Functionalised Bilayer Membranes Having Vitamin B_6 Activity as Artificial Tryptophan Synthase

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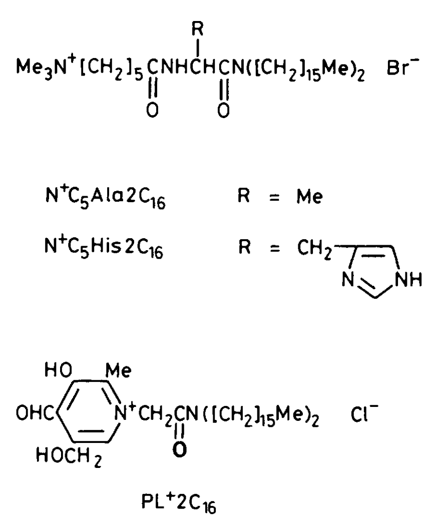
Synthetic bilayer vesicles having vitamin B_6 activity markedly enhanced the β-replacement reaction of serine with indole to afford tryptophan, showing turnover behaviour, in aqueous media under mild conditions.

Development of artificial enzymes having vitamin B_6 activity is an attractive subject in view of non-enzymatic syntheses of α-amino acids and their mutual transformations.[1] As we have reported recently, the single-walled bilayer vesicle formed with a synthetic peptide lipid, *N*,*N*-dihexadecyl-N^α-[6-(trimethylammonio)hexanoyl]-L-histidinamide bromide ($N^+C_5His2C_{16}$), and a hydrophobic vitamin B_6 derivative, 1-(*N*,*N*-dihexadecylcarbamoylmethyl)-2-methyl-3-hydroxy-4-formyl-5-hydroxymethylpyridinium chloride (PL^+2C_{16}), effectively catalyses the transamination reaction of α-amino acids with α-keto acids in the presence of copper(II) ions, exhibiting high substrate selectivity.[2] In the present work, the identical vesicular catalyst was found to behave as an artificial tryptophan synthase which produces tryptophan (Trp) by the β-replacement reaction of L-serine (L-Ser) with indole under mild conditions.

Snell *et al.* previously reported that a small amount of Trp, 0.6—0.8% yield based on the substrates, was obtained from Ser and indole (0.05 mol dm^{-3} each) in the presence of pyridoxal (PL) (0.02 mol dm^{-3}) and aluminium(III) ions



(0.002 mol dm^{-3}) under severe reaction conditions at 100 °C.[3] Recently, Breslow and his co-workers obtained Trp in a somewhat higher yield (still only a few percent) by using a pyridoxal-bound β-cyclodextrin and β-chloroalanine in place of PL and Ser, respectively, under otherwise identical conditions.[4]

In the present work, we evaluated the catalytic efficiency of the single-walled vesicle formed with PL^+2C_{16} and a synthetic lipid, *N*,*N*-dihexadecyl-*N*$^{\alpha}$-[6-(trimethylammonio)hexanoyl]-L-alaninamide bromide ($N^+C_5Ala2C_{16}$) or $N^+C_5His2C_{16}$ for the β-replacement reaction. The reaction was initiated by adding L-Ser to the vesicular solution containing indole at 30.0 °C, and products were analysed by h.p.l.c. on a column of TSK gel ODS-120T with methanol–aqueous Tris buffer (0.01 mol dm^{-3}, pH 8.0) (1 : 1 v/v) as eluant. The results are summarized in Figure 1. Under the present conditions, the extent of the β-replacement catalysed by PL in homogeneous aqueous solution is extremely low, even in the presence of copper(II) ions; yields of Trp based on PL^+2C_{16} for 210 h of incubation were 2.9 and 8.0% for metal-free and copper(II)-catalysed systems, respectively (Figure 1, lines A and B). On the other hand, the bilayer vesicle formed with PL^+2C_{16} and $N^+C_5Ala2C_{16}$ drastically accelerated the reaction (Figure 1, line C), presumably owing to an efficient attack of the hydrophobic indole molecule on the intermediate Schiff's base, (**2**) in Scheme 1, which is derived from the aldimine Schiff's base, (**1**) in Scheme 1, by β-elimination. The imidazolyl group of $N^+C_5His2C_{16}$ enhanced the reaction further because of its general acid–base catalysis (Figure 1, line E). Copper(II) ions exercised a co-ordination effect in a manner observed previously[2] and accelerated the reaction in all the present vesicular systems (Figure 1, lines D and F). As a whole, the bilayer catalyst composed of PL^+2C_{16}, $N^+C_5His2C_{16}$, and copper(II) ions exhibited the highest activity for the β-replacement reaction, showing turnover behaviour.

The direct hydrolysis of intermediate (**2**) afforded the β-elimination products, pyruvate and ammonia (Scheme 1), and the extent of the reaction was evaluated by h.p.l.c. analysis of the fluorescent 3-methylquinoxalin-2-ol, which was derived from pyruvate by reaction with *o*-phenylenediamine,[5] on a column of TSK gel ODS-120T with acetonitrile–aqueous tetrapropylammonium bromide (0.1 mol dm^{-3})–sodium phosphate buffer (0.1 mol dm^{-3}; pH 7.0)–water (5 : 4 : 18 : 13 v/v) as eluant. The results show that the β-replacement prevails over the β-elimination reaction in the vesicular system

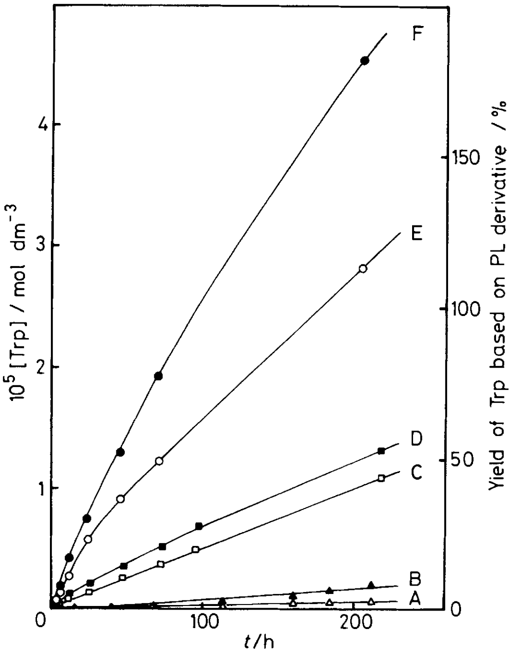
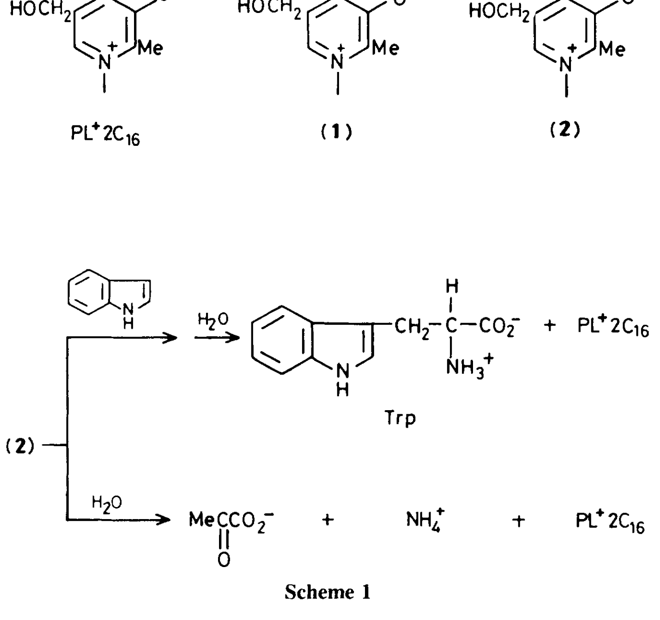


Figure 1. Time courses for β-replacement reaction of L-Ser (5.0×10^{-3} mol dm^{-3}) with indole (5.0×10^{-3} mol dm^{-3}) as catalysed by pyridoxal derivatives (2.5×10^{-5} mol dm^{-3}) in aqueous acetate buffer (2.5×10^{-2} mol dm^{-3}; μ 0.04 mol dm^{-3} with KCl) at pH 5.0 and 30.0 °C: A and B, PL; C and D, PL^+2C_{16} in $N^+C_5Ala2C_{16}$ (1.0×10^{-3} mol dm^{-3}); E and F, PL^+2C_{16} in $N^+C_5His2C_{16}$ (1.0×10^{-3} mol dm^{-3}). Ethylenediaminetetra-acetic acid (1.0×10^{-4} mol dm^{-3}) was added to A, C, and E. $Cu(ClO_4)_2$ (5.0×10^{-5} mol dm^{-3}) was added to B, D, and F.



Scheme 1

composed of PL^+2C_{16}, $N^+C_5His2C_{16}$, and copper(II) ions; the product ratio of Trp to pyruvate was 7:3 after 20 h of incubation under conditions identical with those for Figure 1. In contrast, the β-elimination reaction of Ser predominates relative to the β-replacement by indole in homogeneous aqueous solutions.[3,6]

In conclusion, it is apparent that the present functionalised bilayer membranes can be utilized as supramolecular catalyst assemblies for simulation of the enzymatic reaction catalysed by the vitamin B_6-dependent tryptophan synthase.

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