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Artificial Glutamate Mutase Composed of Hydrophobic Vitamin B_{12} and Synthetic Bilayer Membrane

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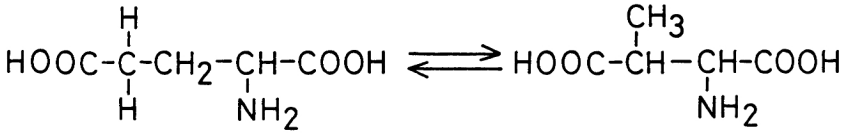
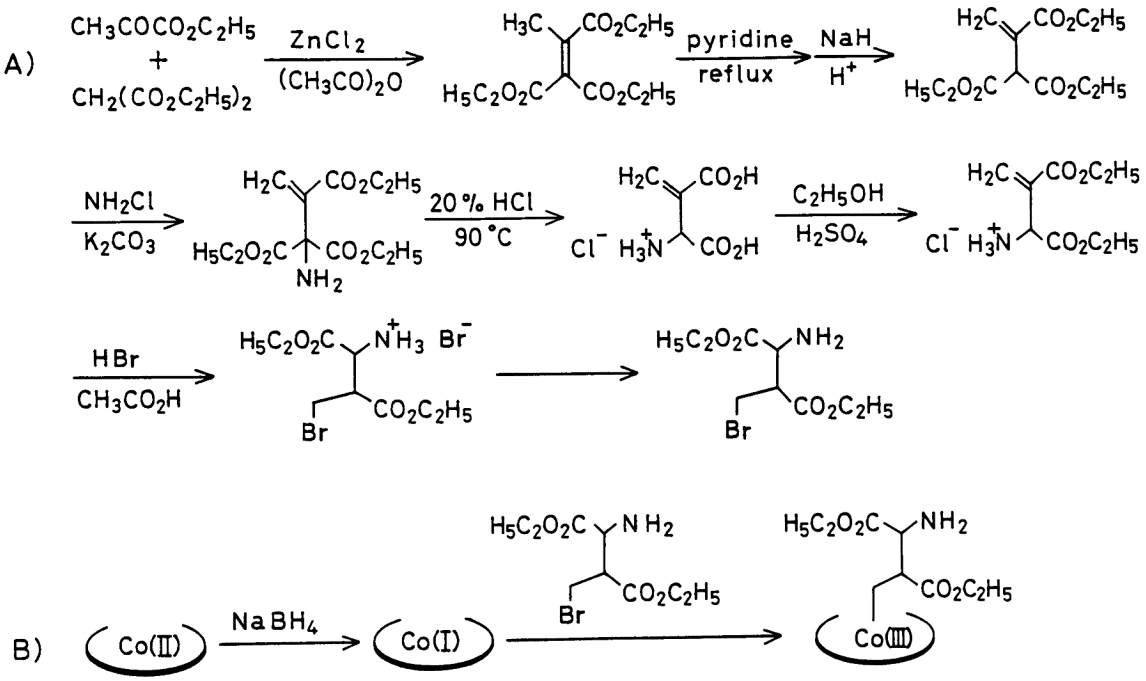
The alkylated hydrophobic vitamin B_{12}, prepared by the reaction between heptapropyl cobyrinate and diethyl bromomethylaspartate, afforded diethyl glutamate, the rearrangement product, in single-compartment vesicles under anaerobic photolysis conditions.

Vitamin B_{12} is the unique coenzyme, which can form the cobalt—carbon bond in vivo, and exercises various catalytic functions when it is incorporated into diverse apoenzymes. In particular, the isomerization reactions accompanied with carbon-skeleton rearrangements are the most interesting catalytic feature from the viewpoints of organic synthesis and organometallic chemistry. The naturally occurring apoproteins are considered to play inevitably important roles in the isomerization reactions. We have been interested in the roles of the apoproteins which provide relevant reaction sites for vitamin B_{12} and investigated various reactions of hydrophobic vitamin B_{12} derivatives in an octopus azaparacyclophane having eight hydrocarbon chains and single-compartment vesicles of peptide amphiphiles, as apoprotein models.[1-4] Those studies demonstrated the first successful examples of methylmalonyl-CoA mutase-like reactions.[4] Glutamate mutase, which is one of the vitamin B_{12}-dependent enzymes capable of causing the carbon-skeleton rearrangement, catalyzes the reversible interconversion of L-glutamate and threo-β-methyl-L-aspartate (Eq. 1). However, any successful performance of the model reaction for this enzymic catalysis has not been reported so far. In this communication, we report on the conversion of the methylaspartic skeleton into the glutamic one by employing a hydrophobic vitamin B_{12} derivative in single-compartment vesicles.

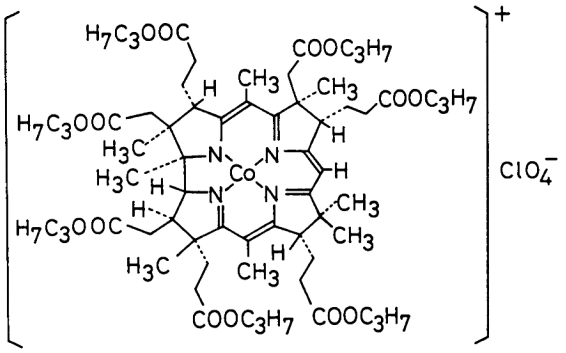


(1)

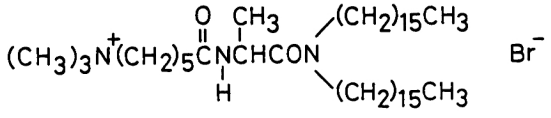
Diethyl bromomethylaspartate, a model substrate for glutamate mutase, was prepared in reference to the method reported previously[5] as shown in Scheme 1. This model substrate was purified by gel-filtration chromatography on columns of Sephadex LH-20 and Toyopearl HW-40 Superfine with methanol. Found: C, 38.09; H, 5.75; N, 5.21%. Calcd for $C_9H_{16}BrNO_4$: C, 38.31; H, 5.68; N, 4.97%. Then, the corresponding alkylated complex, $(CO_2C_2H_5)(NH_2)CHCH(CO_2C_2H_5)CH_2$-Cob(III)7C$_3$ester, was prepared by the reaction of [Cob(II)7C$_3$ester]ClO_4[1] with diethyl bromomethylas-



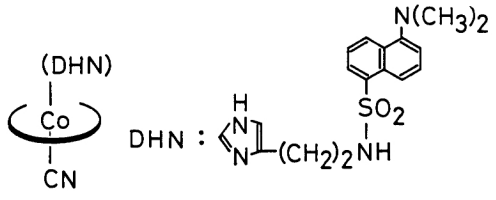
Scheme 1.



[Cob(III)7C$_3$ester]ClO$_4$



N^+C_5Ala 2C_{16}



[(CN)(DHN)Cob(III)7C$_3$ester]ClO$_4$

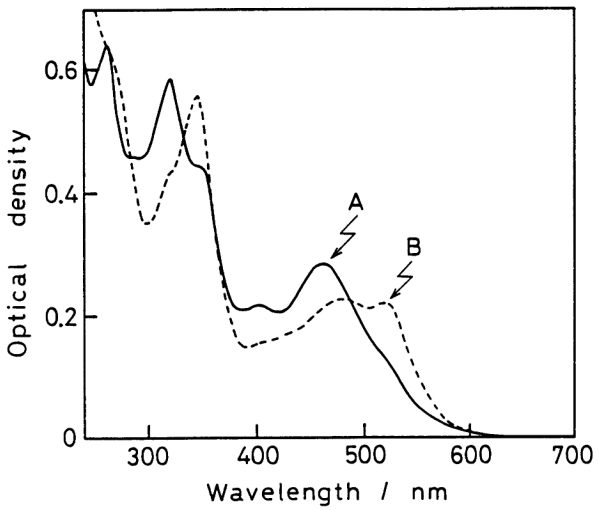
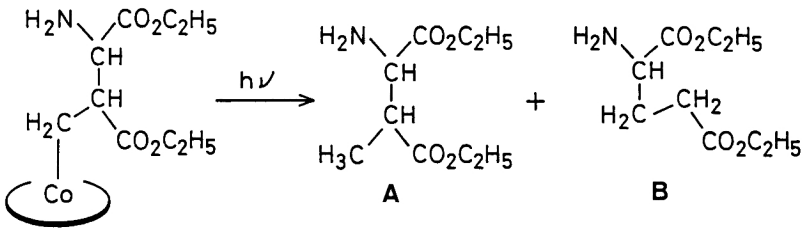


Fig. 1. Electronic spectral change for the aerobic photolysis of the alkylated hydrophobic vitamin B_{12} in dichloromethane at 20.0 ± 0.1 °C: A, $(CO_2C_2H_5)(NH_2)CHCH(CO_2C_2H_5)CH_2$-Cob(III)-7C$_3$ester ($2.7 \times 10^{-5}$ mol dm^{-3}); B, after irradiation with a 500-W tungsten lamp for 1 min from a distance of 10 cm.



(2)

Table 1. Product analyses for the photolysis of $(CO_2C_2H_5)(NH_2)$-$CHCH(CO_2C_2H_5)CH_2$-Cob(III)7C3ester in various media at 20 °C[a)]

Medium	Yield/% A	Yield/% B
Methanol	83 ± 5	0
Benzene	78 ± 2	Trace
$N^+C_5Ala2C_{16}$[b)]	66 ± 5	14 ± 3

a) A solution containing $(CO_2C_2H_5)(NH_2)CHCH(CO_2C_2H_5)CH_2$-Cob(III)-7C3ester ($5.0 \times 10^{-5}$ mol dm^{-3}) was irradiated with a 500-W tungsten lamp for 1 h from a distance of 30 cm under anaerobic conditions. Products were analyzed by GLC. b) $N^+C_5Ala2C_{16}$ (5.0×10^{-3} mol dm^{-3}) in phosphate—borate buffer (0.05 mol dm^{-3}, pH 9.2).

partate and sodium tetrahydroborate in reference to the method reported previously (Scheme 1, B).[6)] The alkylated complex showed an electronic spectrum characteristic of a complex with the cobalt—carbon bond and converted into the corresponding tervalent cobalt complex upon cleavage of the cobalt—carbon bond by aerobic photolysis (Fig. 1).

The reaction was carried out under anaerobic irradiation with the visible light in a manner as described previously,[3,4)] and the products, diethyl methylaspartate (A) and diethyl glutamate (B) (Eq. 2), were analyzed by GLC. The product analyses for the reaction in various media are summarized in Table 1. These analytical results indicate that the conversion of methylaspartate into glutamate takes place much favorably in the molecular aggregate of $N^+C_5Ala2C_{16}$. This is the first example of the glutamate mutase-like reaction as mediated by a vitamin B_{12} model system. On the other hand, the rearrangement product (B) was scarcely detected in homogeneous solutions of methanol and benzene.

In order to clarify the role of the molecular aggregate as the reaction site in such rearrangement process, the microenvironmental property around the hydrophobic vitamin B_{12} placed in the $N^+C_5Ala2C_{16}$ vesicle was evaluated by employing [(CN)Cob(III)7C3ester]ClO_4 coordinated at the residual axial site with a dansylhistamine, 2-(4-imidazolyl)-N-[5-(dimethylamino)-1-naphthalenesulfonyl]ethylamine, [[(CN)(DHN)Cob(III)7C3ester]ClO_4] as a fluorescent probe in a manner as described previously.[4)] The microscopic polarity is reflected on its fluorescence maximum. The $N^+C_5Ala2C_{16}$ vesicle provided a microenvironment for the dansyl moiety that is equivalent to the medium polarity (Z-value = 82 kcal mol^{-1}; 1 cal = 4.184 J) between methanol and ethanol at 20.0 ± 0.1 °C. Since the hydrophobic vitamin B_{12}

itself is placed in a microenvironment equivalent to dichloromethane in medium polarity in the vesicle (Z-value = 64 kcal mol^{-1}) on the basis of the absorption maximum of the hydrophobic vitamin B_{12},[1] the dansyl moiety seems to be placed in the vesicular surface closer to the bulk aqueous phase. Such a result indicates that not only the corrin ring of the hydrophobic vitamin B_{12} but also a hydrophobic fragment bound to the complex is much desolvated in the vesicle. In addition, the phase transition temperature for the vesicle observed by the differential scanning calorimetry did not undergo any change upon incorporation of the hydrophobic vitamin B_{12} into it. Thus, the hydrophobic vitamin B_{12} is not placed in the nonpolar interior domain composed of aliphatic double-chains but in the semipolar barrier domain composed of amino acid residues (so-called hydrogen belt). When [(CN)(DHN)-Cob(III)7C_3ester]ClO_4 was incorporated into the vesicle, large fluorescence polarization (P) values (0.10—0.23) were obtained for the temperature range of 40—5 °C. This apparently indicates that the molecular motion of the complex placed in the vesicle is markedly repressed, since the P-values in methanol and benzene were evaluated to be 0.005—0.006 and 0.02—0.04, respectively, for the identical temperature range. Thus, the microenvironmental effect provided by the vesicle is quite different from those by simple organic solvents which solubilize the complex homogeneously. Such behavior is quite analogous to that observed in the presence of an octopus cyclophane in place of the vesicle.[4] Consequently, the motional repression of the alkylated hydrophobic vitamin B_{12} in the vesicle must result in enhancement of the isomerization reaction.

The present study demonstrates the first successful example of an artificial glutamate mutase. The isomerization reaction accompanied with the carbon-skeleton rearrangement comes from both motional repression and desolvation effects operating on the alkylated cobalt complex in the vesicle. Although the migrating group in the enzymic reaction is known to be glycine,[7] we have not confirmed as yet which group does migrate in the present system, glycine or the ester group. In any case, however, it became clear that the vesicle is effective as an apoenzyme model for functional simulation of the vitamin B_{12}-dependent enzymes.

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