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# CATALYTIC FUNCTION OF A HOST-GUEST COMPLEX DERIVED FROM OCTOPUS AZAPARACYCLOPHANE AND HYDROPHOBIC VITAMIN $B_{12}$ AS A HOLOENZYME MODEL

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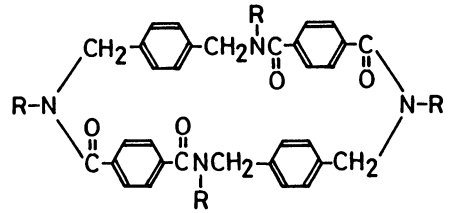
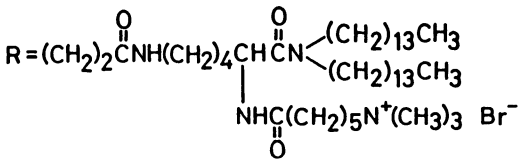
The isomerization reaction accompanied with carbon-skeleton rearrangement, as typically catalyzed by the vitamin $B_{12}$-dependent methylmalonyl-CoA mutase, was simulated by employing the host-guest complexes formed with an octopus azaparacyclophane having eight hydrocarbon chains and hydrophobic vitamin $B_{12}$ derivatives.

We have recently clarified that octopus-like cyclophanes, derived from a tetraaza[3.3.3.3]paracyclophane by introducing flexible hydrocarbon chains, provide effective intramolecular hydrophobic binding sites for various hydrophobic guest molecules and exercise the induced-fit guest-binding behavior originated from both hydrophobic and electrostatic interactions.[1,2] An octopus cyclophane having eight hydrocarbon chains, APC($C_2$Lys2$C_{14}$)$_4$, acts as a cationic host in acidic aqueous media and strongly bind neutral and anionic guest molecules; formation constants



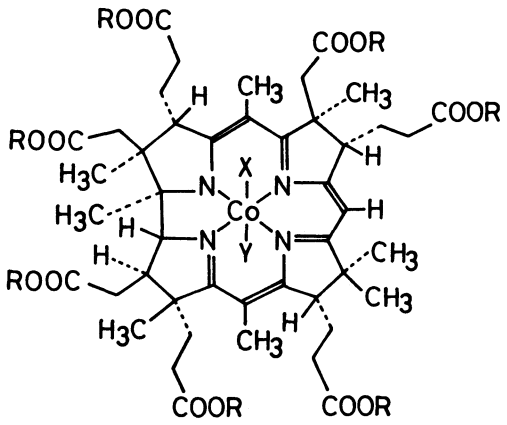
$R=(CH_2)_2CNHCHCN(CH_2)_{13}CH_3$, $(CH_2)_{13}CH_3$; $(CH_2)_4NH_3^+$ $Cl^-$

APC($C_2$Lys2$C_{14}$)$_4$



$R=(CH_2)_2CNH(CH_2)_4CHCN(CH_2)_{13}CH_3$, $(CH_2)_{13}CH_3$; $NHC(CH_2)_5N^+(CH_3)_3$ $Br^-$

APC[$C_2$Lys($C_5N^+$)2$C_{14}$]$_4$



(CN)$_2$Cob(III)7$C_3$ester
X=Y=CN, R=$C_3H_7$

for the 1:1 complexes being $10^5$—$10^6$ $mol^{-1}$ $dm^3$.[2] Now, we prepared another octopus cyclophane bearing quaternary ammonium moieties, APC[$C_2$Lys($C_5N^+$)$2C_{14}$]$_4$,[3] which may behave as an effective cationic host over the whole pH range in aqueous media. In this communication, we report on the reaction behavior of hydrophobic vitamin $B_{12}$ derivatives[4] incorporated into APC[$C_2$Lys($C_5N^+$)$2C_{14}$]$_4$.

The guest-binding ability of APC[$C_2$Lys($C_5N^+$)$2C_{14}$]$_4$ and the microenvironmental properties of its hydrophobic cavity, reflected on polarity parameter [$E_T$(30)] and microviscosity (fluorescence polarization, P), in neutral aqueous media (pH 8.0) were essentially identical with those demonstrated by APC($C_2$Lys$2C_{14}$)$_4$ at pH 6.0.[5] Although a hydrophobic vitamin $B_{12}$, heptapropyl dicyanocobyrinate [$(CN)_2$Cob(III)-$7C_3$ester] was insoluble in water, this Co(III) species was readily and completely solubilized by incorporation into APC[$C_2$Lys($C_5N^+$)$2C_{14}$]$_4$ at the 1:1 molar ratio as confirmed by electronic absorption spectroscopy. The microenvironmental polarity around $(CN)_2$Cob(III)$7C_3$ester in the host molecule was evaluated on the basis of a correlation of the α-band wavelength of the Co(III) chelate with the solvent polarity parameter (Fig. 1);[4] the $E_T$(30) value (42.5 kcal $mol^{-1}$) is nearly equivalent to that provided by acetone (42.2 kcal $mol^{-1}$).[6] Thus, the guest molecule seems to be placed in the sufficiently desolvated hydrophobic cavity. The following cationic and nonionic water-insoluble vitamin $B_{12}$ derivatives were also incorporated into APC[$C_2$Lys($C_5N^+$)$2C_{14}$]$_4$ quantitatively at the 1:1 molar ratio; heptapropyl cobyrinate perchlorate [[Cob(II)$7C_3$ester]$ClO_4$], heptapropyl cobyrinate [Cob(I)$7C_3$-ester], and its alkylated complex [[R-Cob(III)$7C_3$ester]$ClO_4$]. This means that the hydrophobic molecular recognition exercised by the host overcomes the electrostatic repulsion between the host and these hydrophobic guests.

Alkylation of Cob(I)$7C_3$ester with methyl 2-methyl-3-bromopropanoate was monitored by the spectrophotometric means in a manner as described previously[4] under the following conditions: in an aqueous phosphate—borate buffer (0.05 mol $dm^{-3}$) at pH 9.2 and 20.0 °C under argon atmosphere; APC[$C_2$Lys($C_5N^+$)$2C_{14}$]$_4$, $4.0 \times 10^{-5}$ mol $dm^{-3}$; Cob(I)$7C_3$ester, $2.0 \times 10^{-5}$ mol $dm^{-3}$; $(CH_3OCO)(CH_3)CHCH_2Br$, $6.1 \times 10^{-4}$ mol $dm^{-3}$. The pseudo-first-order rate constant ($k_{obsd}$, $1.7 \times 10^{-2}$ $s^{-1}$) is about three times as large as the $k_{obsd}$ value ($5.8 \times 10^{-3}$ $s^{-1}$) obtained for the reaction of heptamethyl cobyrinate [Cob(I)$7C_1$ester][7] with the same alkyl halide in the absence of the host molecule under otherwise the identical conditions. This may come from increased local concentrations of the two reactants through formation of the ternary complex composed of APC[$C_2$Lys($C_5N^+$)$2C_{14}$]$_4$, Cob(I)$7C_3$ester, and the alkyl halide.

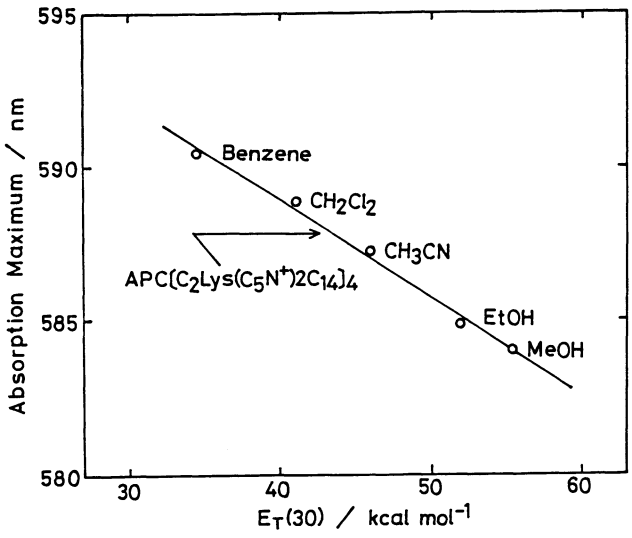
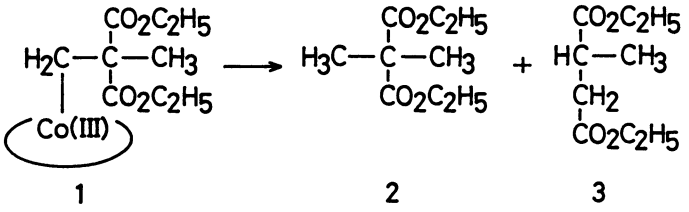



Fig. 1. Solvent effect on electronic transition (α-band) of $(CN)_2$-Cob(III)$7C_3$ester.

Photolysis of the alkylated hydrophobic vitamin $B_{12}$ in the presence of APC[$C_2$Lys($C_5N^+$)$2C_{14}$]$_4$ under aerobic

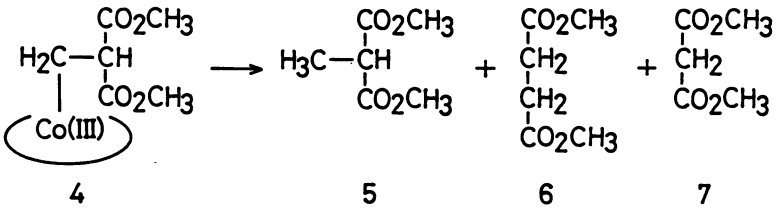
conditions at pH 3.8 afforded the Co(II) species at a 35 ± 5% fraction of the total cobalt species formed; confirmed quantitatively by means of electronic absorption and ESR measurements.[8] On the other hand, the Co(III) species was exclusively produced in homogeneous aqueous solution. The Co(III) was formed through homolytic cleavage of the cobalt—carbon bond of the alkylated Co(III) species, followed by oxygen attack on the naked (solvation free) Co(II) species. The result implies that such oxygen attack was suppressed to a significant extent in the hydrophobic cavity of the host molecule.

One of the most interesting reactions catalyzed by vitamin $B_{12}$-dependent enzymes is the isomerization reaction, which results in the carbon-skeleton rearrangement, as typically performed by methylmalonyl-CoA mutase. We examined the reaction of hydrophobic vitamin $B_{12}$ derivatives bearing relevant alkyl ligands, which were incorporated into the hydrophobic cavity of $APC[C_2Lys(C_5N^+)2C_{14}]_4$ in aqueous media, under anaerobic conditions in the dark. As shown in Table 1, the




1 2 3

Scheme 1.



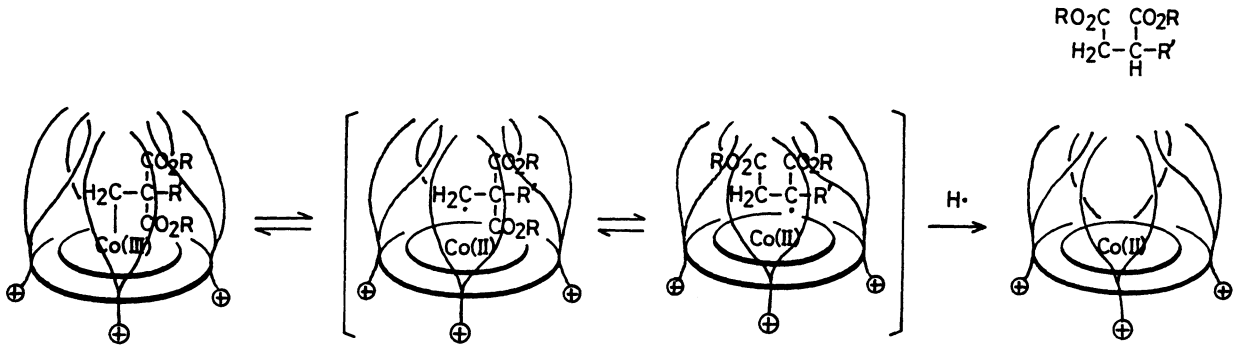

4 5 6 7

Scheme 2.

Table 1. Product analyses for the thermal decomposition reactions of complexes **1** and **4** under anaerobic conditions in the dark at 30 °C[a]

| Medium | Yield/%[b] | | Yield/%[c] | | |
|---|---|---|---|---|---|
| | **2** | **3** | **5** | **6** | **7** |
| $APC[C_2Lys(C_5N^+)2C_{14}]_4$[d] | 75 | 13 | 60 | 6.0 | 12 |
| Methanol[e] | 88 | 0 | 86 | 0 | 1.7 |
| 1-Butanol | 85 | Trace | | | |
| Cyclohexanol | 87 | Trace | | | |
| Benzene[e] | 86 | 1.2 | 86 | 0.9 | 1.5 |

a) After incubation for 48 h, analyzed by GLC. b) Reactant, **1**. c) Reactant, **4**. d) Host : guest = 1:1 ($5.0 \times 10^{-5}$ mol $dm^{-3}$ each) in phosphate—borate buffer (0.05 mol $dm^{-3}$, pH 9.2). e) Taken from Ref. 9.




Scheme 3.

hydrophobic Co(III) species with the 2,2-bis(ethoxycarbonyl)propyl (Scheme 1, **1**) and 2,2-bis(methoxycarbonyl)ethyl (Scheme 2, **4**) moieties afforded the corresponding isomerization products, 1,2-bis(ethoxycarbonyl)propane (**3**) and 1,2-bis(methoxycarbonyl)ethane (**6**), respectively, in significant yields in the presence of APC-$[C_2Lys(C_5N^+)2C_{14}]_4$.[9] Such isomerization products were scarcely obtained in homogeneous organic solutions, and the reduction products without migration of the ester groups (**2** and **5**) were mainly given in high yields. We have clarified that homolytic cleavage of the cobalt—carbon bond of the alkylated Co(III) species occurs under such reaction conditions in the light of electronic absorption and spin-trapping measurements.[9] Accordingly, the hydrophobic cage effect provided by the cyclophane seems to stabilize the intermediate radical pair and enhances the isomerization reaction (Scheme 3).

In conclusion, it became apparent that the host-guest complex formed with the octopus cyclophane, APC$[C_2Lys(C_5N^+)2C_{14}]_4$, and a hydrophobic vitamin $B_{12}$ derivative acts as an effective vitamin $B_{12}$-dependent holoenzyme model. A large and flexible hydrophobic cavity provided by the host molecule may retain large potentiality in constituting an apoenzyme-like recognition site exercising the induced-fit function.

References

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3) Found: C, 67.65; H, 10.36; N, 6.93%. Calcd for $C_{216}H_{392}Br_4N_{20}O_{16}$: C, 67.47; H, 10.28; N, 7.29%.
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9) For experimental details, see: Y. Murakami, Y. Hisaeda, T. Ohno, and Y. Matsuda, Chem. Lett., submitted for publication.

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