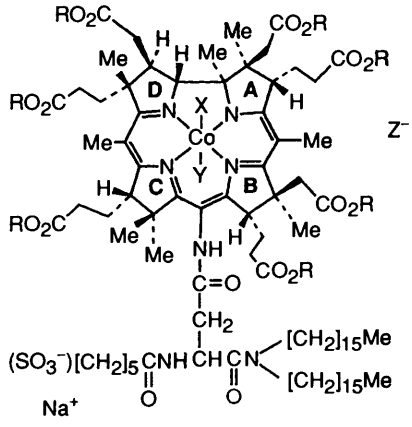
‡ Corrinoids were nitrated at the 10-position with nitronium tetrafluoroborate and the nitrated products were reduced with sodium tetrahydroborate in dry methanol under anaerobic conditions to afford **9** and **10**. Complex **9**: $\lambda_{max}(CH_3OH)$/nm 312, 378 and 616 (Found: C, 57.3; H, 6.7; N, 8.5. $C_{54}H_{74}CoN_7O_{14}\cdot\frac{3}{2}H_2O$ requires: C, 57.34; H, 6.86; N, 8.67%). Complex **10**: $\lambda_{max}(CH_3OH)$/nm 313, 377 and 615 (Found: C, 62.2; H, 7.7; N, 7.6. $C_{68}H_{102}CoN_7O_{14}\cdot\frac{1}{2}H_2O$ requires: C, 62.37; H. 7.93; N, 7.49%).

§ A dry dichloromethane solution (5 cm^3) of $(SO_3^-)C_5Asp2C_{16}$ (150 mg, 0.19 mmol) and *N,N'*-dicyclohexylcarbodiimide (DCC; 40 mg, 0.19 mmol) was stirred for 0.5 h at 0 °C and then **9** (213 mg, 0.19 mmol) was added to the solution. The resulting mixture was stirred for 6 h at 0 °C and overnight at room temperature. The crude product was purified by TLC on silica gel (Kieselgel 60) with methanol–dichloromethane (6:1 v/v) as the eluent to afford a purple powder: yield 115 mg (32%); $\lambda_{max}(CH_2Cl_2)$/nm 281, 309, 316, 371, 420, 509, 548 and 584 (Found: C, 61.0; H, 8.2; N, 6.7. $C_{96}H_{153}CoN_9NaO_{20}S\cdot H_2O$ requires: C, 61.16; H, 8.29; N, 6.69%).

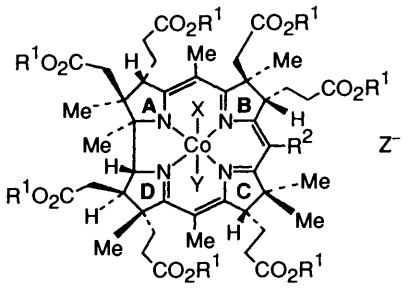
¶ Yield 34.8%; $\lambda_{max}(CH_2Cl_2)$/nm 281, 309, 316, 371, 422, 506, 551 and 585 (Found: C, 63.5; H, 8.7; N, 6.2. $C_{110}H_{181}CoN_9NaO_{20}S\cdot H_2O$ requires C, 63.47; H, 8.86; N, 6.06%).

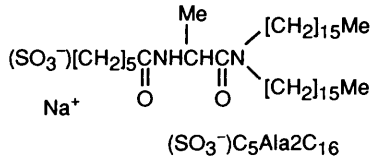
|| Complex **4**: yield 70%; $\lambda_{max}(CH_2Cl_2)$/nm 266, 301, 318, 418 and 469 (Found: C, 59.0; H, 8.0; N, 4.8. $C_{103}H_{168}ClCoN_7NaO_{28}S$ requires: C, 58.85; H, 8.06; N, 4.66%).

** Complex **8**: yield 56.3%; $\lambda_{max}(CH_2Cl_2)$/nm 268, 300, 319, 420 and 474 (Found: C, 61.3; H, 8.6; N, 4.4. $C_{117}H_{196}ClCoN_7NaO_{28}S$ requires: C, 61.14; H, 8.60; N, 4.27).

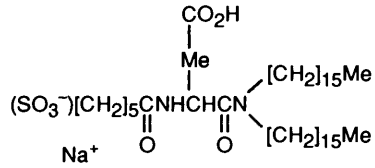


1 R=Me, X=Y=CN, Z=None
2 R=Me, X=CN, Y=H_2O, Z=ClO_4
3 R=Me, X=Y=None, Z=ClO_4
4 R=Me, X=$CH_2C(Me)(CO_2Et)_2$, Y=H_2O, Z=ClO_4
5 R=Pr, X=Y=CN, Z=None
6 R=Pr, X=CN, Y=H_2O, Z=ClO_4
7 R=Pr, X=Y=None, Z=ClO_4
8 R=Pr, X=$CH_2C(Me)(CO_2Et)_2$, Y=H_2O, Z=ClO_4





$(SO_3^-)C_5Ala2C_{16}$



$(SO_3^-)C_5Asp2C_{16}$

11 R^1=Pr, R^2=H, X=Y=CN, Z=None
12 R^1=Pr, R^2=H, X=CN, Y=H_2O, Z=ClO_4
13 R^1=Pr, R^2=H, X=$CH_2C(Me)(CO_2Et)_2$, Y=H_2O, Z=ClO_4
14 R^1=Me, R^2=$NHCOCH_3$, X=Y=CN, Z=None

Table 1 Product analyses for photolysis of alkylated hydrophobic vitamin B_{12} derivatives in vesicles at 20.0 °C under anaerobic conditions[a]

Alkylated hydrophobic vitamin B_{12}	Yield[b] (%)		
	A	B	B/A
4	55.5	9.94	0.18
8	44.5	20.5	0.46
13	74.1	9.13	0.12

[a] Medium: a dispersion sample of $(SO_3^-)C_5Ala2C_{16}$ (5.0×10^{-3} mol dm^{-3}) in aqueous phosphate buffer (20 cm^3; 1 mmol dm^{-3}, μ 0.01 with KCl, pH 7.0) was sonicated with a probe-type sonicator at 30 W for 15 min. A solution containing an alkylated hydrophobic vitamin B_{12} (1.0×10^{-4} mol dm^{-3}) was irradiated with a 500 W tungsten lamp at a distance of 30 cm under an argon atmosphere for 1 h. [b] Products were analysed by GLC. Total yields are less than 100% largely due to losses during extraction and evaporation treatments.

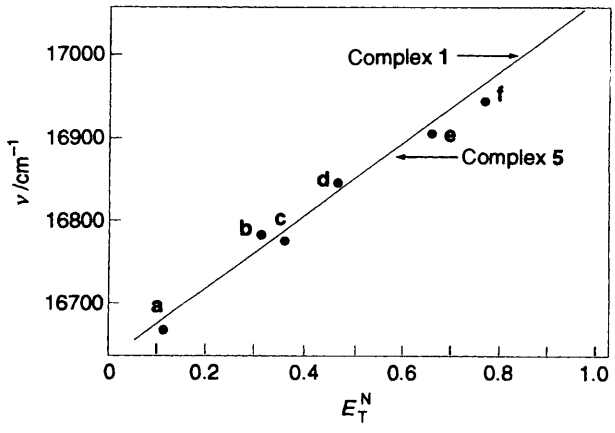


Fig. 1 Correlation between α-band wavenumber for hydrophobic vitamin B_{12} derivatives and solvent polarity parameter (E_T^N): **a**, benzene; **b**, dichloromethane; **c**, acetone; **d**, acetonitrile; **e**, ethanol; **f**, methanol; a reference correlation line was drawn on the basis of **a**–**f** values obtained with **14**. Arrows indicate the polarity parameters for **1** and **5** in the single-walled vesicle.

C_5Ala2C_{16}. The formation ratio of the rearranged product [**B** in eqn. (1)] *vs.* the simple reduction product [**A** in eqn. (1)] from **4**, which has seven peripheral methyl ester groups around the corrin skeleton, is somewhat larger than that from **13** which does not bear a lipid species covalently. It must be noted, however, that the product ratio (**B**/**A**) observed for **8**, which has seven peripheral propyl ester groups, markedly increased as compared with those for **4** and **13**.

The microenvironmental properties around the hydrophobic vitamin B_{12} derivatives placed in the bilayer aggregate were examined by electronic absorption and fluorescence polarization spectroscopy. The microenvironmental polarity experienced by complexes **1** and **5** was evaluated when the complexes were incorporated into the synthetic bilayer membrane with attention to α-bands of the complexes (Fig. 1). The microenvironmental polarity experienced by **1** is between water and methanol, while that experienced by **5** is between ethanol and acetonitrile. This result indicates that the complexes are significantly desolvated when incorporated into the vesicle. In order to obtain further information as regards the microenvironmental effect on molecular motion of the hydrophobic vitamin B_{12} in the vesicle, complexes **2** and **6** coordinated with dansylhistamine at the residual axial site of cobalt were adopted as fluorescent probes. Large fluorescence polarization (P) values were observed for **2** and **6** as compared with the simple hydrophobic vitamin B_{12} (**12**) over a wide temperature range (Fig. 2).[3] These results clearly indicate that the hydrophobic vitamin B_{12} derivative covalently bound to the lipid molecule is subjected to marked motional repression in the bilayer membrane as compared with the simple hydrophobic vitamin B_{12} without lipid linkage.

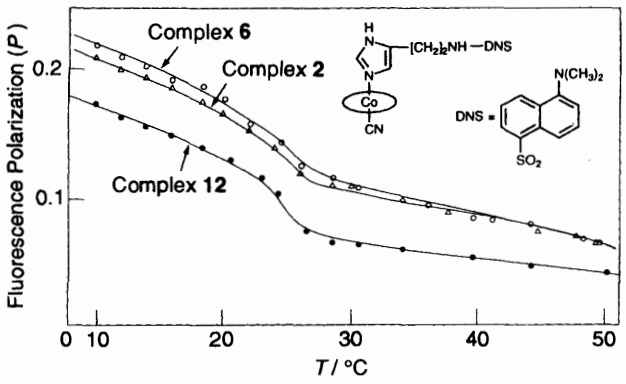


Fig. 2 Correlations of fluorescence polarization (P) for the dansylhistamine moiety bound to complexes **2**, **6** and **12**, incorporated into the $(SO_3^-)C_5Ala2C_{16}$ vesicle, with temperature, as measured in aqueous phosphate buffer (0.01 mol dm^{-3}; μ 0.1 with KCl) at pH 7.0

In conclusion, the high mediator efficiency performed by the hydrophobic vitamin B_{12} in the carbon-skeleton rearrangement must come from both desolvation and motional repression effects furnished by the single-walled vesicle in a manner similar to those observed previously.[3]

References

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