A CONCEPTUAL SYNTHETIC MEMBRANE SYSTEM FOR ACTIVE TRANSPORT

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ABSTRACT: An active transport system with a synthetic membrane has been proposed. The system that effectuates the transport is composed of (1) host molecules of which association constants with a specific substrate can be reversibly changed by photo-irradiation, a change in pH or temperature control, and (2) switching valves on both surfaces of the membrane. This study shows that an active transport can be achieved from successive passive transport steps and the energy for the transport is simply consumed as those for a change in the association constant of the host molecule, for opening and closing of switching valves and for the control of switching valves (i.e., vectorial control). Active transport, which has been considered to be a complicated biological system, is mimicked by a simple hybrid synthetic membrane system (i.e., hybrid-transport theory).

1. INTRODUCTION

Active transport [1], which is endergonic transport in a plasma membrane, is a characteristic feature in biological systems. Reconstitution of substrate-transport systems into liposomes has been successfully devised in recent years. D-glucose transporter from human erythrocytes [2], Ca^{2+}-ATPase from sarcoplasmic reticulum [3], and amino acid transporter [4-7] were incorporated into lipid vesicles for the reconstitution by several investigators [2-7]. Most of these researches, which are mainly studied by biochemists and biophysicists, focused on the solubilization, reconstitution and purification of transporter or kinetic investigations of substrate uptake. Basic mechanisms of active transport are still not clear and can not be applied for the construction of an active transport of substrates with synthetic membrane systems, since e.g., the control of flip-flop movement of proteins is rather difficult in such systems and transport of a large quantity of specific substrates is required with synthetic membranes.

Ion channels [8,9], uptake of ions [10-12] and facilitated transport [13-16] in synthetic membranes were extensively studied as a replication of transport in living membranes. Photo-modulated ion channels based on covalent coupling of two gramicidin A monomers were studied by Stankovic et al [8]. They found that the trans-isomer formed characteristic ion channels into a new flickering channel type. The transport mechanism was, however, passive transport in this case.

The transport of ions against their concentration gradient (uphill transport) has been studied in synthetic membranes [17,18]. The ion flux of uphill transport across the synthetic ion-exchange membrane was explained in the past as active transport by a proton jump mechanism and by ring opening/closing reactions of the carrier. The mechanism of the uphill transport was, however, explained by a simple transport theory in conjunction with Donnan equilibrium and Goldman's or Henderson's assumption by Higa et al [19].

The characteristics of active transport are vectorial movement and high specificity of substrate and endergonicity (i.e., flux is not governed by electrochemical potential gradient) [20].

An active transport system composed of synthetic membrane is conceptually developed in this study. The membrane that effectuates active transport is assumed to consist of (1) host molecules of which association constants with a specific substrate can be
changed by photo-irradiation, a change in pH or temperature control, and (2) switching valves on both surfaces of the membrane. The present study shows that an active transport can be achieved by two successive passive transports intervened in by the change in the association constant of the host. The energy is simply consumed as those for a change in the association constant of the host molecules, for opening and closing of switching valves and for control of switching valves (i.e., vectorial control). Biological active transport, which has been considered to be a complicated system, can be mimicked by a simple hybrid synthetic membrane system (i.e., hybrid-transport theory).

2. THEORY

Fig. 1 shows a model of the present system for active transport in the synthetic membranes. The active transport of substrates is performed using a permeation cell which consists of two chambers separated by a membrane. The cell volume of side 0 and 1 is \( V_0 \) and \( V_1 \), and the concentration in \( V_0 \) and \( V_1 \) is \( C_0 \) and \( C_1 \), respectively. Membranes considered in this study consist of (1) host molecules of which association constants with the specific substrate can be reversibly changed by photo-irradiation, pH change or temperature control, and (2) switching valves (SW0 and SW1, see Fig. 1) on membrane surfaces. The volume of the membrane is \( V_m \), and the concentration of the host molecules in the membrane is \( C_H \). The association constants defined by Eq. 1 are \( K_{on} \) or \( K_{off} \), depending on the controlling conditions (e.g., under photo-irradiation or non-irradiation).

\[
K_i = \frac{C^B_i}{C^F_i + C^B_i} \quad (i = \text{on or off}) \quad (1)
\]

where \( C^F_i \) and \( C^B_i \) are the concentration of free host and free substrate in the membrane, respectively. \( C^B_i \) is the concentration of bound substrates to the host molecules.

In order to emphasize the essential points and deal with an ideal system, the starting assumptions are as follows: (a) the active transport of substrates is generated by initial step and series of four step (i.e. initial step and steps A, B, C and D), (b) concentration of substrates is in equilibrium between solution and membrane on the side where the switching valve is open (i.e. time interval is sufficiently long during each step) and (c) solubility, \( S \), is defined by \( C^F_n = S \cdot C_i(n) \), when the switching valve on side \( i \) (i.e., 0 or 1) is open. \( C_i(n) \) is the concentration of the external solution at side \( i \) (i.e., 0 or 1) in step \( n \). \( C^F_n \) is the concentration of free substrates in the membrane for step \( n \). (d) A host molecule has only one site to bind a substrate.

Each step in the transport of substrates is schematically demonstrated in Fig. 2. There are five steps in Fig. 2. The first step is the initial condition. Steps A, B, C and D are determined from the open or closed conditions of the switching valves on side 0 and 1, and the value of association constant. It is considered that the active transport is achieved from passive transport in each step. Cycle number which consists of a series of four step (i.e. steps A, B, C and D) is defined to be \( m \). \( C_0(n) \) and \( C_1(n) \) for each step are calculated as follows.

(i) initial condition \( (n = 0 \) and \( m = 0 \))

The switching valves on both sides are closed in this step.

\[
C_0(0) = C_0^i \quad (2)
\]

\[
C_1(0) = C_1^i \quad (3)
\]

where \( C_0^i \) and \( C_1^i \) are the initial concentration of substrates for cells in the external solution of side 0 and 1, respectively.

\[
C^F(0) = 0 \quad (4)
\]
Fig. 2. Computational procedures of active transport at $K_{on} = 0$, $K_{off} > 0$, $S = 1$ and $C_{0}(0) = C_{1}(0)$.

(ii) step A ($m = m + 1, n = 4m - 3$)

The switching valves on both sides are closed and the association constant is regulated to be $K_{off}$. Since there is no transport between membrane and external solution, the following equations are obtained.

\[ C_{0}(n) = C_{0}(n - 1) \]
\[ C_{1}(n) = C_{1}(n - 1) \]
\[ C_{m}(n) = C^{F}(n) + C^{B}(n) = C_{m}(n - 1) \]

where $C_{m}(n)$ is the concentration of substrates in the membrane in step $n$. $C^{F}(n)$ is obtained from Eq. 1 and $C^{B}(n) = C_{H}^{P}(n) + C_{H}^{S}(n)$.

\[ C^{B}(n) = \frac{C^{F}(n) \cdot C_{H}^{P} \cdot K_{off}}{1 + C^{F}(n) \cdot K_{off}} \]  (9)

$C^{F}(n)$ is calculated from Eqs. 8 and 9:

\[ C^{F}(n) = \frac{K_{off}(C_{m}(n-1) - C_{H}) - 1 + X}{2 \cdot K_{off}} \]  (10) when $K_{off} \neq 0$

\[ C^{F}(n) = C_{m}(n-1) \]  (11) when $K_{off} = 0$

where $X = [1 + K_{off}(C_{H} - C_{m}(n-1))]^2 + 4K_{off} \cdot C_{m}(n-1)^{1/2}$.

(iii) step B ($n = 4m - 2$)

The switching valve on side 0 is open and the switching valve on side 1 is still closed. The association constant is regulated to be $K_{off}$. $C_{1}(n)$ is, therefore, the same as $C_{1}(n-1)$ in Eq. 12.

\[ C_{1}(n) = C_{1}(n - 1) \]

Since the membrane is in equilibrium with the external solution on side 0, the following equations are obtained:

\[ C_{0}(n) = C_{0}(0) \cdot S \]  (13)
\[ C_{m}(n) = \frac{C^{F}(n) \cdot C_{H} \cdot K_{off}}{1 + C^{F}(n) \cdot K_{off}} \]  (14)

Eq. 15 is given from the mass balance:

\[ (C^{F}(n) + C^{B}(n)) \cdot V_{m} + C_{0}(n) \cdot V_{0} = (C^{F}(n - 1) + C^{B}(n - 1)) \cdot V_{m} + C_{0}(n - 1) \cdot V_{0} = CV_{0}(n) \]

$CV_{0}(n)$ is obtained from Eqs. 13-15:

\[ CV_{0}(n) = \frac{-Y_{2}(n) + (Y_{2}(n)^{2} + 4Y_{1} \cdot CV_{0}(n))^{1/2}}{2Y_{1}} \]  (16) when $K_{off} \neq 0$

\[ CV_{0}(n) = CV_{0}(n)/ (S \cdot V_{m} + V_{0}) \]  (17) when $K_{off} = 0$

where $Y_{1} = S \cdot K_{off} \cdot V_{0} + S^{2} \cdot K_{off} \cdot V_{m}$ and $Y_{2}(n) = S \cdot V_{m} + S \cdot K_{off} \cdot V_{m} \cdot C_{H} + V_{0} - S \cdot K_{off} \cdot CV_{0}(n)$.

(iv) step C ($n = 4m - 1$)

The switching valves on both sides are closed and the association constant is changed to be $K_{on}$. Since there is no transport between membrane and external solution, the following equations are obtained.

\[ C_{0}(n) = C_{0}(n - 1) \]
\[ C_{1}(n) = C_{1}(n - 1) \]
\[ C_{m}(n) = C^{F}(n) + C^{B}(n) = C_{m}(n - 1) \]
\[ C_{m}(n) = C_{H}^{P}(n) + C_{H}^{S}(n) \]

From Eqs. 20 and 21, $C^{B}(n)$ is solved as Eqs. 22 and 23:

\[ C^{B}(n) = \frac{-Z_{1} + (Z_{1}^{2} + 4 \cdot K_{on} \cdot [C^{F}(n-1) + C^{B}(n-1)])^{1/2}}{2 \cdot K_{on}} \]  (22) when $K_{on} \neq 0$

\[ C^{B}(n) = C^{F}(n - 1) + C^{B}(n - 1) \]  (23) when $K_{on} = 0$

where $Z_{1} = 1 + [C_{H} - C^{F}(n - 1) - C^{B}(n - 1)] \cdot K_{on}$.
The switching valve on side 0 is still closed, and the switching valve on side 1 is open. The association constant is regulated to be \( K_{on} \). \( C_0(n) \) is, therefore, the same as \( C_0(n - 1) \) in Eq. 24:

\[
C_0(n) = C_0(n - 1)
\]  \( \text{(24)} \)

Since the membrane is in equilibrium with the external solution on side 1, the following equations are obtained:

\[
C'(n) = C_1(n) \cdot S
\]  \( \text{(25)} \)

\[
C''(n) = \frac{C'(n) \cdot C_0 \cdot K_{on}}{1 + C'(n) \cdot K_{on}}
\]  \( \text{(26)} \)

Eq. 27 is given from the mass balance.

\[
(C'(n) + C''(n)) \cdot V_m + C_1(n) \cdot V_1 =
(C'(n-1) + C''(n-1)) \cdot V_m + C_1(n-1) \cdot V_1 = CV_1(n)
\]  \( \text{(27)} \)

\( C_0(n) \) is calculated from Eqs. 25-27:

\[
C_0(n) = \frac{-W_1(n) + (W_2(n))^2 + 4 \cdot W_1 \cdot CV_1(n))^{1/2}}{2 \cdot W_1}
\]  \( \text{when } K_{on} \neq 0 \)  \( \text{(28)} \)

\[
C_0(n) = \frac{CV_1(n)}{S \cdot V_m + V_1}
\]  \( \text{when } K_{on} = 0 \)  \( \text{(29)} \)

where \( W_1 = S \cdot K_{on} \cdot V_1 + S^2 \cdot K_{on} \cdot V_m \) and \( W_2(n) = S \cdot V_m + S \cdot K_{on} \cdot V_H + V_1 - S \cdot K_{on} \cdot CV_1(n) \).

### 3. RESULTS AND DISCUSSION

#### 3.1 Model Membranes

Some model membranes were selected for the understanding of the hybrid-transport theory developed in this study. In order to emphasize the essential points, the model calculations performed in this study are confined to the following conditions: \( C_0(0) = C_0(0) \), \( S = 1 \), \( V_0 = V_1 \), \( V_m = 10^{-3} \text{ dm}^3 \) and \( K_{on} = 0 \). The values of \( K_{off} \) were chosen to be 0.01, 1.0, 10 or 10000 \( \text{mol}^{-1} \text{ dm}^3 \) (M\(^{-1}\)). Parameters for the 16 models addressed in this study are summarized in Table 1. Calculations were performed using a 16 bit personal computer (PC-9801 VX, NEC Corp.) with Nag Basic (86) language on MS-DOS ver. 3.10 (Microsoft Corp.).

#### 3.2 Concentration Change

Dependence of \( C_0 \) and \( C_1 \) on \( m \) in the A-1 model is shown in Fig. 3. \( m \) indicates a cycle of simulations. It is found that \( C_0 \) decreases with increasing number of simulation cycles (i.e. \( m \)) while \( C_1 \), on the contrary, increases with increasing number of cycles. It is suggested that the active transport of substrates can be demonstrated in the simple hybrid membrane in the synthetic system as this model. Fig. 4 shows dependence of \( C^F(n) \) and \( C^B(n) \) on the number of cycles. It is observed that the pumping movements of \( C^B \) contributes to the transfer of substrates from the \( C_0 \) side to the \( C_1 \) side.

Fig. 5 shows dependence of \( C_0 \) on the cycles for the models having \( V_0 = 10 \text{ V}_m \) (model A-1), 100 \text{ V}_m \) (model A-2) and 1000 \text{ V}_m \) (model A-3) on the conditions of \( C_0(0) = 1 \text{ mol dm}^{-3} \), \( C_H = 2 \text{ M} \) and \( K_{off} = 10000 \text{ M}^{-1} \). It is found that \( C_0(\infty) \) becomes less than 0.01 M in any models, although many cycles of pump-
Separation factors for 16 models at \( m = \infty \) are also summarized in Table 1. It is found that the A-1 model shows a very high separation factor (i.e. \( \alpha(\infty) = 948.5 \)).

Fig. 7 shows the dependence of the separation factor on the cycles for models having \( K_{off} = 0.01, 1, 100 \) and \( 10000 \) \( \text{M}^{-1} \) on the conditions of \( C_0(0) = 1 \text{M}, C_H = 2 \text{M} \) and \( V_0 = 10 \text{ V}_m \). It is found that the separation factor increases with increasing cycles for the models having a larger \( V_0 \). It requires only 20 cycles for model A-1 to give a constant \( C_0 \) (i.e. \( C_0(\infty) \)), although 2000 cycles are necessary for model A-3 to give a constant \( C_0 \).

### 3.3 Separation Factors

Separation factor, \( \alpha(m) \), which is a measure of the separation of solutes between cells of side 0 and side 1 on the \( m \) cycles, is expressed by

\[
\alpha(m) = \frac{C_1(4m)/C_0(4m)}{C_1(0)/C_0(0)}
\]

Fig. 6 shows dependence of \( C_1 \) on the cycles for A-1, A-2 and A-3 models. \( C_1(\infty) \) is found to be higher than 1.90 M (\( \approx C_0(0) + C_1(0) \)) in any models.

#### Table 1 Model Membranes and Transport Parameters in Active Transport

<table>
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<th>Membranes</th>
<th>( C_0/M )</th>
<th>( C_H/M )</th>
<th>( K_{off}/\text{M}^{-1} )</th>
<th>( V_0/\text{dm}^3 )</th>
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the models having $K_{off} \geq 1$. The separation factor at $m = \infty$ is observed to increase with increasing $K_{off}$ in these models.

Fig. 8 shows the dependence of separation factor ($\alpha(\infty)$) on $C_0(0)$ for the models transport having $K_{off} = 0.01$, $1$, $100$, and $10000$ $M^{-1}$ on the conditions of $C_0(0) = 1 M$, $V_o = 2 M$, $V_o = 10 V$, and $K_{on} = 0$.

$C_1(\infty) \cdot S = C_0(\infty) + C_0(\infty) \cdot S$

$C_0(0) \cdot V_0 + C_1(0) \cdot V_1$

$= C_0(\infty) \cdot V_0 + C_1(\infty) \cdot V_1 + |C_0(\infty) + C_0(\infty) \cdot S| \cdot V_m$

The above equations are obtained from $C_f(\infty) = C_0(\infty) \cdot S$, $C_f(\infty) + C_f(\infty) = C_f(\infty) \cdot S$ and the mass balance. $C_f(\infty)/C_0(\infty)$ is calculated from Eqs. 31-33:

$C_0(\infty) = \frac{-E_1 + (E_1^2 + 4 \cdot E_2)^{1/2}}{2(V_1 + S \cdot V_m)}$

where $E_0 = (C_0(0) \cdot V_0 + C_1(0) \cdot V_1) \cdot S \cdot K_{off}$. $E_1 = V_0 - V_1 - S \cdot V_m(1 + C_0(0) \cdot K_{off} + E_0$ and $E_2 = (V_1 + S \cdot V_m)(V_0 \cdot C_0(0) \cdot K_{off} + V_0 + E_0)$. Eq. 34 reduces to be simpler formula on the conditions of $V_0 = V_1 = 1$, $V_0 > V_m = 0$, $C_0(0) = C_1(0)$ and $S = 1$:

$C_f(\infty) = \frac{(C_0(0) - C_0(0) \cdot K_{off} + C_0(0) \cdot K_{off} + E_0)^{1/2}}{C_0(0)}$

This equation indicates that $C_f(\infty)/C_0(\infty)$ becomes to be a unity (i.e. $\alpha(\infty)$ becomes to be a unity), when $K_{off} \rightarrow 0$, $C(0) \rightarrow \infty$ or $C_{ni} \rightarrow 0$.

3.4 Association Constant

Several models (e.g. model A-1) have a high association constant of $K_{off} = 10000$ $M^{-1}$. This is taken from the consideration of binding of L-tryptophan to human serum albumin. It is known that the binding of L-tryptophan takes place only at one site of human serum albumin with an association constant of $4.4 \times 10^4$ $M^{-1}$ [20]. The primary association constants in the binding of diazepam and octanoate to the human serum albumin are also reported to be $3.8 \times 10^5$ $M^{-1}$ and $1.6 \times 10^6$ $M^{-1}$, respectively [21]. Model A-1 having the high association constant of $K_{off}$ is not, therefore, found to be an unrealistic model. It may be possible to perform the active transport of L-tryptophan with $\alpha(\infty) > 900$ where racemic tryptophan is used as the initial feed solution and there is no transport of D-tryptophan (i.e. $C_0(\infty) = C_1(\infty)$ for D-tryptophan), if the association constant of human serum albumin is regulated by temperature or pH control (e.g. $K_{off} = 4.4 \times 10^4$ $M^{-1}$ and $K_{on} = 0$).

3.5 Hybrid Membrane for Active Transport

The hybrid membrane that performs active transport must have the host molecules having $K_{off}$ and $K_{on}$ for the condition of $K_{off} \neq K_{on}$ and the switching valves. Since several chemical valves are reported in the literature [22,23], it is easy to construct the
switching valves in the hybrid membrane. Physical valves controlled by a personal computer may be the most realistic parts in the hybrid membranes. Host molecules controlled by external signals are also reported by several researchers (24). Irie and Kato (24) synthesized trans-cis photoisomerizable thioindigos and found that the cis form of the photoresponsive molecular tweezers had a high binding ability to Ag⁺, Hg⁺, Hg²⁺ and Cu²⁺ in comparison to alkali metal ions.

4. CONCLUSION

The present study reveals that the hybrid membrane consists of (1) host molecules of which association constants with the specific substrate can be changed by external signals, and (2) two switching valves on the membrane surfaces. Active transport was definitely observed for the condition of Koff ≈ 1 and C0(0) ≈ Cn/2 in the present calculations. Although each step in the transport theory is governed by passive transport, the substrate diffuses from the low concentration side to the high concentration side, i.e. against its electrochemical potential gradient between the external solution of side 0 and side 1. The energy for the active transport is consumed for a change in association constants of the host molecules, opening and closing of switching valves and control of switching valves (i.e. vectorial control). The active transport, which has been extensively considered to be a complicated system, is demonstrated to be reconstituted in a simple hybrid-membrane in the synthetic system.

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