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Selective Transport of the Salts of Amino Acid Esters through an Organic Liquid Membrane with Antamanide as a Carrier

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Antamanide was prepared by the 9-fluorenylmethyloxycarbonyl (Fmoc)-based solid-phase method, and the transport of L-Phe–OMe and L-Leu–OMe through an organic liquid membrane mediated by the synthetic antamanide was examined. It was found that antamanide has an ability to transport L-Phe–OMe·HCl twice as efficiently as L-Leu–OMe·HCl. Affinity between the phenyl groups present in both the host and guest may be responsible for the difference.

Keywords——antamanide; ionophore; carrier; amino ester salt; selective transport; solid-phase method; cyclization

Many cyclic compounds, such as crown ethers, cryptands, polyamines and cyclic peptides are known to form complexes with cations.[1] For example, antamanide, *cyclo*(–Ala–$(Pro)_2$–Val–$(Phe)_2$–$(Pro)_2$–$(Phe)_2$–) isolated from extracts of the *Amanita phalloides*,[2,3] is a typical ionophore for Na^+, Li^+ or K^+.[4,5] In addition, chiral macrocycles exhibit chiral recognition in the process of complex formation with primary amine salts.[6]

We examined whether antamanide has an ability to form a complex with Phe–OMe, since there is a possibility that interaction may take place between the host molecule and a guest with an analogous Phe substituent. To examine this possibility, we conducted selective transport experiments through an organic liquid membrane[7] using antamanide as a host.

Antamanide was prepared by the solid-phase method,[8] followed by cyclization with water-soluble carbodiimide. As a guest compound, L-Phe–OMe was selected, and L-Leu–OMe was also used for comparison.

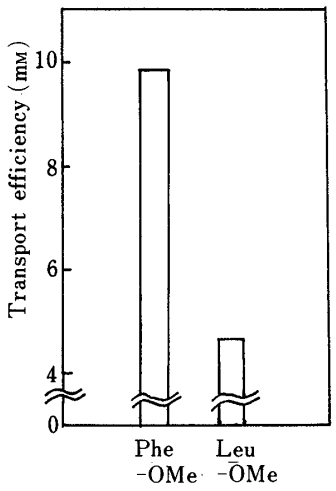

Fig. 1. Graphical Representation of Transport of L-Phe–OMe·HCl and L-Leu–OMe·HCl through a Liquid Membrane

Transport conditions: aqueous source phase, 0.1 M amino acid methyl ester hydrochloride and 0.2 M $LiPF_6$ in 2.5 ml of 0.08 M HCl; aqueous receiving phase, 2.5 ml of 0.1 M HCl; organic phase, antamanide (2.5 mM) in 10 ml of $CHCl_3$. The organic phase was stirred at 400 rpm with a magnetic stirrer at 25 °C for 24 h.

Transport of these amino acid methyl ester salts through a liquid membrane was measured using an apparatus described previously.[7] A mixture of an amino acid methyl ester salt and $LiPF_6$ in 0.08 M HCl aqueous solution was used as the source phase, and the ammonium cation transferred into chloroform by complexation with synthetic antamanide was released to a receiving phase of 0.1 M HCl aqueous solution. After 24 h, the concentration of transferred amino acid methyl ester salt in the receiving phase was determined by gas-liquid chromatographic analysis after converting the salt to the corresponding *N*-trifluoroacetyl (Tfa) ester derivative. The net values of transport, *i.e.*, average values of the differences between apparent and blank test transport in several runs, are shown in Fig. 1.

In these comparative experiments, it was found that antamanide has an ability to transport L-Phe–OMe·HCl twice as efficiently as L-Leu–OMe·HCl. Affinity between the phenyl groups present in both the host and guest may be responsible for the difference.

Experimental

The melting point of antamanide was measured with a Yanaco MP-S3 apparatus and is uncorrected. Fast atom bombardment mass spectra (FAB-MS) were recorded on a Nihon Denshi mass spectrometer, model JMS DX-303, JMS FAB-09, with a JMS DA-5000 computer system. For measurement, each sample was dissolved in a matrix of glycerol and the solution was bombarded with a beam of neutral Xe atoms at an energy of 3 keV.

Synthesis of Antamanide——Antamanide[2] was synthesized by cyclization of a linear decapeptide, H–Ala–Pro–Pro–Val–Phe–Phe–Pro–Pro–Phe–Phe–OH (**1**),[9] which was prepared by the Fmoc-based solid-phase method.[8] Fmoc–Phe–OH was loaded onto the 4-hydroxymethylphenoxyacetylpolyacrylamide resin[10] (2.0 g) by the pentafluorophenyl ester (Pfp) method[11] using a catalytic amount of 4-dimethylaminopyridine. This Fmoc–Phe–resin (Phe content, 0.04 mmol/g) was placed in a reaction column and the peptide chain was elongated to the decapeptide corresponding to the entire amino acid sequence of linear antamanide by using an automated LKB-4170 peptide synthesizer. Each synthetic cycle consisted of (i) deprotection with 20% piperidine in dimethylformamide (DMF, 10 min) and (ii) coupling with Fmoc–amino acid Pfp in the presence of 1-hydroxybenzotriazole monohydrate (HOBt) (0.5 mmol each, 6.25 eq) in DMF for 1 h. The final peptide resin was treated with trifluoroacetic acid (TFA)–H_2O (95 : 5, 20 ml) at room temperature for 2 h, then the solution was filtered. After evaporation of TFA *in vacuo*, the residue was dissolved in H_2O and lyophilized to give **1**·TFA as a powder. FAB-MS *m/z*: 1165 $[(M+H-TFA)^+]$.

Cyclization of **1**: An ice-chilled solution of **1**·TFA (0.11 g), *N*-methylmorpholine (0.02 ml) and HOBt (0.13 g) in anhydrous DMF (100 ml) was added to 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (0.16 g) in anhydrous DMF (200 ml) over a period of 3 h, then the mixture was stirred at room temperature for 10 d. The solvent was removed by evaporation *in vacuo*, and the residue was treated with a small amount of ethyl acetate and 10% citric acid. The resulting powder was collected by filtration, washed with 10% citric acid, 10% sodium bicarbonate and water, and recrystallized from acetone–water; yield 64.3 mg (65.5%), mp 172—173 °C (lit.[12] 169—171 °C), FAB-MS *m/z*: 1147 $[(M+H)^+]$, $[\alpha]_D^{22}$: −142° (*c* = 1, methanol) (lit.[13] $[\alpha]_D^{20}$: −148° (*c* = 1, methanol)).

Transport of L-Phe–OMe·HCl and L-Leu–OMe·HCl——As reported,[7] a glass tube (1.6 cm i.d.) was placed in a cylindrical tube (2.6 cm i.d.) to separate two aqueous phases. The outer source phase contained 0.1 M amino acid methyl ester hydrochloride and 0.2 M $LiPF_6$ in 2.5 ml of 0.08 M HCl and the inner receiving phase contained 2.5 ml of 0.1 M HCl. The organic phase, consisting of 2.5 mM antamanide in 10 ml of $CHCl_3$, was placed at the bottom of the cylindrical tube and stirred at 400 rpm with a magnetic stirrer at 25 °C. After 24 h, an aliquot of the receiving phase (0.5 ml) was withdrawn, and lyophilized. The residue was dissolved in TFA (0.2 ml), and then trifluoroacetic anhydride (0.5 ml) was added. The solution was permitted to stand at room temperature for 1.5 h, and then evaporated *in vacuo* at about 15 °C. The oily residue was dissolved in ethyl acetate (1 ml), and subjected to gas liquid chromatographic analysis using a Shimadzu GC-7AG apparatus (hydrogen flame ionization detector) equipped with a column (l = 1.6 m, i.d. = 3 mm) of 2% cyclohexanedimethanol succinate on Gas Chrom Q (80—100 mesh) which was developed with N_2 as a carrier gas at a flow rate of 50 ml/min. Quantitative analysis was performed by using *n*-paraffin as an internal standard (*n*-docosane for Tfa–Phe–OMe, *n*-octadecane for Tfa–Leu–OMe).

References and Notes

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