Transport of D-Glucosamine through a Supported Liquid Membrane System Using Lipophilic Aldehyde as a Carrier

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Synopsis. Transport of amino compound relevant to biology, D-glucosamine, was carried out with a supported liquid membrane using a lipophilic aldehyde, 4-octadecyloxybenzaldehyde, as a carrier. In the present membrane transport system, a reversible organic reaction, Schiff base formation reaction, between amino group in D-glucosamine and aldehyde group in carrier was utilized as a molecular recognition reaction.

Transport of neutral substrates relevant to biology is an interesting and important subject from a viewpoint of both the simulation of a biological system and the development of a novel separation technique for such compounds. There were, however, few successful approach to artificial membranes for transport of neutral compounds.[1-7] The authors gave attention to Schiff base formation reaction as a molecular recognition reaction for amino compounds or compounds with aldehyde moiety. In the present article, transport of D-glucosamine through a supported liquid membrane containing 4-octadecyloxybenzaldehyde was investigated.

Experimental

Transport Experiments. The experimental procedures are described elsewhere in detail.[5-7] A porous polymer membrane, Ultipor N_{66} (Pall Trinity Micro Corp., 1.25×10^{-2} cm thickness, 80% porosity, 0.2×10^{-4} cm pore size) was employed for the support membrane. The transport experiments were carried out at 25 °C. The change in the concentration of D-glucosamine (GlcN) on the R-side was measured by Elson-Morgan reaction.[8]

Results and Discussion

Time Dependence of GlcN Transport. An example of the time course change of the transport is shown in Fig. 1. After the induction period (Stage I), the GlcN concentration increased linearly for 2.5 h (Stage II). At around t=5.5 h, the slope of the straight line became smaller (Stage III). From this profile, the straight line in Stage II was thought to be the flux mediated by ODBA and that in Stage III as the flux in the stage that ODBA was inactivated and scarcely showed an ability to mediate the GlcN transport. This inactivation of ODBA is estimated to be due to the fission of the formed Schiff base in the incorrect position, which is different from -CH=N- linkage, as is well-known in the catalytic reaction of pyridoxal-enzyme.

In order to ascertain in the speculation for Stage III, the control experiments were carried out. In Fig. 2, the flux value in Stage III for each transport experiment and the fluxes for control experiments, are shown as a function of GlcN concentration on the L-side. This led to the conclusion that the transport flux for Stage III in Fig. 1 is due to the non-mediated transport, and that for Stage II is the sum of fluxes due to the non-mediated transport and the mediated one.

Transport experiments of D-glucose (Glc), which has no amino group in the molecule, were carried out through the membrane containing ODBA and that without ODBA under the condition that the concentration of ODBA in the membrane was 1.5×10^{-5} mol cm^{-3},

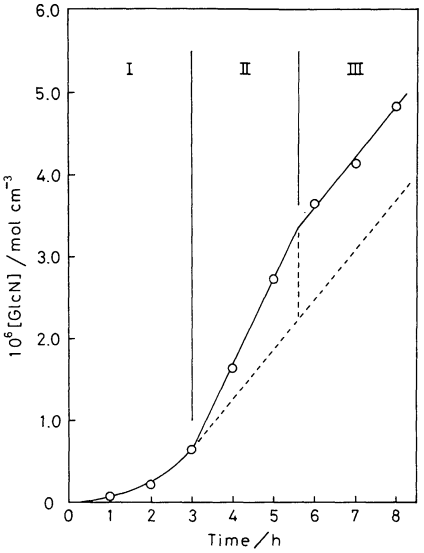

Fig. 1. Time-transport curves of GlcN through the liquid membrane. ($[C]_0=1.0\times10^{-5}$ mol cm^{-3}; $[GlcN]=7.5\times10^{-5}$ mol cm^{-3}).

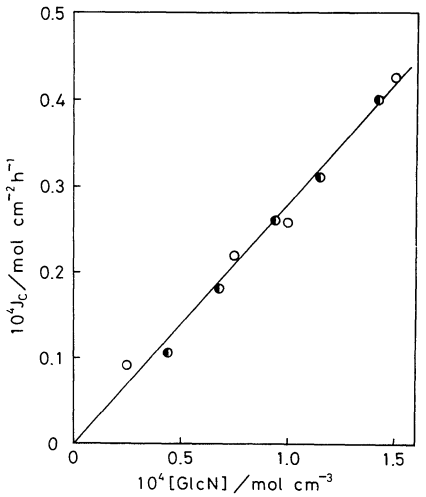

Fig. 2. Relationship between non-mediated flux and GlcN concentration. (O, $[C]_0=0$ mol cm^{-3}; ◐, $[C]_0=1.0\times10^{-5}$ mol cm^{-3}; the line was calculated employing the value $P=3.49\times10^{-3}$ cm^2 h^{-1}).

that of Glc on the L-side was 1.0×10^{-4} mol cm^{-3}, and the other conditions were similar to those for GlcN transport. The concentration of Glc on the R-side was measured by the phenolsulfuric acid reaction.[9,10] Fluxes for both transport experiments were found to be equal values of 5.89×10^{-5} mol cm^{-2} h^{-1}. This also suggests that an amino group in the transported substrate contributes to the facilitated transport of amino compounds through the present membrane.

Concentration Dependence of GlcN Transport. In order to study the transport mechanism, the relationship between GlcN concentration on the L-side and GlcN flux transported from the L-side to the R-side, and the effect of ODBA concentration on GlcN transport were investigated. The results are summarized in Fig. 3. Each transport experiment, of which ODBA concentration was 1.0×10^{-5} to 2.0×10^{-5} mol cm^{-3}, gave a straight line passing through the origin. The slopes of the straight lines increased with the increase of ODBA concentration in the membrane.

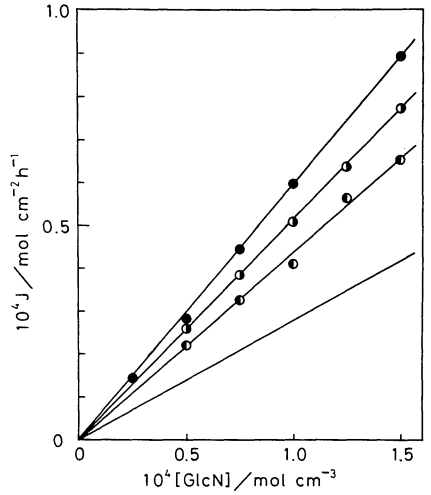

Fig. 3. Effect of GlcN concentration on fluxes. (—, non-mediated flux; -◐-, $[C]_0=1.0\times10^{-5}$ mol cm^{-3}; -◑-, $[C]_0=1.5\times10^{-5}$ mol cm^{-3}; -●-, $[C]_0=2.0\times10^{-5}$ mol cm^{-3}; the lines were calculated employing the values $P=3.49\times10^{-3}$ cm^2 h^{-1} and $kK=1.98\times10^2$ mol^{-1} cm^5 h^{-1}).

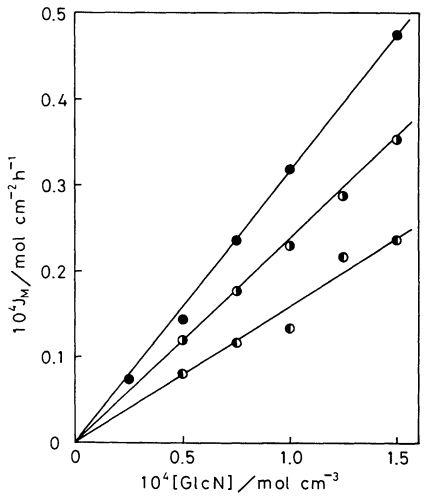

Fig. 4. Relationship between carrier-mediated flux and GlcN concentration. (◐, $[C]_0=1.0\times10^{-5}$ mol cm^{-3}; ◑, $[C]_0=1.5\times10^{-5}$ mol cm^{-3}; ●, $[C]_0=2.0\times10^{-5}$ mol cm^{-3}; the lines were calculated employing the values $P=3.49\times10^{-3}$ cm^2 h^{-1} and $kK=1.98\times10^2$ mol^{-1} cm^5 h^{-1}).

Figure 4 shows the effect of GlcN concentration on the mediated flux, which is the total flux minus the non-mediated flux. In a general mediated transport, the flux reaches an assymptotic limit as the substrate concentration in the feed is increased: that is, a typical Michaelis–Menten profile represented by Eq. 1 is observed.

$$J_M = (k/l)(K[C]_0[GlcN])/(1 + K[GlcN]) \quad (1)$$

where k is the apparent diffusion coefficient, l is the membrane thickness, K denotes the complex formation constant between OBDA and GlcN, $[C]_0$ is the carrier concentration in the membrane, and [GlcN] denotes the GlcN concentration on the L-side. In Fig. 4, however, such a saturation profile was not observed and we can conclude that K[GlcN] is negligibly small compared with unity. Therefore, the mediated flux in this case is expressed by

$$J_M = (kK/l)[C]_0[GlcN] \quad (2)$$

Equation 3 can be derived from Eq. 2 in order to obtain the constant value of kK.

$$J_M/[GlcN] = (kK/l)[C]_0 \quad (3)$$

The data in Fig. 5 showed a straight line and the constant value of kK was determined to be 1.98×10^2 mol^{-1} cm^5 h^{-1} from the slope. For reference, the permeability coefficient for non-mediated flux of GlcN was evaluated to be 3.49×10^{-3} cm^2 h^{-1} ($J_C=(P/l)$[GlcN]).

Transport Mechanism. In the present membrane transport system, the transport mechanism of GlcN with ODBA can be thought to be similar to that of tryptophan (Trp) previously reported.[5-7] The tentative

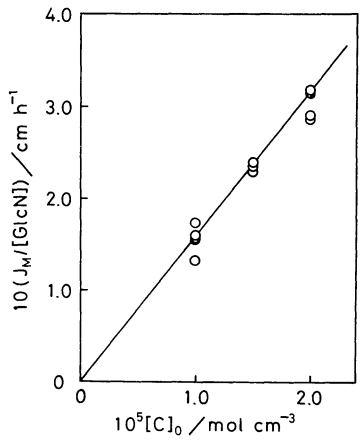

Fig. 5. Relationship between $J_M/[GlcN]$ and $[C]_0$.

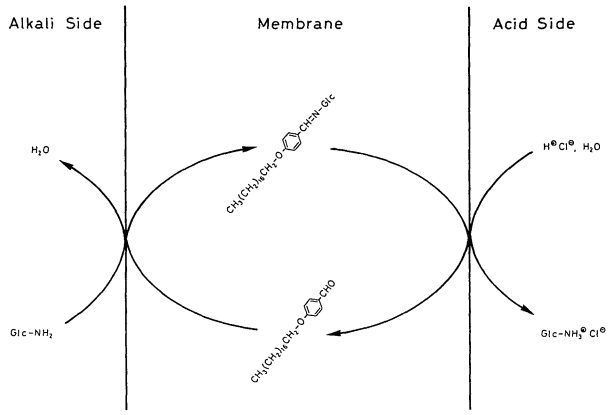

Fig. 6. Tentative mechanism for transport of GlcN.

mechanism is shown in Fig. 6. At the L-side-liquid membrane (LM) interface, GlcN interacts with carrier (ODBA), and the formed complex is extracted into LM. GlcN-ODBA (carrier) Schiff base complex diffuses across LM, and at the R-side-LM interface, it is decomposed to release GlcN into the R-side. Regenerated carrier, ODBA, then diffuses back to the L-side-LM interface and the process is repeated. In the present study, the turnover number for each transport experiment was 330—470.

An amino group in GlcN released into the R-side is impossible to be converted to Schiff base with carrier. GlcN transported to the R-side, as a result, could not be back-transported. Namely, the presence of proton (HCl) on the R-side led to the determination of transport direction.

The present membrane transport system should be applicable to other amino compounds, and moreover, uphill transport of such organic compounds may also be attained.

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