Facilitated transport of amino acids by fixed-site jumping

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Amino acids are transported with high fluxes through plasticized cellulose triacetate membranes containing large amounts of quaternary tetraalkylammonium salts; similar results are obtained using analogous polymer-supported liquid membranes.

The main aim in facilitated transport research is to develop selectively permeable, high-flux membranes with long-term durability. A major impediment to the industrial exploitation of this technology is the problem of membrane instability.[1] Recently, we discovered that plasticized cellulose triacetate (CTA) membranes containing large amounts of trioctylmethylammonium chloride (TOMAC) are selectively permeable to small, neutral carbohydrates.[2] The plastic films, which were originally designed as anion exchange membranes,[3] exhibit high saccharide fluxes and high membrane stabilities. Mechanistic studies produced evidence in favour of a fixed-site jumping mechanism, where the TOMAC acts as fixed 'stepping stones' and the sugar permeates through the membrane by jumping from one fixed-site to another.[2,4] These findings raise the possibility that facilitated transport through plasticized membranes by fixed-site jumping is a general way of improving membrane stability while still retaining high and selective permeability. As a consequence we have investigated the facilitated transport of other hydrophilic solutes through lipophilic CTA membranes. This report focuses on the neutral amino acids, phenylalanine, leucine and alanine.

Previous work has shown that liquid organic membranes incorporating TOMAC act as anion exchange membranes for amino acids at basic pH.[5,6] Under these conditions (relatively low concentration of TOMAC dissolved in the liquid membrane), transport occurs by membrane-diffusion of lipophilic ion-pairs of TOMA cation and amino acid anion.[7] At neutral pH very little of the amino acid is present in its anionic form, and transport enhancement is very weak.[8,9] We now report that amino acid transport can be greatly enhanced if the membrane contains enough TOMAC to induce fixed-site jumping.

Two related membranes were examined: plasticized CTA films and polymer-supported liquid membranes.† The plasticized films were prepared by evaporating a chloroform solution of CTA, 2-nitrophenyl octyl ether (2-NPOE) and TOMAC in a flat-bottomed glass petri dish.[3] The resulting plastic film (*ca.* 50 μm thick, depending on composition) was peeled off, trimmed and clamped into a cylindrical transport cell.† The supported liquid membranes were solutions of TOMAC dissolved in 2-NPOE that were absorbed by thin sheets of microporous polypropylene (Celgard 2500™, 25 μm thick). A selection of the transport fluxes observed with the plasticized CTA films is listed in Table 1. Even at neutral pH, the amino acid fluxes are very high, whereas the membranes are only weakly permeable to metal cations and hydrophilic organic cations such as dopamine.

Table 1 Non-competitive transport through cellulose triacetate membranes at neutral pH

Solute[a]	Flux/10^{-8} mol m^{-2} s^{-1} TOMAC present[b]	TOMAC absent[c]
L-Phenylalanine	1590	0.12
L-Leucine	520	0.12
L-Alanine	290	0.76
Dopamine	25	0.31
NaCl[d]	12	0.81

[a] Initially source contained 100 mM solute. Both aqueous phases contained 100 mM sodium phosphate, pH 7.3, T = 298 K. [b] Membrane: 0.10 g CTA, 0.20 g 2-NPOE, 0.20 g TOMAC. [c] Membrane: 0.10 g CTA, 0.20 g 2-NPOE. [d] Source: 100 mM NaCl in deionised water; receiving: deionised water.

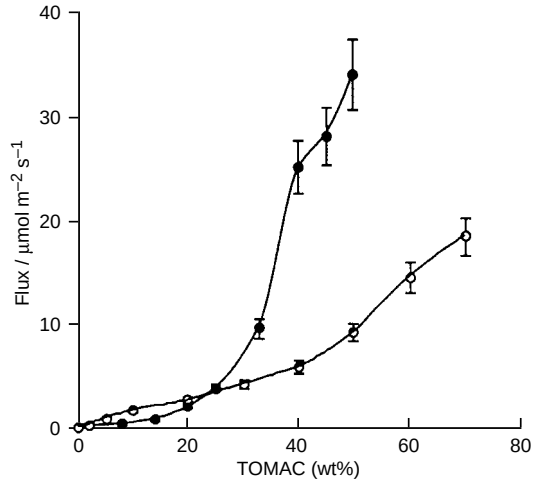

Fig. 1 Initial phenylalanine flux *versus* wt% TOMAC in (●) plasticized membrane that also included CTA (0.1 g) and 2-NPOE (0.2 g) and (○) liquid membrane of 2-NPOE supported by a 25 μm thick sheet of Celgard 2500™. Both aqueous phases contained sodium phosphate (60 ml, 100 mM, pH 7.3), T = 298 K. The source phase also contained phenylalanine (100 mM). To account for changes in flux due to changes in membrane thickness, the fluxes have been multiplied by the ratio (membrane thickness in presence of TOMAC)/(membrane thickness in absence of TOMAC).

Plots of phenylalanine flux *versus* wt% of TOMAC for both membranes with aqueous phases at neutral pH are shown in Fig. 1. The profiles do not exhibit the linear relationship that is typically found with transport systems that operate by carrier-diffusion.[4,12] Rather the profiles are non-linear, with the flux increasing dramatically at high TOMAC levels. This is indicative of a transport process that uses fixed-site jumping (Fig. 2).[4] In the case of the CTA films there is clear evidence of a percolation threshold at around 20% TOMAC. We postulate that below the threshold concentration, flux is negligible because the distance between the relatively immobile quaternary ammonium sites is too great (> *ca.* 15 Å) to allow solute

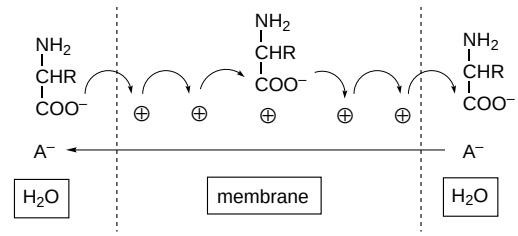

Fig. 2 A transport process using fixed-site jumping

jumping and the high membrane viscosity greatly inhibits transport by carrier-diffusion. In the case of the supported liquid membrane, the flux *versus* percentage of TOMAC is first-order until about 40% TOMAC and then increases at a higher power. The difference in curve shape is attributed to a change in the relative contributions of the two transport pathways, *i.e.* carrier-diffusion at lower TOMAC concentrations and fixed-site jumping at higher TOMAC levels. The lower viscosity associated with a liquid membrane makes carrier-diffusion a more favourable pathway compared to a plasticized membrane, and the TOMAC concentration has to reach 40% before any flux enhancement due to fixed-site jumping is observed.

Amino acid flux through the CTA films is dependent on membrane thickness. Stacking multiple membranes together or preparing a single membrane of additional thickness lowers the flux in a linear fashion. Additional transport mechanism studies are ongoing, however, three pieces of evidence implicate an anion exchange process. (i) When the source phase but not the receiving phase contains sodium phosphate (100 mM, pH 7.3), phenylalanine flux is very low, but when the situation is reversed, (*i.e.* receiving phase contains sodium phosphate and the source phase does not) the flux is quite high. (ii) A plot of phenylalanine flux as a function of pH shows very low flux at pH < 5.5 (the isoelectric point for phenylalanine) and dramatically increasing flux after pH 5.5. At pH 10 the flux is five times higher than at pH 7.3. (iii) Changing the TOMA counter-anion from chloride to phosphate leads only to a 30% drop in initial flux, whereas changing the counter-anion to lipophilic bis(2-ethylhexyl)phosphate results in complete loss of phenylalanine permeability.

Previous work by others has shown that a crown–boronic acid mixture is a better transporter of phenylalanine through bulk, liquid membranes than TOMAC at neutral pH.[8] With CTA films containing high concentrations of transporting agent we find that TOMAC is superior. It appears that the crown–boronic acid mixture uses a carrier-diffusion process and, unlike TOMAC, does not switch to a fixed-site jumping mechanism at high membrane concentrations. A similar conclusion was recently reached by Lamb concerning metal cation transport mediated by crown carriers.[12] We have also examined the facilitated transport of dopamine through plasticized membranes using crown–boronic acid mixtures that are known to be effective dopamine carriers in supported liquid membranes.[13] Again there is no evidence for the onset of fixed-site jumping at high carrier concentrations. Thus, it remains to be seen if this remarkable transport effect, which we attribute to fixed-site jumping (*i.e.* the solute is rapidly relayed along a sequence of relatively immobile carriers), can be duplicated with transport agents other than quaternary alkylammonium salts.[4]

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Footnotes and References

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† The transport cell consisted of two identical, water-jacketed cylindrical halves (half-cell volumes 60 ml, membrane area 16 cm^2) that were stirred by turbines which in turn were driven by externally situated magnets (ref. 10). The side of the plastic film exposed to the air during the evaporation process was placed facing the source solution. Amino acid concentrations were determined by ninhydrin assay (ref. 11). Phenylalanine concentrations were also monitored spectrometrically at $\lambda = 262$ nm ($\varepsilon = 139$ l mol^{-1} cm^{-1}) as were the dopamine levels ($\lambda = 279$ nm, $\varepsilon = 2675$ l mol^{-1} cm^{-1}). Metal cation concentrations were determined by conductivity or inductively coupled plasma emission. All CTA films were prepared from CTA (0.10 g) and 2-NPOE (0.20 g). In the absence of TOMAC the films were 30 μm thick. Addition of TOMAC thickened the films up to 75 μm for a membrane containing 50 wt% TOMAC.

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