Analysis of Facilitated Oxygen Transport in a Liquid Membrane of Hemoglobin

Xue-si Chen, Hiroyuki Nishide,* and Eishun Tsuchida*

Department of Polymer Chemistry, Waseda University, Shinjuku, Tokyo 169

(Received August 7, 1995)

A liquid membrane containing bovine hemoglobin (Hb) as an oxygen carrier was prepared, and oxygen permeation through the membrane was measured by the electrochemical reduction of the permeated oxygen. The oxygen permeation was facilitated, as analyzed by a dual-mode model, to give permeation parameters of the membranes such as the diffusion coefficient (D_{Hb}) of the mobile carrier Hb and the solution-diffusion coefficient of oxygen (D_{O2}). The facilitated permeation was the product of the Hb concentration ($[Hb]_0$) and D_{Hb} in the membrane solution. D_{Hb} decreased with $[Hb]_0$ or the solution viscosity; the maximum of the oxygen permeability (6.2×10^{-8} cm^3(STP)cm $cm^{-2}s^{-1}$ $cmHg^{-1}$ at the feed stream oxygen pressure of 0.54 cmHg) was observed at $[Hb]_0 = 12.8$ g dl^{-1}, where the facilitation factor and the oxygen/nitrogen permselectivity were ca. 10 and 18, respectively.

Hemoglobin (Hb) is the most efficient and well-studied oxygen carrier at room temperature. For example, Roughton analyzed the diffusion of oxygen in red blood cells and described the characteristics of oxygen-transport by Hb.[1,2] The Hb solution had first been examined by Scholander[3] for air separation by a procedure in which the Hb solution is retained in the pores of a microporous cellulose acetate membrane. The aqueous Hb solution was immobilized in the microporous support by capillary action, and the permeated gases were measured by a vacuum gauge and a micro gas analyzer. The oxygen transport through the Hb membrane involved two processes:[3] One pathway was a simple physical permeation of oxygen through the solvent (water) called the dissolution–diffusion process, and the other was the specific transport of oxygen mediated by Hb as an oxygen carrier, called facilitated transport.

His report was the first demonstration of the facilitated transport process for air separation.[3] In the Hb liquid membrane, the oxygen flux at low oxygen partial pressure in the feed stream was eight times greater than that of a pure water membrane at steady state. This difference was ascribed to the facilitated transport with Hb. However, the fluxes of gases through the membrane were not presented in that permeation test. Further experiments were conducted by Wittenberg,[4] who measured by a gas chromatography method the fluxes of oxygen and nitrogen through a Hb solution immobilized in a Millipore membrane as a function of gas partial pressure (>2 cmHg, 1 cmHg = 1333.22 Pa) on the feed side of the membrane. The transport mechanism involving Hb as a mobile carrier and the reversible oxygen-binding reaction by Hb were described. Kreuzer and Hoofd[5] collected and scrutinized the pertinent numerical data, particularly the diffusion coefficients of oxygen and Hb, from the viewpoint of respiration physiology. But, the diffusion coefficients of oxygen and Hb have not been estimated directly from a permeation measurement with the Hb liquid membrane, and the facilitated transport or the contribution of Hb as a mobile carrier in the membrane has not been fully analyzed.

In this research, we first applied an electrochemical method to measure the oxygen flux permeating through a liquid membrane containing Hb. We used bovine hemoglobin as Hb, since it is abundant as waste in the cattle industry at the present time. The data of facilitated oxygen transport in the Hb liquid membrane were analyzed using a dual-mode transport model. A survey of the important parameters such as the diffusion coefficients of oxygen and Hb was conducted.

Experimental

Preparation of Concentrated Hemoglobin Solution. Hemoglobin (Hb) was purified and concentrated using an organic solvent method.[6] For example, bovine blood (200 ml) was washed twice with saline solution and separated by centrifugation (3000 G, 10 min). The concentrated red blood cell suspension (25 g dl^{-1}, 80 ml) was shaken with $CHCl_3$ (16 ml) for 5 min to homolyze the cells and then centrifuged (3000 G, 20 min). The aqueous solution containing Hb was evaporated at 30 °C for 30 min to remove the residual $CHCl_3$. The precipitate of other proteins was removed by centrifugation (3000 G, 20 min) and filtration with a 0.45 μm acetate cellulose membrane (Toyo Roshi Co.). Concentrated Hb solution (25 g dl^{-1}, 40 ml) was obtained (<5% methemoglobin), stored in a refrigerator (4 °C), and used within 5 d.

Membrane Preparation. The liquid membrane of the Hb solution was prepared by immersing a microporous flat-sheet membrane (nitrocellulose filter: Advantec, Toyo Roshi Co.; pore diameter, 0.45 μm; porosity, 0.78; weight, 5.2 mg cm^{-2}; thickness, 145 μm) into 25 mM phosphate buffer solution (M = mol dm^{-3}) (pH = 8.0) of Hb. The Hb solution was quickly taken up into the microporous membrane and held by capillary action within the pores.

Electrochemical Measurement of Oxygen Permeation. Molecular oxygen is electrochemically reduced to OH^- (in principle, $O_2+2H_2O+4e^-\rightarrow4OH^-$) on a carbon electrode in alkaline solution. This electrochemical reduction of oxygen is specific and

very rapid under the appropriate potential at the electrode.

The measurement of the reduction current of the permeated oxygen was carried out using the apparatus[7] described in Fig. 1. A carbon paste containing Mn_5O_8, a platinum wire, and Hg/HgO were used as the working, auxiliary, and reference electrodes, respectively. The electrolyte was an aqueous solution of KOH (10 wt%). The liquid membrane, in the form of a 25 mm diameter flat sheet, was placed at the feed side of the carbon electrode, which was coated with a porous perfluorocarbon polymer film, under a nitrogen atmosphere. The potential was kept constant at −0.4 V by a dual potentiogalvanostat (Nikko Keisoku DPG-3). Feed gas mixtures were saturated with water at 25 °C, to reduce evaporization loss of water from the solvent in the membranes, and passed through the feed side of the cell at a rate of 60 ml min^{-1}. The total gas pressure was kept at 1 atm. Before the measurement, nitrogen was passed through the cell and a potential was applied to the electrodes until the current reached zero to remove the oxygen contained in the cell such as that in the electrolyte solution, carbon electrode, or spaces in the cell. The oxygen permeation through the membrane was measured at various feed stream oxygen partial pressures at 25 °C. The oxygen permeation through the membrane reached steady state within 6 min in the measuring process.

Permeability of oxygen through the membrane mounted in front of the electrode (Fig. 1) is measured as the reduction current of oxygen according to Eq. 1[7] at the steady state permeation.

$$P_{O2} = \frac{I}{n \times 1.602 \times 10^{-19}} \times \frac{22400}{6.02 \times 10^{23}} \times \frac{l}{A p_{O2} \delta} \qquad (1)$$

Here, P_{O2} (cm^3(STP)cm $cm^{-2}s^{-1}$ $cmHg^{-1}$) is the permeability coefficient of oxygen through a membrane whose thickness is l (cm) and whose area is A (cm^2). I (amperes) is the reduction current at the carbon electrode. p_{O2} is the oxygen partial pressure in the feed stream because oxygen pressure at the product side is kept at zero in this experiment due to the rapid reduction of the permeated oxygen. n is a constant of effectively reduced electrons at the carbon electrode (in this experiment, with Mn_5O_8 as a catalyst) and is determined to be n=2.9 for this experimental condition. δ is the porosity of the microporous membrane.

Oxygen-Binding Measurement. Oxygen-binding to Hb in the solution was measured by a spectral change in the visible absorption using a spectrophotometer (Shimadzu Model UV-2100) as shown in Fig. 2. The visible absorption spectrum (λ_{max}=554 nm) of deoxy Hb was reversibly changed to the spectrum (λ_{max}=541 and 576 nm) of oxy Hb (HbO_2) with isosbestic points at 521, 548, 568, 584 nm in response to the oxygen partial pressure in the atmosphere. Inactive methemoglobin concentration was determined also spectroscopically on the basis of the cyanomethemoglobin method.[8]

Viscosity Measurement. The viscometric measurement was performed with a cone-plate-rotation viscometer (Vismetron VS-AK, Shibaura System Co.) at a shear rate of 11.5 s^{-1} at 25 °C.

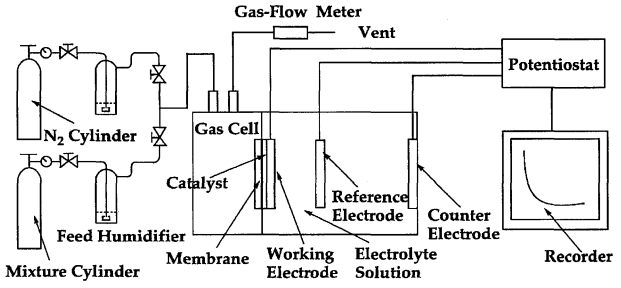

Fig. 1. Flow system for the flux measurement.

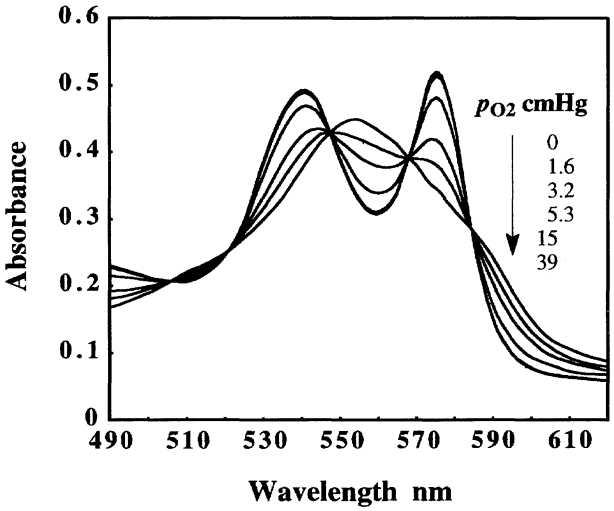

Fig. 2. Spectral change in the oxygen-binding to Hb in the solution at 25 °C. $[Hb]_0$=0.05 g dl^{-1}.

Results and Discussion

Facilitated Oxygen Transport in the Liquid Membrane. As shown in Fig. 3, the reaction of Hb with oxygen provides the following specific transport in addition to physical dissolution–diffusion transport. A mobile carrier of oxygen, here Hb, is dissolved in the liquid membrane that separates the feed-air stream side from the permeated or product stream side. The feed stream is maintained at a sufficiently high oxygen pressure so that the oxygen carrier absorbs specifically oxygen and converts to its oxy form at the feed stream–membrane interface. The permeated stream is kept at a sufficiently low oxygen pressure (by being evac-

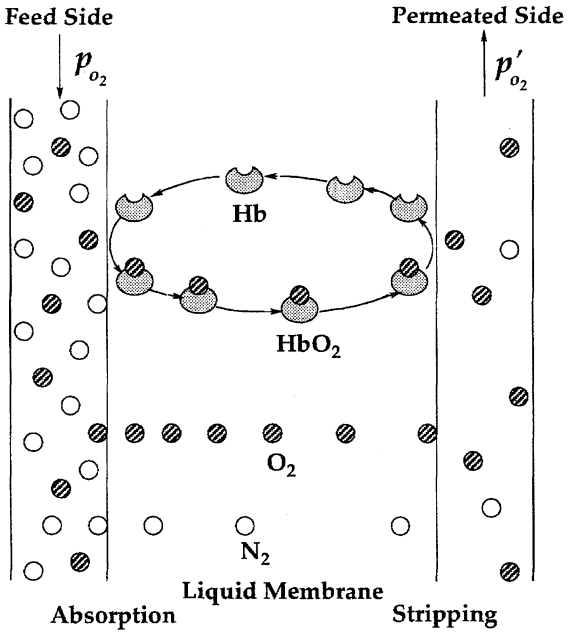

Fig. 3. Schematic diagram of facilitated oxygen transport through the Hb liquid membrane.

uated or with sweep inert gas) to strip oxygen or maintain the carrier in its deoxy form at the membrane–permeated stream interface. The oxygen carrier thus acts as a shuttle, picking up oxygen at the feed stream–membrane interface, diffusing across the membrane as the oxy form, releasing oxygen to the permeated stream and then diffusing back to the feed stream–membrane interface to repeat the process. Because the carrier is specific for oxygen, the rate of oxygen transport is enhanced with no effect on the rate of nitrogen transport, resulting in a considerably higher oxygen enrichment of the permeated stream than is possible in the absence of the carrier. Such a process of selective transport across membranes facilitated by carriers is called facilitated transport or carrier-mediated transport.[9,10]

The chemically specific but reversible reaction of Hb with an oxygen molecule is the following:

$$\mathrm{Hb} + \mathrm{O_2} \overset{K}{\rightleftharpoons} \mathrm{HbO_2} \tag{2}$$

$$K = \frac{[\mathrm{HbO_2}]}{([\mathrm{Hb}]_0 - [\mathrm{HbO_2}])p_{\mathrm{O2}}} \tag{3}$$

K, $[\mathrm{Hb}]_0$, $[\mathrm{HbO_2}]$, and p_{O2} are the equilibrium constant of the oxygen-binding reaction, the total Hb concentration in the liquid membrane, oxy Hb ($\mathrm{HbO_2}$) concentration, and oxygen partial pressure at the atmosphere or the feed stream, respectively. Equation 3 is converted to the following equation:

$$[\mathrm{HbO_2}] = \frac{[\mathrm{Hb}]_0 K p_{\mathrm{O2}}}{1 + K p_{\mathrm{O2}}} \tag{4}$$

The permeability coefficient, whose unit is $\mathrm{cm^3(STP)\,cm\,cm^{-2}\,s^{-1}\,cmHg^{-1}}$, is defined as the product of the diffusivity and the solubility in the membrane for dense membranes including liquid membranes, which is often represented for the facilitated transport as a dual-mode permeation.[11—13]

$$P_{\mathrm{O2}} = kD_{\mathrm{O2}} + \frac{[\mathrm{Hb}]_0 K D_{\mathrm{Hb}}}{1 + K p_{\mathrm{O2}}} \tag{5}$$

Here, P_{O2} and D_{Hb} are the permeability coefficient of oxygen across the membrane and the diffusion coefficient of Hb ($\mathrm{cm^2\,s^{-1}}$), respectively. Because D_{Hb} is a constant at a given temperature and Hb concentration, P_{O2} steeply increases with the decrease in p_{O2}.

Figure 4 shows a typical result revealing two pathways of oxygen flux through the Hb membrane. The solid line represents the oxygen flux for the control liquid membrane containing inactive methemoglobin (metHb) or the flux of physical dissolution–diffusion permeation which is proportional to the partial pressure difference of oxygen across the membranes. The open symbols represent the total flux (involving the facilitated flux) across the Hb membrane. The partial flux over the solid line (the shaded area in Fig. 4) is ascribed to the flux of facilitated transport; it steeply increased at low oxygen partial pressure and saturated at higher oxygen partial pressure of the feed stream.

Figure 5 shows the oxygen-binding equilibrium curve or oxygen-saturation curve for Hb in the solution, which was measured spectroscopically on the same Hb solution used in the membrane preparation. The equilibrium curve indicates that Hb acts as a reversible oxygen-binding site and is appropriate for analysis by the Langmuir isotherm. The latter gave both the effective Hb fraction ($([\mathrm{Hb}]_0 - [\mathrm{metHb}])/[\mathrm{Hb}]_0$) as the oxygen carrier and the equilibrium constant of the oxygen-binding reaction (K in Eq. 2): the effective Hb fraction in the solution was 0.97±0.02 (the effective Hb concentration was almost equal to $[\mathrm{Hb}]_0$) and K=3.1 $\mathrm{cmHg^{-1}}$. The flux of facilitated transport in Fig. 4 corresponds to the oxygen-binding curve in Fig. 5, which also supports the contribution of Hb as the oxygen carrier. The flux reached 3.2×10^{-6} $\mathrm{cm^3(STP)\,cm^{-2}s^{-1}}$ at $[\mathrm{Hb}]_0$=12.8 $\mathrm{g\,dl^{-1}}$.

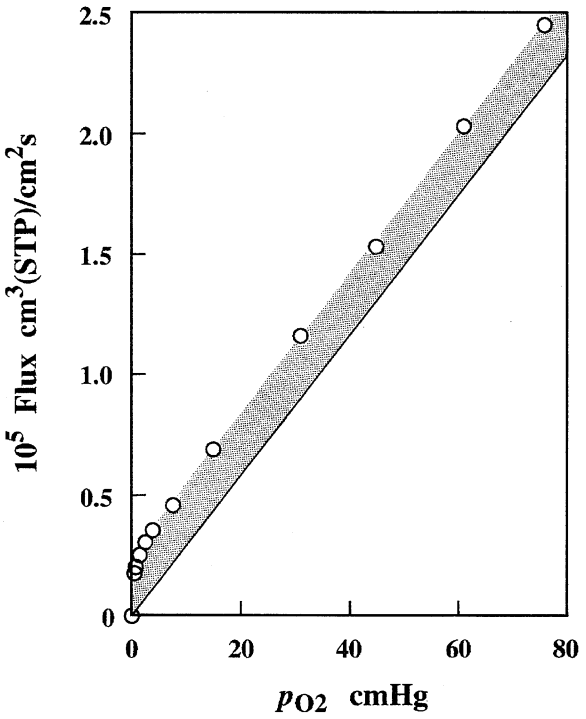

Fig. 4. Oxygen plain flux (lower solid line) for the control membrane and the facilitated flux (plots). $[\mathrm{Hb}]_0$=12.8 $\mathrm{g\,dl^{-1}}$.

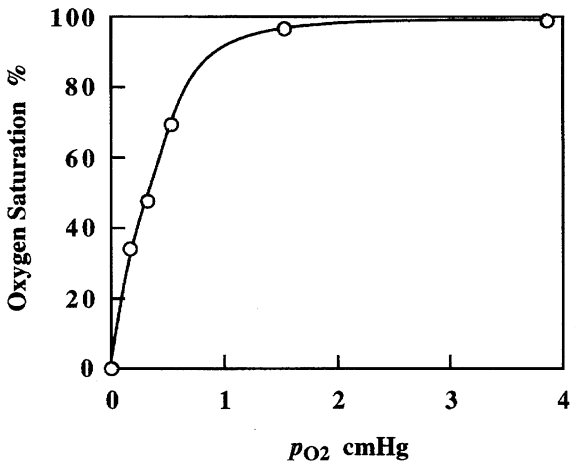

Fig. 5. Oxygen-binding equilibrium or oxygen-saturation ($[\mathrm{HbO_2}]/[\mathrm{Hb}]_0$) curve for Hb in the solution at 25 °C.

From Eq. 5 the facilitated flux (J_{Hb}) can be represented as:

$$J_{Hb} = \frac{[Hb]_0 K D_{Hb}}{1 + K p_{O2}} \times \frac{p_{O2}}{l\delta} \quad (6)$$

while p_{O2} is large enough, the expression becomes a constant:

$$J_{Hb} = \frac{[Hb]_0 D_{Hb}}{l\delta} \quad (7)$$

thus, the value of J_{Hb} is determined by $[Hb]_0$, D_{Hb}, δ (the porosity), and l (the thickness of the membrane).

The relationship between J_{Hb} and the Hb concentration in the membrane $[Hb]_0$ is shown in Fig. 6, J_{Hb} was enhanced to a maximum at $[Hb]_0$=12.8 g dl^{-1} and decreased linearly with the increasing $[Hb]_0$ beyond the maximum. That is, the rate of facilitated diffusion is approximately proportional to $[Hb]_0$ at the lower concentration and declines at the higher concentration.

Figure 7 shows the effect of feed stream oxygen pressure (p_{O2}) on the permeability (P_{O2}) through the Hb solution membranes. The oxygen permeability significantly increased with a decrease in p_{O2}, as seen in Fig. 7a. On the other hand, P_{O2} for the control and inactive metHb membrane was independent of p_{O2} and was not enhanced at the low p_{O2}. This permeation behavior is in accordance with the facilitated or carrier-mediated transport in this membrane, which supports the following analysis using a dual-mode transport model, as represented by Eq. 5.

Figure 7b shows a clear facilitation effect for the membrane with $[Hb]_0$=12.8 g dl^{-1}. The facilitation factor (the ratio of P_{O2} at low p_{O2} (here p_{O2}=0.53 cmHg) to P_{O2} at higher p_{O2} (>30 cmHg)) was over 10. It is predicted that the O_2/N_2 selectivity reached 18 at the low p_{O2}.

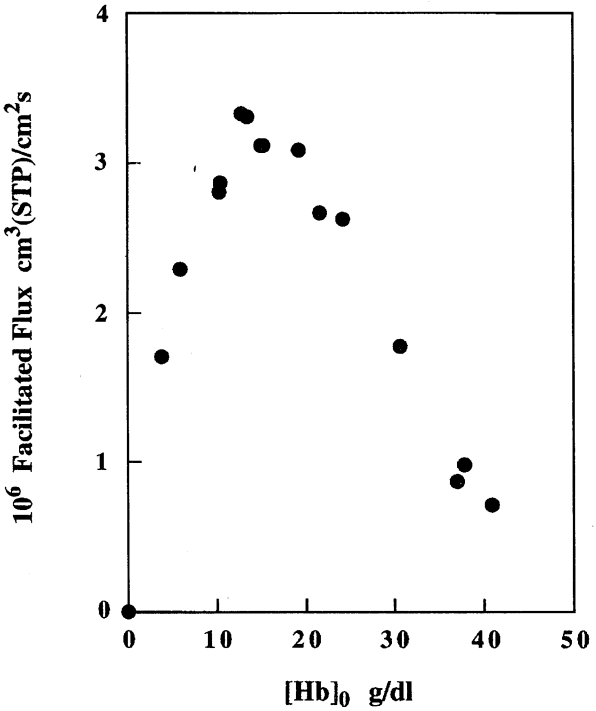

Fig. 6. Effect of the Hb concentration ($[Hb]_0$) on the facilitated O_2 flux through the Hb solution membrane.

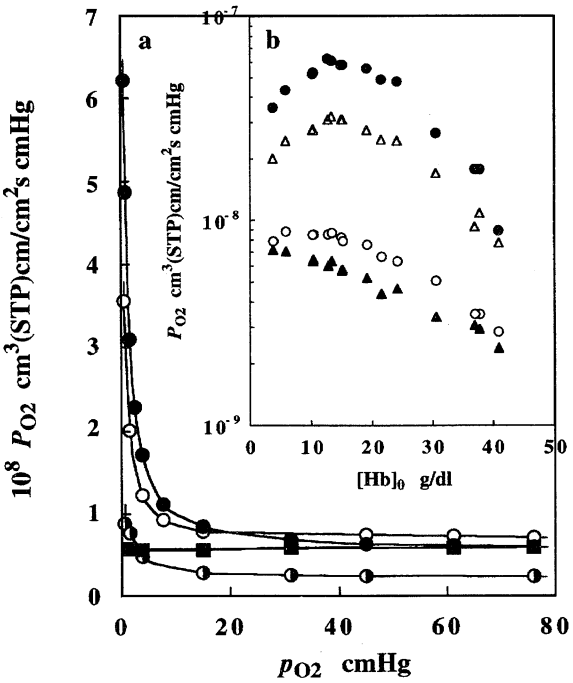

Fig. 7. Effect of upstream oxygen pressure (p_{O2}) on the permeation coefficient (P_{O2}) through the Hb solution membrane (a) and effect of the Hb concentration ($[Hb]_0$) on P_{O2} (b); $[Hb]_0$ in (a), ○: 3.81, ●: 12.8, ◑: 40.9 g dl^{-1}; ■: metHb control 13.2 g dl^{-1}; p_{O2} in (b), ●: 0.54 cmHg, △: 1.5 cmHg, ○: 15 cmHg, ▲: 76 cmHg.

Analysis of Facilitated Oxygen Transport. The facilitated transport data (P_{O2} at various p_{O2} and $[Hb]_0$) were appropriate for the analysis in terms of the dual-mode transport model (represented by Eq. 5). P_{O2} was plotted against $[Hb]_0 K/(1+Kp_{O2})$, as shown in Fig. 8. The linear relationship at any $[Hb]_0$ supports the validity of the analysis using the dual-mode transport model.

The diffusion coefficient (D_{Hb}) of Hb through the membrane was estimated from the slope of the linear relationship

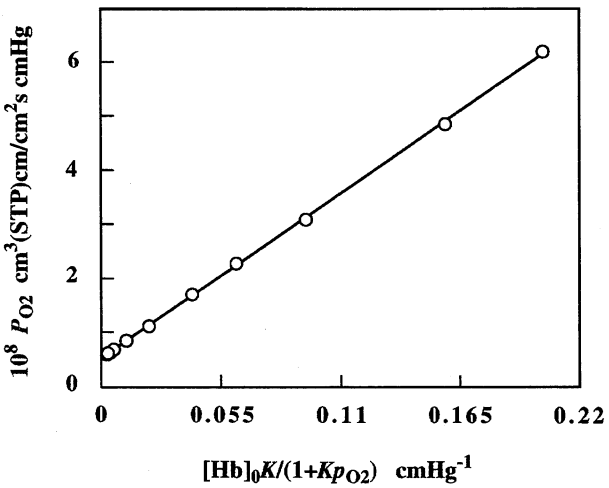

Fig. 8. Calculation of permeation coefficient (kD_{O2}) and hemoglobin facilitated coefficient (D_{Hb}) from the dual-mode model $[Hb]_0$=12.8 g dl^{-1}.

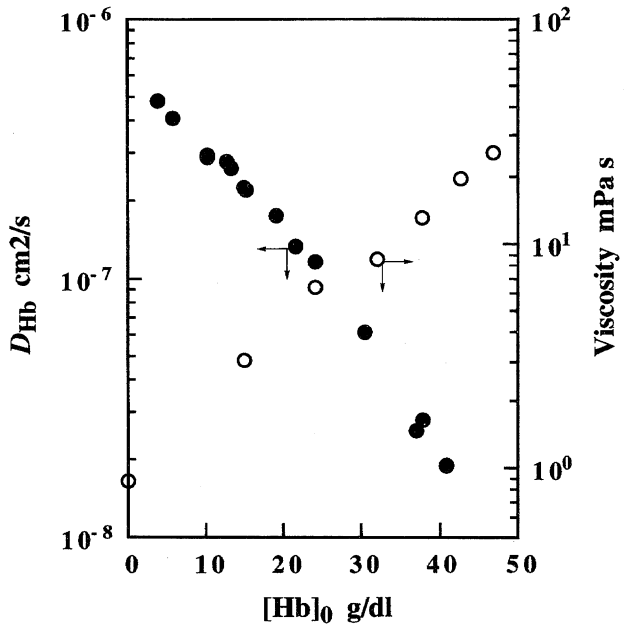

Fig. 9. Diffusion coefficient of Hb (D_{Hb}) (●) and viscosity (○) of the Hb solution at 25 °C.

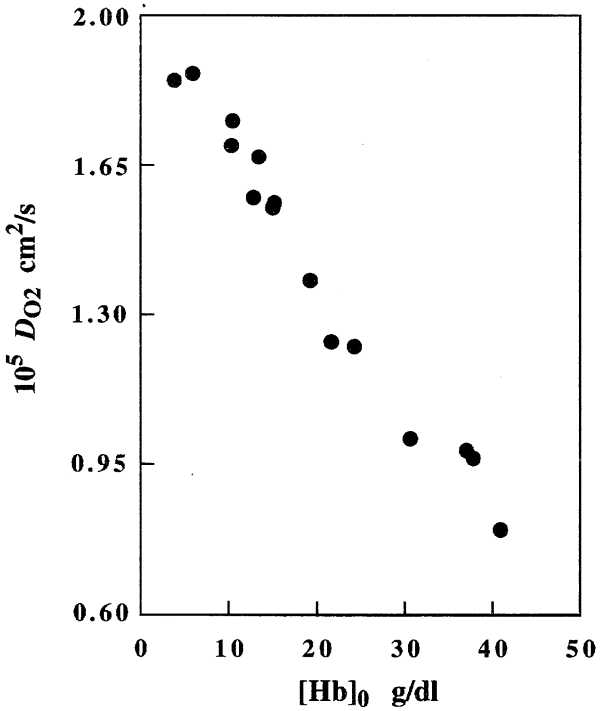

Fig. 10. Effect of the Hb concentration ($[Hb]_0$) on the diffusion coefficient of oxygen (D_{O2}) in the Hb solution membrane.

in Fig. 8. The effect of $[Hb]_0$ on D_{Hb} is shown in Fig. 9. We see that D_{Hb} logarithmically decreases with the increase in $[Hb]_0$ in the membrane. So we consider that the viscosity of the Hb solution tremendously retards the diffusion of Hb and that Hb acts as a mobile carrier of oxygen in the membrane.

The viscosity of the Hb solution was measured and the valves are also given in Fig. 9. The viscosity increased with $[Hb]_0$ in the range of the Hb concentration used for the liquid membrane in this experiment. This result supports the above consideration regarding the significant decrease in D_{Hb} with $[Hb]_0$ and the role of Hb as a mobile carrier in the membrane.

The permeability of oxygen for the physical diffusion transport in the liquid membrane was estimated from the intercept of the linear relationship in Fig. 8. The physical permeability decreases with $[Hb]_0$. Oxygen solubility in the water portion in the membranes at any $[Hb]_0$ valve was calculated under the following assumption:

$$k = k_{H_2O}(100 - [Hb]/\rho_{Hb})/100 \quad (8)$$

where ρ_{Hb} and k_{H_2O} are the density of hemoglobin in the solid state and the oxygen solubility of pure water at 25 °C, respectively. ρ_{Hb}=1.78 g cm^{-3} (measured in this experiment) and k_{H_2O}=3.66×10^{-4} cm^3(STP)/(cm^3 of water) cmHg[5] were substituted in Eq. 5 to give the diffusion constant of oxygen in the membrane D_{O2}. This D_{O2} almost linearly decreases with $[Hb]_0$, as shown in Fig. 10. Facilitated transport with a liquid membrane containing a mobile carrier could provide an elegant method for air separation to improve both the flux and the permselectivity. However, the viscosity increases with the carrier concentration in the membrane is an unavoidable problem which must be overcome before the mobile carrier/liquid membrane can enhance its facilitation efficiency.

This work was partially supported by a Grant-in-Aid for Scientific Research No. 05555243 from the Ministry of Education, Science, Sports and Culture. X. Chen thanks the Monbusho for a Scholarship which supported his stay in the laboratory.

References

1) F. J. W. Roughton, *Proc R. Soc. London, Ser. B*, **B111**, 1 (1932).
2) A. Klug, F. Kreuzer, and F. J. W. Roughton, *Helv. Physiol. Pharmacol. Acta*, **14**, 121 (1956).
3) P. F. Scholander, *Science*, **131**, 585 (1960).
4) J. B. Wittenberg, *J. Biol. Chem.*, **241**, 104 (1966).
5) F. Kreuzer and L. J. C. Hoofd, *Respir. Physiol.*, **8**, 280 (1970).
6) H. Sakai, S. Takeoka, H. Yokohama, Y. Seino, H. Nishide, and E. Tsuchida, *Protein Exp. Purif.*, **4**, 563 (1993).
7) T. Suzuki, H. Yasuda, H. Nishide, X. Chen, and E. Tsuchida, *J. Membr. Sci.*, in press (1995).
8) L. Tentori and A. M. Salvati, "Methods in Enzymology," ed by E. Antonini, L. R. Bernardi, and E. Chiancone, Academic Press, New York (1981), Vol. 76, pp. 707—715.
9) J. D. Way and R. D. Noble, "Membrane Handbook," ed by W. S. Winston and K. K. Sirkar, Van Nostrand Reinhold, New York (1992), pp. 833—866.
10) H. Nishide and E. Tsuchida, "Polymers for Gas Separation," ed by N. Toshima, VCH Publishers, New York (1992), pp. 183—219.
11) D. R. Paul and W. J. Koros, *J. Polym. Sci., Polym. Phys. Ed.*, **14**, 675 (1976).
12) R. M. Barrer, *J. Membr. Sci.*, **18**, 25 (1984).
13) H. Nishide, T. Suzuki, H. Kawakami, and E. Tsuchida, *J. Phys. Chem.*, **98**, 5084 (1994).