A Synthetic Bilayer Membrane Functionalised with Hydrophobic Vitamin B_{12} as an Artificial Glutamate Mutase

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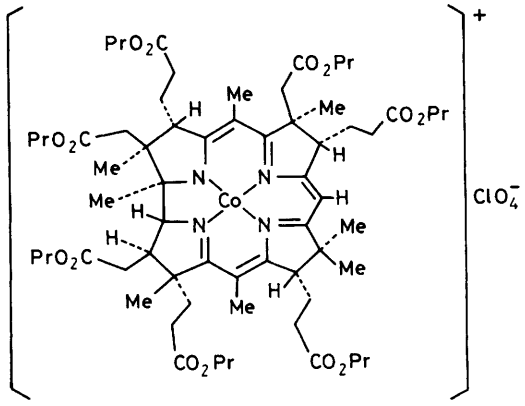
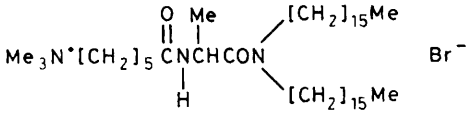
Heptapropyl cobyrinate perchlorate catalysed the conversion of diethyl β-methylaspartate into diethyl glutamate in single-compartment vesicles in the presence of vanadium trichloride as a co-catalyst under aerobic irradiation conditions.

Vitamin B_{12} exercises various catalytic functions when it is incorporated into diverse apoenzymes under physiological conditions.[1] The isomerization reactions, which result in carbon-skeleton rearrangements, are of particular interest from the viewpoints of synthetic organic and organometallic chemistry. Thus, clarification of the reaction mechanisms involved has become a challenging area of bioinorganic research, and relevant model reaction systems have been developed.[2] We have investigated the carbon-skeleton rearrangement reactions by electrochemical means, and have found that they proceed *via* anionic intermediates.[3] Scheffold *et al.* have utilized vitamin B_{12} derivatives and model cobalt complexes extensively as radical-generating reagents for syntheses of natural products.[4] Although no persuasive evidence has yet been provided for the catalytic role of coenzyme B_{12} in the enzymes concerned, it is generally considered that coenzyme B_{12} acts to convert a substrate into the corresponding radical species. The formation of such radical species can be readily achieved by photolysis of substrates bound to vitamin B_{12} by a cobalt–carbon bond, regardless of the real mechanism of action of coenzyme B_{12}-dependent enzymes.

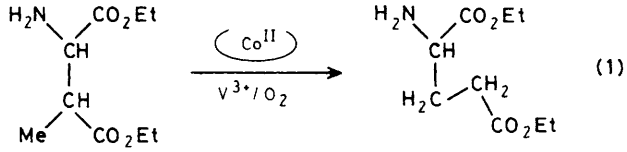
We have been interested in the catalytic activity of vitamin B_{12} in the hydrophobic microenvironments provided by the apoenzymes concerned. From this viewpoint, we have been dealing with artificial holoenzyme systems composed of hydrophobic vitamin B_{12} derivatives and relevant apoenzyme models.[5] We have reported that the β-methylaspartate skeleton is converted into glutamate in single-compartment vesicles *via* formation of a hydrophobic vitamin B_{12} derivative, produced by the reaction of heptapropyl cobyrinate in the Co^{I} state with diethyl β-bromomethylaspartate; the Co^{I} species was derived from heptapropyl cobyrinate perchlorate (**1**) by reaction with sodium tetrahydroborate.[6] The isomerization which accompanied carbon–skeleton rearrangement can be



(**1**)

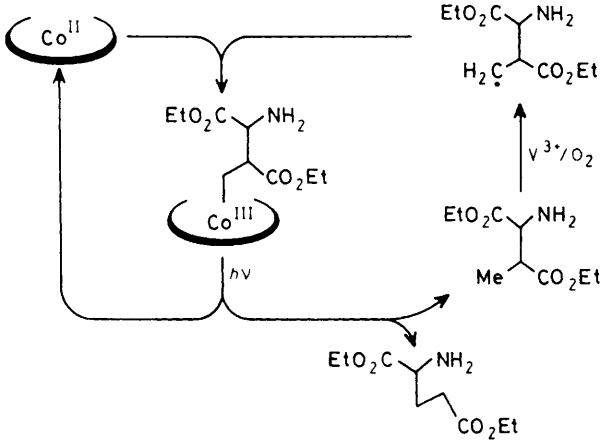


(2)



(1)

Scheme 1



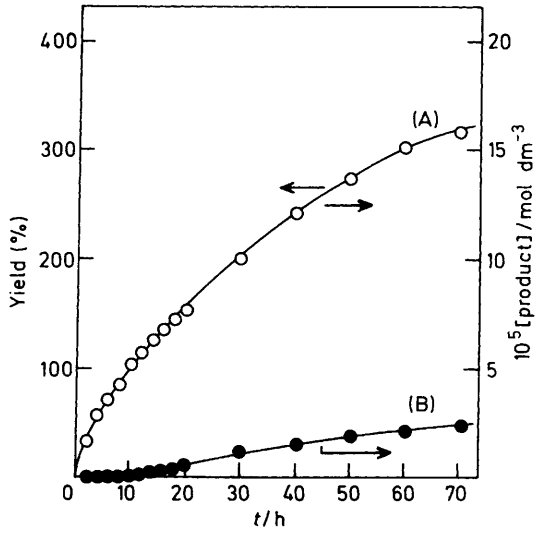


Figure 1. Conversion of diethyl β-methyl-DL-aspartate into diethyl glutamate in the vesicle (**2**) under aerobic irradiation conditions at 20 ± 2 °C: (A) [salt (**1**)] 5.0 × 10^{-5} mol dm^{-3} [yield based on the amount of (**1**); for detailed conditions, see text]; (B) without the hydrophobic vitamin B_{12} (**1**).

attributed to both motional repression and desolvation effects on the alkylated cobalt complex in the bilayer vesicles.[5,6] In the next stage of our investigation, we required an effective process for activation of substrates in order to set up a real catalytic system.

Schrauzer *et al.* developed a new method for synthesis of organocobalamins which employs both molecular oxygen and vanadium(III) ions as oxidising and reducing reagents, respectively.[7] In their method, a wide variety of non-activated organic compounds, including alkanes, can be used as substrates. We repeated this reaction with heptamethyl cobyrinate perchlorate (**1**) and ethyl L-alaninate in the dark. A methanolic solution (30 ml) containing the salt (**1**) (20 mg) and ethyl L-alaninate (10 mg) was mixed with aqueous sodium carbonate buffer (30 ml; 0.02 mol dm^{-3}; pH 11.2), and aqueous 15% (w/w) perchloric acid (5 ml) containing vanadium trichloride (10 mg) was added to the solution. The mixture was stirred vigorously for 5 min at room temperature, air was introduced into it for 2 min, and stirring was continued for a further 1 h. The product was extracted with dichloromethane. The corresponding alkylated complex was isolated as its perchlorate salt by gel-filtration chromatography on a column of Sephadex LH-20 with methanol (yield 20—40%).†

We adopted the same method to set up a true catalytic system for the glutamate mutase-like reaction shown in Scheme 1 [see also equation (1)]. Diethyl β-methyl-DL-aspartate (a mixture of *erythro-* and *threo*-diastereoisomers) was activated with vanadium trichloride and molecular oxygen; the corresponding alkylated complex formed as intermediate then underwent photolysis to afford the rearrangement and reduction products; the latter was re-cycled as substrate.

An aqueous dispersion (20 ml) containing *N*,*N*-dihexadecyl-N^{α}-[6-(trimethylammonio)hexanoyl]-L-alaninamide bromide (**2**) (3.0 × 10^{-3} mol dm^{-3}) was subjected to ultrasound for 2 min with a probe-type sonicator (30 W) at room temperature to give a clear solution of single-compartment vesicles. A methanolic solution (0.2 ml) containing the salt (**1**) (5.0 × 10^{-5} mol dm^{-3}) and diethyl β-methyl-DL-aspartate (3.0 × 10^{-3} mol dm^{-3}) was then added at room temperature. Aqueous 10% (w/w) perchloric acid containing vanadium trichloride (final concentration 0.10 mol dm^{-3}) was added to the resulting solution at room temperature; the final pH was 1.0. The mixture was irradiated with a 500 W tungsten lamp at a distance of 30 cm while air was bubbled through the mixture, maintained at 20 ± 2 °C. Samples were taken out at appropriate intervals for g.l.c. analysis. Diethyl β-methyl-DL-aspartate was converted catalytically into diethyl glutamate in the presence of the hydrophobic vitamin B_{12} derivative (**1**), as shown in Figure 1.‡ However, only a small amount of diethyl glutamate was detected when the reaction was performed without the hydrophobic vitamin B_{12} under otherwise identical conditions. The same catalytic system was successfully applied to conversions of other substrates; *e.g.* diethyl methylmalonate into diethyl succinate. Some control experiments were carried out as follows. Without vanadium trichloride, under otherwise identical conditions, no diethyl glutamate was obtained. In the absence of the single-compartment vesicle, in aqueous media, the hydrophobic vitamin B_{12} was insoluble and diethyl β-methyl-DL-aspartate was also difficult to dissolve. When the reaction was attempted in methanol or benzene without the vesicle-forming lipid, under otherwise identical conditions, no diethyl glutamate was detected by g.l.c.

† Satisfactory elemental analyses (C, H, and N) were obtained.

‡ The yield based on substrate was *ca.* 5% after 70 h.

In conclusion, an artificial glutamate mutase, composed of the cobyrinate derivative (**1**) and the vesicle (**2**), exhibited turnover catalytic behaviour by adopting an effective substrate-activation process.

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References

1 J. M. Pratt, *Chem. Soc. Rev.*, 1985, **14**, 161.
2 B. T. Golding, 'Mechanisms of Action of the B_{12} Coenzyme: Theory and Models,' in 'B_{12},' ed. D. Dolphin, Wiley, New York, 1982, vol. 1, pp. 543—582.
3 Y. Murakami, Y. Hisaeda, T. Ozaki, T. Tashiro, T. Ohno, Y. Tani, and Y. Matsuda, *Bull. Chem. Soc. Jpn.*, 1987, **60**, 311.
4 R. Scheffold, S. Abrecht, R. Orlinski, H.-R. Ruf, P. Stamouli, O. Tinembart, L. Walder, and C. Weymuth, *Pure Appl. Chem.*, 1987, **59**, 363.
5 Y. Murakami, Y. Hisaeda, J. Kikuchi, T. Ohno, M. Suzuki, Y. Matsuda, and T. Matsuura, *J. Chem. Soc., Perkin Trans. 2*, 1988, 1237.
6 Y. Murakami, Y. Hisaeda, and T. Ohno, *Chem. Lett.*, 1987, 1357.
7 A. Maihub, J. W. Grate, H. B. Xu, and G. N. Schrauzer, *Z. Naturforsch., Teil B*, 1983, **38**, 643.