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KINETICS OF PERMEATION OF NUCLEOTIDE THROUGH OIL/WATER INTERFACE IN THE INTERACTION OF NUCLEOTIDE WITH OCTADECYLAMINE AND DODECYL GUANIDINE

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SUMMARY

The transports of tritiated ATP, ADP and AMP from the aqueous to scintillator phase with and without octadecylamine (or dodecyl guanidine) have been studied by the layered scintillation method and a theory suitable for an explanation of the results has been presented.

(1) Transport processes were all expressed by the first order kinetics.

(2) For the simple partitioning of ATP, the reciprocal of the rate constant of the backward permeation was linear with respect to the square of the partition coefficient.

(3) For the transport of nucleotide with chemical reaction, the reciprocal of the rate constant of the backward permeation was linear against the overall partition coefficient of nucleotide.

(4) A theory was presented on the basis of a general diffusion equation by assuming the two-film model with potential energy near the interface.

(5) The theory could explain the dependences of the permeation rates on the partition coefficients.

(6) From the finding that the ratio of the apparent diffusion coefficient in aqueous to scintillator phase was much smaller than unity, the occurrence of an energy barrier at interface was suggested. For the simple partitioning of ATP, the energy barrier was not significant.

INTRODUCTION

A simple method for studying the mass transport through oil/water interface with and without chemical reaction has been devised [1, 12]. The organic scintillator solution containing nonradioactive reactant is layered on the aqueous solution of radioactive reactant just before measurements and the increase in counts due to the radioactive reactant entering the scintillator solution is followed.

In the present paper, this method has been applied to the study of the transports of tritiated ATP, ADP and AMP from the aqueous to scintillator phase with and without octadecylamine (or dodecyl guanidine). The phenomenon simulates the facilitated diffusion, or the carrier-mediated diffusion, of a substance through biological membranes.

A number of studies have been carried out for the mass transport between two different phases and many theories have been proposed [2–10]. However, since the phenomena occur near the interface, it is not easy to understand the nature of mass transport into the different phases across the interface. Thus, for the sake of simplicity, each theory has its own assumption concerning the nature of boundary layer.

The purpose of this paper is to derive a kinetic equation suitable for explaining the experimental results and comparing our theory with other proposed theories.

SYMBOLS

A	Radioactive compound dissolved in the aqueous phase (phase I)
B	Reactant dissolved in the oil phase (phase II)
AB_n	Reaction product produced in the phase II where n is the stoichiometric coefficient
C^0, C, $\bar{C}$	Concentrations of a substance at the oil/water interface, boundary layer and bulk of solution, respectively
$C_A{}^{\mathrm{I}}$, $C_A{}^{\mathrm{II}}$	Concentrations of A in the boundary layers of phases I and II
$C_{AB}{}^{\mathrm{II}}$, $C_t{}^{\mathrm{II}}$	Concentration of AB_n and the sum of concentrations A and AB_n ($C_t{}^{\mathrm{II}} = C_A{}^{\mathrm{II}} + C_{AB}{}^{\mathrm{II}}$) in the boundary layer of phase II
$\bar{C}_A{}^{\mathrm{I}}$, $\bar{C}_A{}^{\mathrm{II}}$, $\bar{C}_{AB}{}^{\mathrm{II}}$, $\bar{C}_t{}^{\mathrm{II}}$	Bulk concentrations
D	Diffusion coefficient of a substance in a boundary layer
D_0	Diffusion coefficient of a substance in a bulk phase
$D_A{}^{\mathrm{I}}$, $D_{A0}{}^{\mathrm{I}}$	Diffusion coefficients of A in the boundary layer and the bulk of the phase I
$D_A{}^{\mathrm{II}}$, $D_{A0}{}^{\mathrm{II}}$	Diffusion coefficients of A in the boundary layer and the bulk of phase II
$D_{AB}{}^{\mathrm{II}}$	Diffusion coefficient of AB_n in the boundary layer of phase II
$D_t{}^{\mathrm{II}}$, $D_{t0}{}^{\mathrm{II}}$	Overall diffusion coefficients of A in the boundary layer and the bulk of the phase II
E	Energy barrier for the diffusion process near the oil/water interface
$E_A{}^{\mathrm{I}}$, $E_A{}^{\mathrm{II}}$	Energy barriers for the diffusion of A in the boundary layers of phases I and II
$E_t{}^{\mathrm{II}}$	Energy barrier for the overall diffusion process of A in the boundary layer of phase II
I_0, I_t, I_∞	Counting rates at time zero, t and infinity, respectively
j	Flux through a boundary layer
$j_A{}^{\mathrm{I}}$, $j_A{}^{\mathrm{II}}$	Fluxes of A through the boundary layers of phases I and II
$j_{AB}{}^{\mathrm{II}}$, $j_t{}^{\mathrm{II}}$	Fluxes of AB_n and sum of $j_A{}^{\mathrm{II}}$ and $j_{AB}{}^{\mathrm{II}}$
k, k^0	Observed permeation rate constants in the presence and absence of B in phase II
k_1, k_2	Forward and backward permeation rates in the equation of first order kinetics when B is present in phase II

k_1^0, k_2^0	Forward and backward permeation rates in the equation of first order kinetics when B is absent in phase II
K_1, K_2	Partition coefficients of A in the absence and presence of B in phase II
K_f	Formation constant of AB_n
$-L_1$, L_2	Positions of the surfaces of boundary layers in phases I and II
m_t, m_∞	Total masses of A in phase II at a time t and infinity
P	Overall permeability coefficient for the permeation process from the aqueous to oil phase
P_A^I, P_A^{II}	Permeability coefficients of A in the boundary layers of phases I and II
P_t^{II}	Overall permeability coefficient of A in the boundary layer of phase II
P_A^{I*}, P_A^{II*}, P_t^{II*}	Permeability factors in P_A^I, P_A^{II} and P_t^{II}, respectively
S	Diffusional cross section
U	Adsorption energy at the interface
α, α^0	Ratios, $P_t^{II*}/P_A^{I*}K_1$ and $P_A^{II*}/P_A^{I*}K_1$
η	Overall free energy of partition
Φ	Reduced potential, the potential energy of a substance divided by RT
Φ_A^I, Φ_A^{II}	Reduced potentials of A in the boundary layers of A in phases I and II
$\Phi_A^{-L_1}$, Φ_A^0	Reduced potentials of A at the surface of the boundary layer of phase I and at the oil/water interface
$\Phi_{AB}^{L_2}$, Φ_{AB}^{II}, Φ_{AB}^0	Reduced potentials of AB_n at the surface of the boundary layer of phase II, in the boundary layer of phase II and at the oil/water interface, respectively
Φ_t^{II}, Φ_t^0, $\Phi_t^{L_2}$	Overall reduced potentials of A in the surface of boundary layer of phase II, at the oil/water interface and the surface of the boundary layer of phase II, respectively

THEORY

The following assumptions are made for the mass transport with chemical reactions across the oil/water interface.

(1) The reactant B, which can bind with the radioactive compound A of the aqueous phase, and the reaction product AB_n (n is the stoichiometric coefficient) are present only in the oil phase. The radioactive compound migrates from the aqueous (phase I) to the oil or scintillator phase (phase II).

(2) The schematic diagram of boundary layers is illustrated in Fig. 1. The coordinate of the interface is taken as the origin and the boundary layers are bounded at $-L_1$ in phase I and L_2 in phase II. The concentration of each component in the boundary layers and bulk phases is illustrated in Fig. 1. Furthermore, for the sake of simplicity we assume that the concentrations of B may be regarded as a constant throughout the boundary layer and bulk of phase II including the interface because the concentration of B is present in much greater excess than that of A

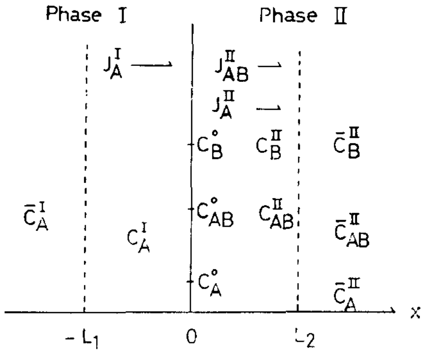

Fig. 1. Schematic diagram of boundary layers. The concentration of A in the boundary layer of the phase I, the concentration of A, B, and AB_n in the boundary layer of the phase II are denoted as C_A^I, C_A^{II}, C_B^{II} and C_{AB}^{II}, respectively. These concentrations in the bulk phases are assumed constant everywhere and denoted as $\bar{C}_A^I$, $\bar{C}_A^{II}$, $\bar{C}_B^{II}$ and $\bar{C}_{AB}^{II}$, respectively. Similarly, the concentrations of A, B and AB_n at the interface are denoted as C_A^0, C_B^0 and C_{AB}^0, respectively. According to assumption II, the concentration of B is constant throughout phase II including the interface, i.e., $C_B^0 = C_B^{II} = \bar{C}_B^{II}$.

and AB_n. The diffusion takes place in the boundary layers and the concentration gradient may be ignored in the bulks of phases I and II.

(3) The reaction of A with B occurs very quickly at the interface and within the boundary layer of phase II and hence the transport process is the rate-determining step.

(4) The fluxes within the boundary layers are in a stationary state.

(5) The flux equation of the transport is assumed as follows [11].

$$J = -D\left(\frac{dc}{dx} + C\frac{d\Phi}{dx}\right) = -D\exp(-\Phi)\left\{\frac{d}{dx}C\exp(\Phi)\right\} \tag{1}$$

where D is the diffusion coefficient, C, the concentration of diffusive species and Φ, the reduced potential, i.e., the potential energy divided by RT (R is the gas constant and T, the absolute temperature). The presence of the potential energy near the interface may also be presumed from the existence of the interfacial free energy, the excess free energy at interface. The reduced potential should be taken into account for explaining the equilibrium partitioning.

The solution of Eqn. 1 for the flux of A, J_A^I, in phase I is given by

$$-J_A^I\int_{-L_1}^{0}\frac{1}{D_A^I}\exp(\Phi_A^I)dx = C_A^0\exp(\Phi_A^0) - \bar{C}_A^I\exp(\Phi_A^{-L_1}) \tag{2}$$

where subscript A refers to component A and superscripts I, o and $-L_1$, to phase I, the oil/water interface and the surface of boundary layer in phase I, respectively. Eqn. 2 may be rewritten as

$$J_A^I = P_A^I\left\{\bar{C}_A^I\exp\left(\frac{\Delta\Phi_A^I}{2}\right) - C_A^0\exp\left(-\frac{\Delta\Phi_A^I}{2}\right)\right\} \tag{3}$$

where P_A^I denotes a permeability coefficient of component A in the boundary layer of phase I,

$$(P_{\mathrm{A}}^{\mathrm{I}})^{-1} = \exp\left(-\frac{\Phi_{\mathrm{A}}^{-\mathrm{L}_1}+\Phi_{\mathrm{A}}^{0}}{2}\right)\int_{-\mathrm{L}_1}^{0}\frac{1}{D_{\mathrm{A}}^{\mathrm{I}}}\exp(\Phi_{\mathrm{A}}^{\mathrm{I}})\mathrm{d}x \tag{4}$$

and $\Delta\Phi_{\mathrm{A}}{}^{\mathrm{I}}$, the difference between the reduced potentials at $-\mathrm{L}_1$ and zero

$$\Delta\Phi_{\mathrm{A}}^{\mathrm{I}} = \Phi_{\mathrm{A}}^{-\mathrm{L}_1}-\Phi_{\mathrm{A}}^{0}. \tag{5}$$

In phase II, the equation for total flux is given as

$$J_{\mathrm{t}}^{\mathrm{II}} = J_{\mathrm{A}}^{\mathrm{II}}+J_{\mathrm{AB}}^{\mathrm{II}} = -D_{\mathrm{A}}^{\mathrm{II}}\left(\frac{\mathrm{d}C_{\mathrm{A}}^{\mathrm{II}}}{\mathrm{d}x}+C_{\mathrm{A}}^{\mathrm{II}}\frac{\mathrm{d}\Phi_{\mathrm{A}}^{\mathrm{II}}}{\mathrm{d}x}\right) -D_{\mathrm{AB}}^{\mathrm{II}}\left(\frac{\mathrm{d}C_{\mathrm{AB}}^{\mathrm{II}}}{\mathrm{d}x}+C_{\mathrm{AB}}^{\mathrm{II}}\frac{\mathrm{d}\Phi_{\mathrm{AB}}^{\mathrm{II}}}{\mathrm{d}x}\right) \tag{6}$$

where subscript AB and superscript II refer to the component AB_n and phase II, respectively. From the assumption of Eqn. 3, it can be considered that the following chemical equilibrium exists not only at the interface, but also in the boundary layer of phase II;

$$\mathrm{A}+n\mathrm{B} \rightleftharpoons \mathrm{AB}_n \tag{7}$$

where n denotes a stoichiometric coefficient.

The formation constant of the reaction product in the oil phase is defined as

$$K_{\mathrm{f}} = C_{\mathrm{AB}}^{\mathrm{II}}/C_{\mathrm{A}}^{\mathrm{II}}(C_{\mathrm{B}}^{\mathrm{II}})^n. \tag{8}$$

The total concentration of A, $C_{\mathrm{t}}{}^{\mathrm{II}}$, in phase II is, therefore, given as follows

$$C_{\mathrm{t}}^{\mathrm{II}} = C_{\mathrm{A}}^{\mathrm{II}}+C_{\mathrm{AB}}^{\mathrm{II}} = C_{\mathrm{A}}^{\mathrm{II}}\{1+K_{\mathrm{f}}(C_{\mathrm{B}}^{\mathrm{II}})^n\}. \tag{9}$$

Thus, the overall partition coefficient of A, K_2, is related to the formation constant by the equation

$$K_2 = K_1\{1+K_{\mathrm{f}}(\bar{C}_{\mathrm{B}}^{\mathrm{II}})^n\} \tag{10}$$

where K_1 represents the partition coefficient of A for the simple partition in which the component B is absent in the scintillator phase.

Eqn. 6 can be reduced to

$$J_{\mathrm{t}}^{\mathrm{II}} = -D_{\mathrm{t}}^{\mathrm{II}}\left(\frac{\mathrm{d}C_{\mathrm{t}}^{\mathrm{II}}}{\mathrm{d}x}+C_{\mathrm{t}}^{\mathrm{II}}\frac{\mathrm{d}\Phi_{\mathrm{t}}^{\mathrm{II}}}{\mathrm{d}x}\right) \tag{11}$$

where $D_{\mathrm{t}}{}^{\mathrm{II}}$ and $\mathrm{d}\Phi_{\mathrm{t}}{}^{\mathrm{II}}/\mathrm{d}x$ are given by

$$D_{\mathrm{t}}^{\mathrm{II}} = \{D_{\mathrm{A}}^{\mathrm{II}}+K_{\mathrm{f}}(C_{\mathrm{B}}^{\mathrm{II}})^n D_{\mathrm{AB}}^{\mathrm{II}}\}/\{1+K_{\mathrm{f}}(C_{\mathrm{B}}^{\mathrm{II}})^n\} \tag{12}$$

and

$$\frac{\mathrm{d}\Phi_{\mathrm{t}}^{\mathrm{II}}}{\mathrm{d}x} = \frac{1}{D_{\mathrm{A}}^{\mathrm{II}}+K_{\mathrm{f}}(C_{\mathrm{B}}^{\mathrm{II}})^n D_{\mathrm{AB}}^{\mathrm{II}}}\frac{\mathrm{d}}{\mathrm{d}x}\{D_{\mathrm{A}}^{\mathrm{II}}\Phi_{\mathrm{A}}^{\mathrm{II}}+K_{\mathrm{f}}(C_{\mathrm{B}}^{\mathrm{II}})^n D_{\mathrm{AB}}^{\mathrm{II}}\Phi_{\mathrm{AB}}^{\mathrm{II}}\}, \tag{13}$$

respectively. From Eqn. 11, we derive

$$J_{\mathrm{t}}^{\mathrm{II}} = P_{\mathrm{t}}^{\mathrm{II}}\{C_{\mathrm{t}}^{0}\exp(\Delta\Phi_{\mathrm{t}}^{\mathrm{II}}/2)-\bar{C}_{\mathrm{t}}^{\mathrm{II}}\exp(-\Delta\Phi_{\mathrm{t}}^{\mathrm{II}}/2)\} \tag{14}$$

where $\Delta\Phi_{\mathrm{t}}{}^{\mathrm{II}}$ is defined as

$$\Delta\Phi_t^{II} = \Phi_t^0 - \Phi_t^{L_2} \tag{15}$$

and P_t^{II} expresses a total permeability of A in the boundary layer of phase II.

$$(P_t^{II})^{-1} = \exp\left(-\frac{\Phi_t^{L_2}+\Phi_t^0}{2}\right)\int_0^{L_2}\frac{1}{D_t^{II}}\exp(\Phi_t^{II})\mathrm{d}x. \tag{16}$$

By the assumption of a stationary state, $j_t^{II} = j_A^{I}$, we have from Eqns. 3 and 14

$$P_t^{II}\left\{C_t^0\exp\left(\frac{\Delta\Phi_t^{II}}{2}\right) - \bar{C}_t^{II}\exp\left(\frac{-\Delta\Phi_t^{II}}{2}\right)\right\} = P_A^{I}\left\{\bar{C}_A^{I}\exp\left(\frac{\Delta\Phi_A^{I}}{2}\right) - C_A^0\exp\left(\frac{-\Delta\Phi_A^{I}}{2}\right)\right\} \tag{17}$$

This can be solved with respect to C_A^0 under the assumption of chemical equilibrium at interface as follows:

$$C_A^0 = \frac{C_t^0}{1+K_f(C_B^{II})^n}$$
$$= \frac{P_A^{I}\bar{C}_A^{I}\exp(\Delta\Phi_A^{I}/2) + P_t^{II}\bar{C}_t^{II}\exp\left(-\frac{\Delta\Phi_t^{II}}{2}\right)}{P_t^{II}\{1+K_f(C_B^{II})^n\}\exp(\Delta\Phi_t^{II}/2) + P_A^{I}\exp(-\Delta\Phi_A^{I}/2)} \tag{18}$$

In obtaining Eqn. 18, C_B^{II} was assumed constant not only throughout phase II but also at the interface. This may be assumed since $\bar{C}_B^{II}$ is in much greater excess than $\bar{C}_A^{II}$.

Accordingly, by substituting Eqn. 18 into Eqn. 14, we finally get the equation for the mass transport in the form

$$J_t^{II} = k_1\bar{C}_A^{I} - k_2\bar{C}_t^{II} \tag{19}$$

where k_1 and k_2 refer to the forward and backward rate constants, respectively. The rate constants can be expressed by

$$\frac{1}{k_2} = \frac{K_2}{P_A^{I*}} + \frac{1}{P_t^{II*}} = \frac{K_2}{k_1} \tag{20}$$

where

$$k_1/k_2 = K_2 = \{1+K_f(\bar{C}_B^{II})^n\}\exp(\Delta\Phi_A^{I}+\Delta\Phi_t^{II}) \tag{21}$$

Eqn. 21 should be the same as Eqn. 10, hence

$$K_1 = \exp(\Delta\Phi_A^{I}+\Delta\Phi_t^{II}) \tag{22}$$

Each of P_A^{I*} and P_t^{II*} in Eqn. 20 may be regarded as a part of the permeation factor of the radioactive material in the films of phases I and II, respectively, and given as follows:

$$\frac{1}{P_A^{I*}} = \int_{-L_1}^{0}\frac{1}{D_A^{I}}\exp(\Phi_A^{I} - \Phi_A^{-L_1})\mathrm{d}x \tag{23}$$

and

$$\frac{1}{P_t^{II*}} = \int_0^{L_2} \frac{1}{D_t^{II}} \exp(\Phi_t^{II} - \Phi_t^{L_2}) dx \tag{24}$$

respectively.

In obtaining Eqns. 23 and 24, the reduced potentials $\Phi_A^{-L_1}$ and $\Phi_t^{L_2}$ were assumed to be constant for the sake of simplicity.

Eqn. 19 may also be written in the form (see ref. 12)

$$J_t^{II} = P\{\bar{C}_A^{I} \exp(\eta/2) - \bar{C}_t^{II} \exp(-\eta/2)\} \tag{25}$$

where P denotes the overall permeability of radioactive material and is related to k_1 and k_2 by the following relation

$$\frac{1}{P} = (k_1 k_2)^{-\frac{1}{2}} = \frac{K_2^{\frac{1}{2}}}{P_A^{I*}} + \frac{1}{P_t^{II*} K_2^{\frac{1}{2}}} \tag{26}$$

and η is given by

$$\eta = \ln K_2 = \ln k_1/k_2 . \tag{27}$$

In a previous paper [1], it was shown that Eqn. 19 leads to the relations

$$J_t^{II} = k(m_\infty - m_t)/S \tag{28}$$

and

$$k = S\left(\frac{k_1}{V^I} + \frac{k_2}{V^{II}}\right) \tag{29}$$

where m_t denotes the total amount of A entering phase II at time t, m_∞, the equilibrium amount, k, the observed rate constant, S, the diffusional cross section and V^I and V^{II}, the volume of phase I and II, respectively. Combining Eqn. 29 with Eqns. 20 and 21 we have

$$\frac{K_2 V^{II} + V^I}{V^I V^{II}} \cdot \frac{S}{k} = \frac{K_2}{P_A^{I*}} + \frac{1}{P_t^{II*}} \tag{30}$$

which is equivalent to Eqn. 20 since the left hand side becomes k_2^{-1}.

We now make a further assumption for the reduced potential Φ, the nature of which is of a short range force [12]. Φ_A^{I} and Φ_t^{II} may be put as the same as $\Phi_A^{-L_1}$ and $\Phi_t^{L_2}$, respectively, except for those at the interface, Φ_A^0 and Φ_t^0, respectively. Then Eqns. 23 and 24 may be reduced to

$$P_A^{I*} = D_A^I/L_1 \tag{31}$$

and

$$P_t^{II*} = D_t^{II}/L_2 \tag{32}$$

respectively, because the contribution of Φ_A^0 and Φ_t^0 may be ignored in the integrals of Eqns. 23 and 24.

The diffusion coefficient D near the interface may be written as (see ref. 12)

$$D = D_0 \exp(U - E)/RT \tag{33}$$

where D_0 denotes the diffusion coefficient in bulk phase, U, the adsorption energy and E, the energy barrier near the interface. Since in the present study, the adsorption energies U, at the interface (refer to phases I and II) can be denoted by $RT\Delta\Phi_A{}^I$ and $RT\Delta\Phi_t{}^{II}$, respectively, the ratio of Eqn. 31 to Eqn. 32 becomes

$$P_t^{II*}/P_A^{I*} = D_t^{II}L_1/D_A^I L_2 = (D_{t0}^{II} L_1/D_{A0}^I L_2)\exp\left\{(\Delta\Phi_A^I+\Delta\Phi_t^{II})+\frac{E_A^I-E_t^{II}}{RT}\right\} \tag{34}$$

where $D_{t0}{}^{II}$ and $D_{A0}{}^I$ denote the diffusion coefficients in the bulk phases. Furthermore, by comparing Eqn. 34 with Eqn. 22, we have

$$P_t^{II*}/P_A^{I*} = \alpha K_1 \tag{35}$$

where

$$\alpha = (L_1 D_{t0}^{II}/L_2 D_{A0}^I)\exp(E_A^I - E_t^{II})/RT \tag{36}$$

We obtain from Eqns. 30 and 35

$$\frac{K_2V^{II}+V^I}{V^IV^{II}}\frac{S}{k} = \frac{1+\alpha K_1K_2}{P_t^{II*}} = \frac{1}{P_A^{I*}}\left(K_2+\frac{1}{\alpha K_1}\right) \tag{37}$$

For the simple permeation without chemical reaction, Eqns. 22, 32, 35, 36 and 37 reduce to

$$K_1 = \exp(\Delta\Phi_A^I+\Delta\Phi_A^{II}) \tag{38}$$

$$P_A^{II*} = D_A^{II}/L_2 \tag{39}$$

$$P_A^{II*}/P_A^{I*} = \alpha^0 K_1 \tag{40}$$

$$\alpha^0 = (L_1 D_{A0}^{II}/L_2 D_{A0}^I)\exp(E_A^I - E_A^{II})/RT \tag{41}$$

and

$$\frac{K_1V^{II}+V^I}{V^IV^{II}}\frac{S}{k^0} = \frac{1+\alpha^0 K_1^2}{P_A^{II*}} = \frac{1}{P_A^{I*}}\left(K_1+\frac{1}{\alpha^0 K_1}\right), \tag{42}$$

respectively, where the superscript 0 refers to the simple permeation. Comparing Eqn. 22 with Eqn. 38, we have $\Delta\Phi_A{}^{II} = \Delta\Phi_t{}^{II}$. This is a consequence of the assumptions that $C_B{}^{II}$ and K_f are constant in the phase II.

EXPERIMENTAL AND RESULTS

The procedure and the samples used in the present study were already described elsewhere [1]. The experiments were carried out at 20±1 °C.

The rate constant k was determined according to the equation (see ref. 12):

$$\ln(I_\infty - I_t) = \ln(I_\infty - I_0) - kt \tag{43}$$

where I_0, I_t and I_∞ denote the counting rate at time zero, t and infinity, respectively.

Eqn. 43 leads to Eqn. 28, since the counting rate is proportional to the amount of the radioactive material entering phase II.

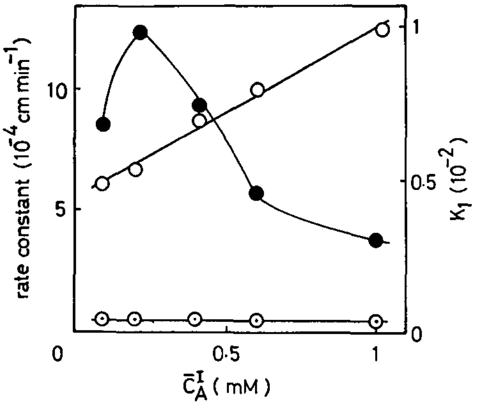

Fig. 2. The rate constant and partition coefficient as a function of the concentration of ATP, $\bar{C}_A{}^I$. ○, K_1; circle with dot, $k_1{}^0$; ●, $k_2{}^0$.

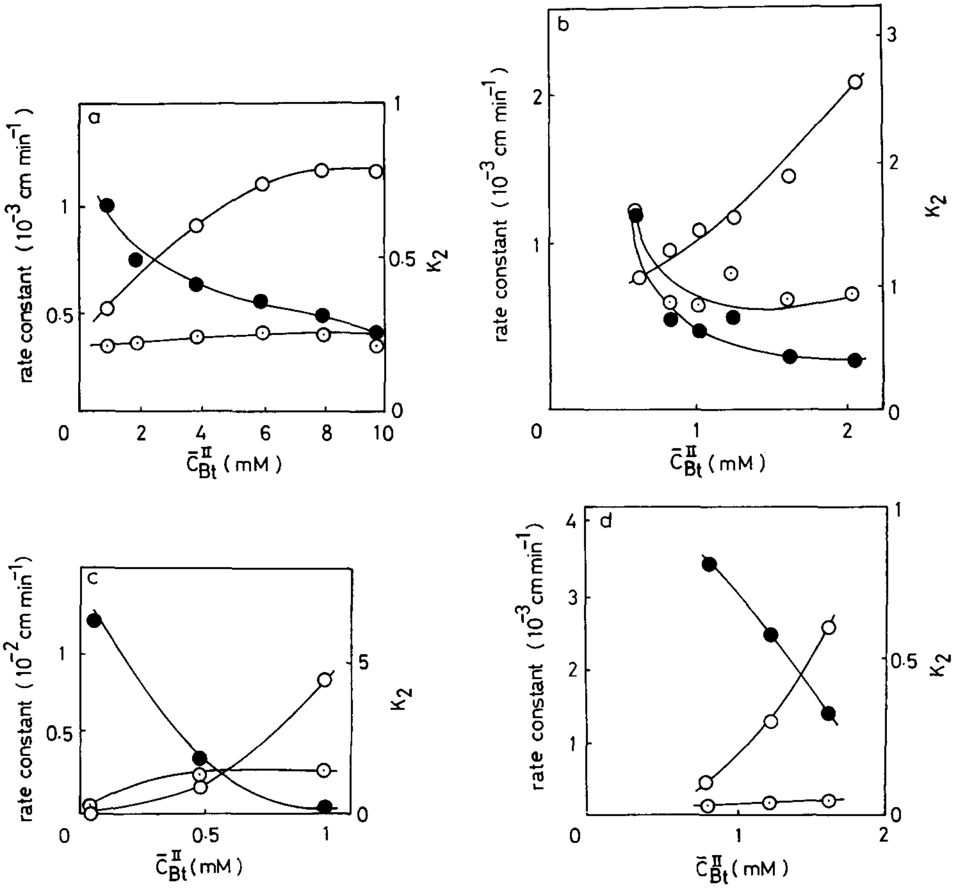

Fig. 3. The rate constant and partition coefficient as a function of the total reactant concentration, $\bar{C}_{Bt}{}^{II} = \bar{C}_B{}^{II} + n\bar{C}_{AB_n}{}^{II}$. ○, K_2; circle with dot, k_1; ●, k_2. Initial concentration of nucleotide is kept constant at 1 mM. (a) ATP-octadecylamine (b) ATP-dodecyl guanidine (c) ADP-octadecylamine (d) AMP-dodecyl guanidine

Figs. 2 and 3 show the results for the permeation rate of nucleotides in the absence and presence respectively of the reactant B. Rate constants for the simple partitioning of ADP and AMP could not be observed because of a too fast attainment of the equilibrium. Fig. 3 indicates that the backward rate constant, k_2 changes with the reactant concentration whereas the forward one, k_1 is almost independent of the concentration of the reactant. The relationship between the rate constant and the partition coefficient is expressed by Eqn. 37 as shown in Fig. 4. This result indicates

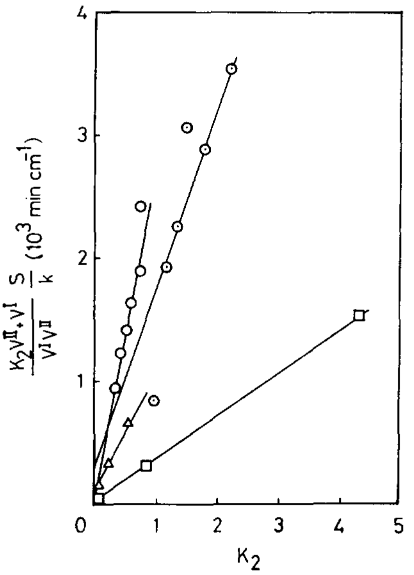

Fig. 4. Plot for a permeation with a chemical reaction according to Eqn. 37. ○, ATP-octadecylamine; circle with dot, ATP-dodecyl guanidine; □, ADP-octadecylamine; △, AMP-dodecyl guanidine.

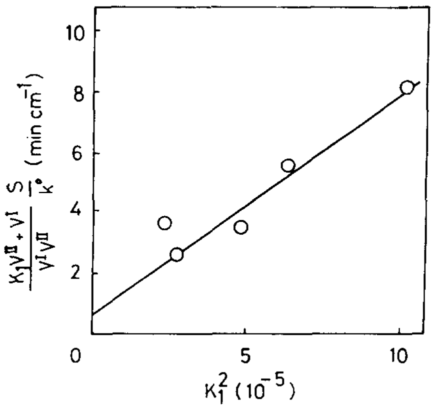

Fig. 5. Plot for a simple permeation of ATP according to Eqn. 42.

TABLE I

CONSTANTS IN RATE EQUATIONS

System	$P_A^{I\star}$ (cm/s)	$P_t^{II\star}$ (cm/s)	$1/\alpha$	$E_A^I - E_t^{II}$ (kcal/mol)
ATP-octadecylamine	$7.47 \cdot 10^{-6}$	$9.42 \cdot 10^{-5}$	$7.97 \cdot 10^{-4}$	4.15
ADP-octadecylamine	$4.58 \cdot 10^{-5}$	$4.01 \cdot 10^{-4}$	$2.21 \cdot 10^{-4}$	4.88
ATP-dodecyl guanidine	$1.33 \cdot 10^{-5}$	$3.78 \cdot 10^{-5}$	$1.70 \cdot 10^{-3}$	3.70
AMP-dodecyl guanidine	$3.78 \cdot 10^{-5}$	$1.02 \cdot 10^{-4}$	$1.26 \cdot 10^{-3}$	3.87
	$P_A^{II\star}$ (cm/s)		$1/\alpha^0$	$E_A^I - E_A^{II}$ (kcal/mol)
ATP	$1.73 \cdot 10^{-6}$		0.667	0.14

that P_A^{I*} and P_t^{II*} are constant. The values of P_A^{I*}, P_t^{II*} and $1/\alpha$ estimated according to Eqn. 37 are given in Table I. In the absence of the chemical reaction, the results obeyed Eqn. 42 as shown in Fig. 5, from which one can estimate the values of P_A^{II*} and $1/\alpha^0$ as cited in Table I.

DISCUSSION

The transport phenomena in a two-phase system are treated on the basis of Fick's equation. The diffusion coefficient of nucleotide was also estimated according to the solution of Fick's equation obtained by the method of Bird et al. [2, 3]. It was found that the diffusion coefficient of nucleotide showed appreciable dependence on the concentration of the reactant B. This is unreasonable since the nucleotide concentration is kept constant. Furthermore, the presence of a reactant in the oil phase suppresses the apparent diffusion coefficient by a factor of one to two as compared with that in the aqueous solution. This may be due to interfacial resistance. The diffusion equation with the interfacial resistance was solved by Scott et al. [7], but it is difficult to apply their solution to the present results because of too many parameters. Davies [9] independently treated the equation for the two-phase system with and without a chemical reaction by taking into account the interfacial resistance. Unfortunately, his solution of the diffusion equation disagrees with the present results that the time course of permeation obeys the first order kinetics.

Thus, a theory is presented on the basis of a generalized diffusion equation as developed in the present paper. The analysis of the results indicates that the present theory is suitable for explaining the experimental observations. Table I indicates that the factor $1/\alpha$ is much smaller than unity. Since the thicknesses of the boundary layers of phases I and II may be regarded as being of the same order, the ratio, L_1/L_2 would be around unity. We may also accept that the diffusion coefficients in the two bulk phases should be near each other and hence the ratio of the diffusion coefficients, D_{t0}^{II}/D_{A0}^{I} should also be near unity. Thus, approximate values of the energy barrier can be estimated according to the equation

$$\alpha = \exp(E_A^I - E_t^{II})/RT \qquad (44)$$

by assuming

$$D^{\mathrm{I}}_{\mathrm{A}0}\,\mathrm{L}_2/D^{\mathrm{II}}_{\iota 0}\,\mathrm{L}_1 = 1. \tag{45}$$

Similarly, we have

$$\alpha^0 = \exp\,(E^{\mathrm{I}}_{\mathrm{A}} - E^{\mathrm{II}}_{\mathrm{A}})/RT \tag{46}$$

and

$$D^{\mathrm{I}}_{\mathrm{A}0}\,\mathrm{L}_2/D^{\mathrm{II}}_{\mathrm{A}0}\,\mathrm{L}_1 = 1. \tag{47}$$

The relations assumed by Eqns. 45 and 47 may not be numerically exact, but they may yield a reasonable order of magnitude of the ratio. The energy barrier evaluated according to Eqns. 44 and 46 is given in Table I. The results indicate that the energy barrier for the simple permeation of ATP is small. In this case, the diffusion coefficient calculated on the basis of the simple diffusion theory [2] was found to be of the order of $10^{-5} \cdot \mathrm{cm}^2$ per s. This value is a reasonable order of magnitude as deduced from the molecular dimension. In this case, therefore, the simple diffusion theory presented by Bird et al. [2] provides only an approximate explanation for the experimental results. However, the simple diffusion theory predicts that the time course of permeation is too fast to measure e.g., as shown experimentally by the permeation of ADP and AMP.

Table I also indicates that the chemical reaction enhances the partitioning of nucleotide in spite of the smaller rate constant for permeation. The smaller rate constant may be due to the accumulation of a reaction product at the interface which suppresses the migration of nucleotide from the aqueous to oil phase.

The phenomena studied in the present paper simulate the carrier-mediated transport or facilitated diffusion of a substance through biological membrane[13, 14]. The theoretical means described might be of use in elucidating the permeation through the membrane when the facilitated diffusion seems to occur. But a suitable procedure and a choice of system would be necessary to determine all the parameters involved in the theory and to elucidate the permeation mechanisms thoroughly when either a model or a real biological membrane is used.

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