CHEMISTRY LETTERS, pp. 1177-1180, 1979. Published by the Chemical Society of Japan

REDOX REACTION DRIVEN BY DIFFUSION OF IONS THROUGH AN ARTIFICIAL LIQUID MEMBRANE

Toshio SHINBO*, Masaaki SUGIURA*, Naoki KAMO**, and Yonosuke KOBATAKE**
* National Chemical Laboratory for Industry, Yatabe, Tsukuba 300-21
** Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo 060

A trans-membrane redox reaction against its redox potential was induced with coupling to diffusion of an anion, when a gradient of anion concentration was imposed across an organic liquid membrane containing dibutyl-ferrocene which separated two solutions of equal redox potential.

In biological membranes, active (or uphill) transport is supported by a chemical reaction, called as "vectorial metabolism"[1]. Several reports have been published concerning with the reverse direction of energy transduction, i.e., chemical reactions are driven by the downhill movement of solutes. Garrahan and Glynn[2] showed that ATP was synthesized from ADP and phosphate in red blood cells when large concentration gradients of Na^+ and K^+ were imposed. Panet and Selinger[3] also described the formation of ATP in sarcoplasmic reticulum via a reverse operation of Ca-ATPase.

Several authors[4-8] have reported the artificial membrane systems where uphill transport of some substances has been achieved. These systems were composed of a liquid membrane or a polymer membrane separating alkaline and acid solutions. The neutralization reaction across the membrane supports the uphill transport of ions. Previously, we reported an active transport of picrate anion through a liquid membrane with coupling to a trans-membrane redox reaction[9]. The fact that active transport occurred in such simple liquid membrane systems suggests the possibility that a chemical reaction is induced by diffusion of ions through an artificial membrane, as is observed in biological membranes. We describe here a membrane system where a redox reaction is driven by diffusion of ions through an organic liquid membrane.

The apparatus used for the present experiment was essentially the same as that described previously[7]. The cell consisted of a cylindrical glass vessel (7 cm i.d., 7 cm in height) containing a central glass wall which separated two aqueous solutions (phase I and II, 50 ml each). The dichloroethane layer (phase III, 100 ml) containing 2×10^{-3} M dibutyl-ferrocene (Fc) lay under these aqueous phases and bridged them across the central separation. All three phases were agitated with a pair of glass stirrers at a speed of 180 min^{-1} and maintained at 25 °C.

The two aqueous phases (I and II) contained initially 0.1 mM ferricyanide and 0.1 mM ferrocyanide, and were buffered with 25 mM sodium borate (pH 8.5). Sodium perchlorate and sodium sulfate were added to phase II and I, respectively, in order to build up gradients of anion concentration within the membrane. The concentration

of sodium perchlorate was 1 M, and that of sodium sulfate was 0.5 M, so that no concentration gradient of sodium ion was present. The rate of the progress of redox reaction across the membrane was followed by monitoring the concentration changes in ferricyanide and ferrocyanide in phase I and II. The determination of ferricyanide concentration was conducted on a spectrophotometer at 420 nm, and that of ferrocyanide concentration by the colorimetric method[10]. The quantity of perchlorate transported from phase II to I was determined by the methyleneblue method[11].

Figure 1 shows the typical data, where the concentrations of ferricyanide and ferrocyanide in both aqueous phases are plotted against time. The ferricyanide concentration in phase I increased with an elapse of time and that in phase II decreased, and finally reached a plateau level. The ferrocyanide concentration in phase I decreased and that in phase II increased, and the sum of the concentrations of ferricyanide and ferrocyanide in the respective aqueous phases was unchanged. In the absence of Fc in phase III, no concentration change of ferricyanide or ferrocyanide was observed. The inset in Fig. 1 shows the concentration ratio of ferricyanide to ferrocyanide, r, as a function of time, revealing that in phase I the follwing reaction occurred; $Fe(CN)_6^{4-} \longrightarrow Fe(CN)_6^{3-} + e^-$, and that in phase II the reverse reaction occurred.

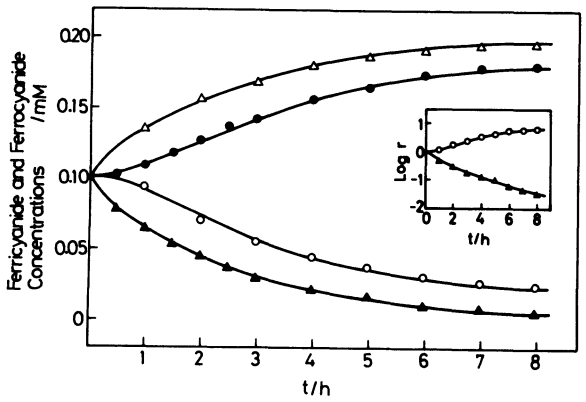

Fig. 1. Plots of the concentrations of ferricyanide and ferrocyanide against time. ●, the ferricyanide concentration in phase I; ○, the ferrocyanide concentration in phase I; ▲, the ferricyanide concentration in phase II; △, the ferrocyanide concentration in phase II. The inset shows the concentration ratio of ferricyanide to ferrocyanide as a function of time. ○, the value of log r in phase I; △, the value of log r in phase II.

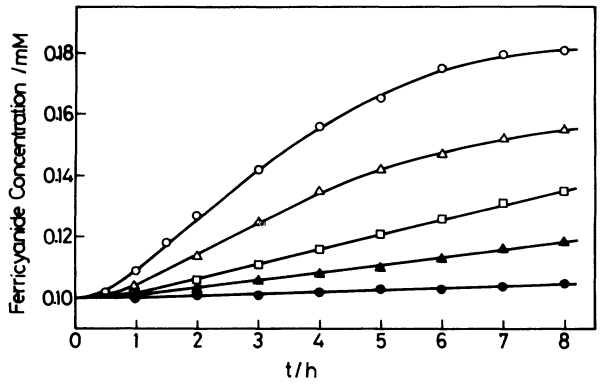

Fig. 2. The dependence of the rate of redox reaction on various anions. ○, sodium perchlorate; △, sodium thiocyanate; □, sodium nitrate; ▲, sodium bromide; ●, sodium chloride.

The rate of oxidation of ferrocyanide in phase I was measured when perchlorate anion in phase II was replaced by various anions. As seen in Fig. 2, the rate of production of ferricyanide decreased in the following order: $ClO_4^- > SCN^- > NO_3^- > Br^- > Cl^-$. This series is often found

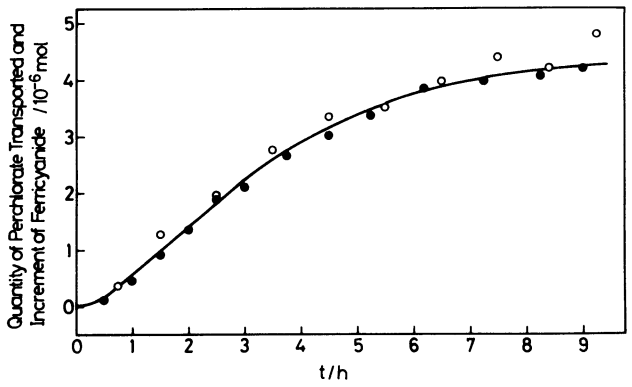

Fig. 3. Simultaneous measurement of the rates of redox reaction and perchlorate transport.

○, the quantity of perchlorate transported from phase II to I; ●, the increment of ferricyanide in phase I.

in the order of selectivity coefficient in a liquid membrane electrode sensitive to anion[12]. It has been shown that the selectivity coefficient is mainly determined by the difference of the standard chemical potentials between aqueous and oil phases, i.e., by the lipophilicity. The results show that lipophilic anions such as ClO_4^- or SCN^- are potent to induce the redox reaction, suggesting that the permeation of anion is necessary. We measured the quantity of perchlorate transported from phase II to I, and the result is shown in Fig. 3. Although quantity of perchlorate transported is found to be a little larger than the amount of ferricyanide produced in phase I, we regard that these two quantities are approximately equal.

We also measured the membrane potentials in these systems. The membrane potentials for combination of sulfate (phase I) and perchlorate, thiocyanate, nitrate, bromide and chloride anions (phase II) were found to be 190, 160, 100, 65 and 40 mV, respectively. The polarity was negative in phase I with respect to phase II, and the potentials were virtually independent of time at least 30 min after initiation of the experiment. The potentials acted in the same direction as the resultant electron flow caused by the trans-membrane redox reaction: The reaction which brings about the production of ferricyanide in phase I and the disappearance of ferricyanide in phase II is equivalent to an electron flow across the membrane from phase I to II. Moreover, the order of the magnitude of the potentials is in accordance with that of electron transfer rate, and hence it is possible that the potential might be responsible for part of the observed rate. To examine the role of the membrane potential in the electron transfer rate, i.e., the rate of the redox reaction, we clamped the membrane potential at 0, 500 and -500 mV (the value is expressed with respect to phase II)[13]. This made no difference to the observed results (data not shown); that is, the membrane potential did not contribute to the rate of redox reaction in this system. This indicates that the trans-membrane redox reaction occurred against its free energy gradient.

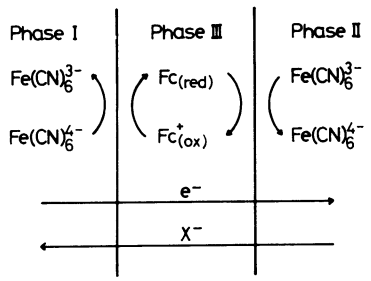

Fig. 4. Diagrammatic representation of coupling between diffusion and redox reaction.

From the results described above, the mechanism of the present system can be visual-

ized as shown diagrammatically in Fig. 4. Before addition of a lipophilic anion (denoted as X^-) into phase II, Fc in phase III is not virtually oxidized by ferricyanide in phase II, because oxidized ferrocene (Fc^+_{ox}) has a positive charge and the production of Fc^+_{ox} breaks the electroneutrality condition in phase III. Note that ferricyanide and ferrocyanide are not able to be counter-ions of Fc^+_{ox} due to their low lipophilicity. When X^- is added to phase II, the redox reaction at the interface between phase II and III begins to proceed owing to the presence of extractable negative charge of X^-. In phase III, an ion pair between Fc^+_{ox} and X^- diffuses toward phase I. The increase of the concentration of Fc^+_{ox} and X^- at the interface between I and III drives the redox reaction, i.e., $Fc^+_{ox} + Fe(CN)^{4-}_6 \longrightarrow Fc_{red} + Fe(CN)^{3-}_6$, which is the reverse reaction occurring at the interface between phase II and III. In addition, X^- is liberated from phase III to I due to the production of neutral Fc_{red}. Fc_{red} produced migrates toward phase II and oxidized there. These processes are repeated. As a result of these processes, X^- diffuses from phase II to I, and the trans-membrane redox reaction, i.e.,

$$Fe(CN)^{4-}_{6,I} + Fe(CN)^{3-}_{6,II} \longrightarrow Fe(CN)^{3-}_{6,I} + Fe(CN)^{4-}_{6,II}$$

takes place via Fc, where subscripts I and II represent phase I and II, respectively.

References

(1) P. Mitchell, Biol. Rev., 41, 445 (1966).
(2) P. J. Garrahan and I. M. Glynn, J. Physiol., 192, 237 (1967).
(3) R. Panet and Z. Selinger, Biochim. Biophys. Acta, 255, 34 (1972).
(4) J. H. Moore and R. S. Schechter, Nature, 222, 476 (1969).
(5) J. P. Behr and J. M. Lehn, J. Am. Chem. Soc., 95, 6108 (1973).
(6) E. M. Choy, E. F. Evans and E. L. Cussler, J. Am. Chem. Soc., 96, 7085 (1974).
(7) M. Sugiura and T. Shinbo, Bull. Chem. Soc. Jpn., 52, 684 (1979).
(8) T. Shimidzu, M. Yoshikawa, M. Hasegawa and H. Chiba, Kobunshi Ronbunshu, 34, 753 (1977).
(9) T. Shinbo, K. Kurihara, Y. Kobatake and N. Kamo, Nature, 270, 277 (1977).
(10) M. Avron, in Experimental Plant Physiology (ed. by A. San Pietro) p.21 (C. V. Mosby Co., St. Louis, 1974).
(11) T. Ozawa, in Shinjikkenkagakukoza Bunsekikagaku Vol. 1 (ed.by K. Nakano and Y. Yoshino) p.247 (Maruzen Co., Tokyo, 1976).
(12) N. Lakshminarayanaiah, in Membrane Electrode, p.254 (Academic Press, New York, 1976).
(13) A. Imamura, in Seitaimaku Jikkengijutsu (ed.by T. Onishi) p.301 (Nankodo, Tokyo, 1967).

(Received June 26,1979)