A Migrating Group in a Glutamate Mutase Model Reaction Mediated by a Functionalised Bilayer Membrane

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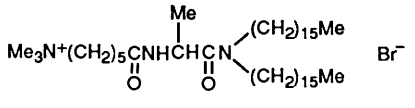
The alkylated hydrophobic vitamin B_{12}, prepared by the reaction of heptapropyl cobyrinate with diethyl β-bromomethyl-β-deuterio-DL-aspartate, affords diethyl γ-deuterioglutamate, the glycyl-migrated product, in the single-compartment vesicle of *N,N*-dihexadecyl-N^{α}-[6-(trimethylammonio)hexanoyl]-L-alaninamide bromide under anaerobic photolysis conditions.

Vitamin B_{12}-dependent enzymes catalyse various isomerisation reactions, and those accompanied by carbon-skeleton rearrangements are of particular interest in view of their unique catalytic features.[1] The naturally occurring apoproteins, which provide relevant reaction sites for vitamin B_{12}, are considered to play crucial roles in the isomerisation reactions.[2] In this context, we became interested in microenvironmental effects provided by single-compartment vesicles composed of synthetic peptide lipids, *e.g.*, *N,N*-dihexadecyl-N^{α}-[6-(trimethylammonio)hexanoyl]-L-alaninamide bromide, $N^{+}C_5Ala2C_{16}$.[3]

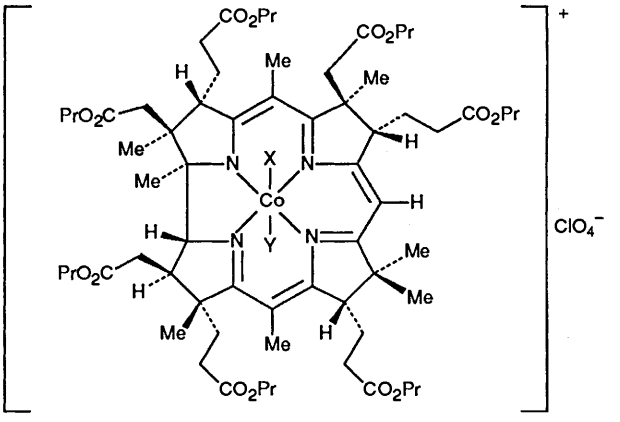


$N^{+}C_5Ala2C_{16}$

We have constructed an artificial holoenzyme composed of a hydrophobic vitamin B_{12} derivative and the $N^{+}C_5Ala2C_{16}$ bilayer vesicle,[4] and successfully carried out a model reaction, that mimics a catalytic function of glutamate mutase, without confirming the specific migrating group.[5] In the present work, we have clarified the primary migrating group in β-methylaspartate as mediated by an artificial glutamate mutase by utilising a deuteriated substrate.

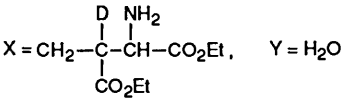
Diethyl β-bromomethyl-β-deuterio-DL-aspartate was prepared by a reaction of diethyl β-methylene-DL-aspartate with deuterium bromide in deuteriated acetic acid with reference to the method reported previously.[6,7] The corresponding alkylated complex, [{$(CO_2Et)(NH_2)CHCD(CO_2Et)CH_2$}$(H_2O)$Cob(III)7$C_3$ester]$ClO_4$, was prepared by a reaction of [Cob(II)-7C_3ester]ClO_4 with diethyl β-bromomethyl-β-deuterio-DL-aspartate and sodium tetrahydroborate after the previous method,[4] and purified by gel-filtration chromatography on a column of Sephadex LH-20 with methanol as eluent; yield 86%: (Found: C, 58.2; H and D, 7.65; N, 4.25. $C_{75}H_{118}DClCoN_5O_{23}$ requires C, 57.95; H and D, 7.8; N, 4.5%); $\lambda_{max}(CH_2Cl_2)$/nm 263, 295, 318sh, 410sh and 455; ν_{max}(KBr)/cm^{-1} 2950 (C–H str.), 1730 (ester C=O str.), 1100 and 620 (ClO_4^- str.).

The isomerisation reaction was carried out under anaerobic irradiation with visible light as follows: $N^{+}C_5Ala2C_{16}$ (1.54 g, 2.0×10^{-3} mol) was dispersed in an aqueous buffer (200 cm^3, phosphate–borate 0.05 mol dm^{-3}; pH 7.2) at room temperature by mechanical Vortex mixing, and the dispersion sample was sonicated at room temperature for 2 min with a probe-type sonicator at 30 W to give a single-compartment vesicle solution, which was subsequently allowed to stand at 0 °C for 5 min. After the solution was deoxygenated with argon gas, a methanol



[Cob(II)7C_3ester]ClO_4 : X = Y = none

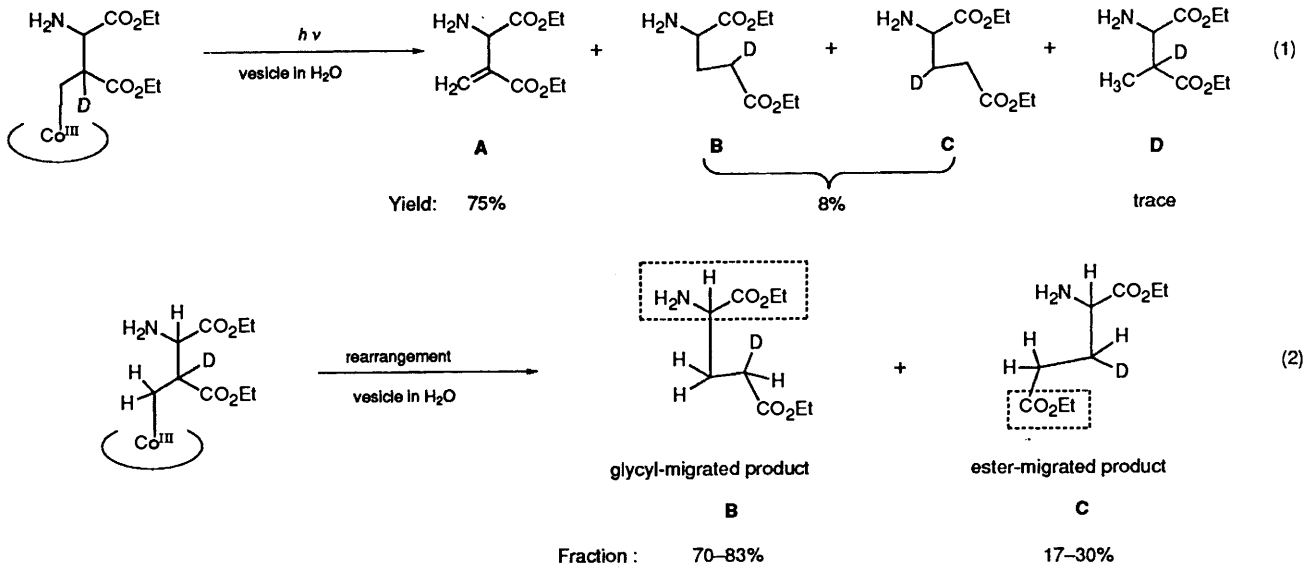
[{$(CO_2Et)(NH_2)CHCD(CO_2Et)CH_2$}$(H_2O)$Cob(III)7$C_3$ester]$ClO_4$:



solution (0.2 cm^3) of the alkylated complex (100 mg, 6.4×10^{-5} mol) was added to it, resulting in the following final concentrations: the alkylated hydrophobic vitamin B_{12}, 3.2×10^{-4} mol dm^{-3}; $N^{+}C_5Ala2C_{16}$, 1.0×10^{-2} mol dm^{-3}.† The resulting solution, maintained at 20.0 °C, was then irradiated with a 500 W tungsten lamp at a distance of 30 cm under argon atmosphere. After *ca.* 2 h the alkylated complex was completely decomposed (as confirmed by electronic absorption spectroscopy), and the products were extracted with dichloromethane (3×20 cm^3). The extract was dried (Na_2SO_4) and evaporated to dryness at room temperature. After $CDCl_3$ (0.6 cm^3) was added to the residue, the solution was evaporated at 50 °C under reduced pressure (below 10^{-3} mmHg). The evaporated fraction containing products and $CDCl_3$ was analysed by GLC and ^1H NMR spectroscopy.

Formation of diethyl β-methylene-DL-aspartate (**A**), diethyl glutamate (**B** and **C**) and diethyl β-methyl-DL-aspartate (**D**) was

† The reaction conditions are somewhat different from those employed in our previous work as regards final concentrations:[4,5b] the alkylated complex, 5.0×10^{-5} mol dm^{-3}; $N^{+}C_5Ala2C_{16}$, 5.0×10^{-3} mol dm^{-3}.



confirmed by GLC analysis,[4] as shown in eqn. (1).* Although diethyl glutamate is the rearrangement product, it was not previously determined which group, glycyl or carboxylic ester, does migrate in the mediator system. In this work, we determined the migrating group in the rearrangement reaction by 500 MHz ^{1}H NMR spectroscopy. Assignments of NMR signals for specific protons of diethyl glutamate were performed by means of the 2D-NMR technique: δ_H(500 MHz, $CDCl_3$) 3.46 (dd, C*H*NH_2), 2.46 (t, $CH_2CH_2CO_2C_2H_5$), 2.07 and 1.85 (each m, $CH_2CH_2CO_2C_2H_5$). The product ratio **B**/**C** was evaluated from ^{1}H NMR signal areas with the aid of a NMR1™ software (New Methods Research, Inc., U.S.A.) on a DEC 5000/200PX workstation. The result given in eqn. (2) indicates that the glycyl group migration predominates in the present artificial enzyme system. Note that the migrating tendency in the real enzymic system is completely specific for glycyl.

Dowd *et al.* observed that the β-methylaspartate–glutamate rearrangement took place readily *via* formation of a ketimine Schiff base derivative of diethyl β-methylaspartate, as mediated by vitamin B_{12}. On this basis, they suggested the possible formation of such a Schiff base intermediate in the corresponding enzymic reaction.[7,8] However, the glycyl group can migrate without the Schiff base formation in the synthetic bilayer membrane used as an apoenzyme model as described above, even though the yield is relatively low under the present conditions.

* Under previous conditions, the products were diethyl glutamate (14 ± 3%) and diethyl β-methyl-DL-aspartate (66 ± 5%).[5b] Because $N^+C_5Ala2C_{16}$ and the alkylated complex were used in higher concentrations in the present work, the major product turned out to be diethyl β-methylene-DL-aspartate.

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