CHEMISTRY LETTERS, pp. 1011-1014, 1978. Published by the Chemical Society of Japan

PHOTOCHEMICAL REACTION IN/ON MEMBRANE LIKE ASSEMBLIES: PHOTOSENSITIZED REDOX REACTION IN/ON LIPOSOME USING ANTHRAQUINONE DERIVATIVE

Yukio SUDO and Fujio TODA

Department of Synthetic Chemistry, Faculty of Engineering,
University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113

Photoreduction of Fast Red A by ascorbate in/on liposome, using anthraquinone derivative with a long hydrocarbon chain, as a sensitizer was studied. In liposome system, the reaction was accelerated and proceeded through the Michaelis-complex.

Chlorophylls in chloroplast are contained in thylakoid membrane as a two-dimentional array, and this orientation is considered important for photosynthesis. Although chlorophyll-sensitized photoreduction of several dyes and NAD (so-called Kranovskii's reaction) in homogeneous system has been studied by several groups[1,2,3,4], little has been reported about the photosensitized redox reaction in/on bilayer membrane.[5,6]

In this paper, we wish to report the photochemical reduction of Fast Red A in/on lecithin bilayer membrane, liposome, sensitized by an anthraquinone derivative with a long hydrocarbon chain. Liposome is amphoteric ionic bilayer membrane of lecithin, and it can orient the anthraquinone derivative with a long hydrocarbon chain.

Anthraquinone is chosen because it is a good sensitizer in the photoredox reaction of organic substrates, and the reaction mechanism is well known.[7,8,9] In addition, quinone in a redox cycle plays an important role in the proton and electron transports through the biological membrane.

The reactions in a liposome system (system L) were carried out in the following way. Lecithin (isolated from egg yolk[10]) with a given amount of stearyl anthraquinone-2-sulfonate (SAQ, preparated in the usual way) was dispersed in the aqueous solution of Fast Red A (FRA, 10^{-4}M), ascorbic acid (ASC, 0.01 M), and KCl (0.01M). The liposome was then prepared by ultrasonicating the dispersion. After degassed by passin N_2 gas, the solution was sealed in a Pyrex tube with a UV cell on the side arm, and was irradiated by a super-high pressure mercury lamp at room temperature. The reaction was determined by measuring the decrease in a absorption band of FRA at 500nm.

As a control experiment, the reaction was carried out without lecithin in an aqueous homogeneous system (system A). Other experimental conditions in the system A were the

same as in the system L, except that anthraquinone-2-sulfonate (AQ) was used instead of SAQ as a sensitizer.

The absorption maxima of AQ in water (system A) were observed at 255, 275, and 330 nm. In the case of SAQ in liposome (system L), the absorption maxima were almost the same as those of AQ in water, but the shape of the absorption was broader.

SAQ is existed in the lipid phase of liposome, since it is insoluble in water and is hydrophobic. Because of the low permeability of ASC in lecithin, ASC is in the aqueous phase. FRA is thought to be existed both in the lipid phase and in the aqueous phase.

This reaction proceeds through the following steps.

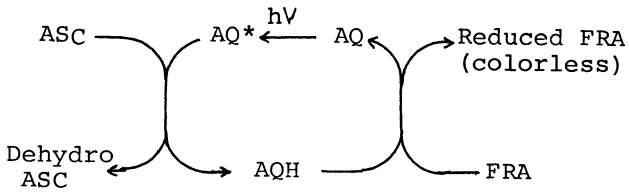
(1) $AQ \xrightarrow{h\nu} AQ^*$

(2) $AQ^* \longrightarrow AQ$

(3) $AQ^* + ASC \longrightarrow AQH + \text{Dehydro ASC}$

(4) $AQH + \text{Dehydro ASC} \longrightarrow AQ + ASC$

(5) $AQH + FRA \longrightarrow AQ + \text{Reduced FRA}$



(AQ* = AQ in the excited state, AQH = reduced AQ; 9,10-dihydroxy anthracene derivative, in sytem L AQ = SAQ, AQH = SAQH)

Without light FRA cannot be reduced by ASC, because the redox potential of FRA is lower than that of ASC. Without AQ it was confirmed by the blank test that FRA did not fade even when it was irradiated.

As shown in Fig.1, in the system A, the reaction rate was constant during irradiation, but it decreased with time in the system L. From this observation and Fig.3, the reaction rate of fading of FRA is given by:

For the system A

$$d[FRA]/dt = -k_0[AQ] \qquad (6)$$

For the system L

$$d[FRA]/dt = -k_1[FRA] \qquad (7)$$

$$= -k_2[FRA][AQ] \qquad (8)$$

where ASC is added in excess and [ASC] is considered constant during reaction.

As shown in Fig.2, the fluorescence of SAQH (or AQH) was observed at 480nm only in the photolyzed solution of the system L.

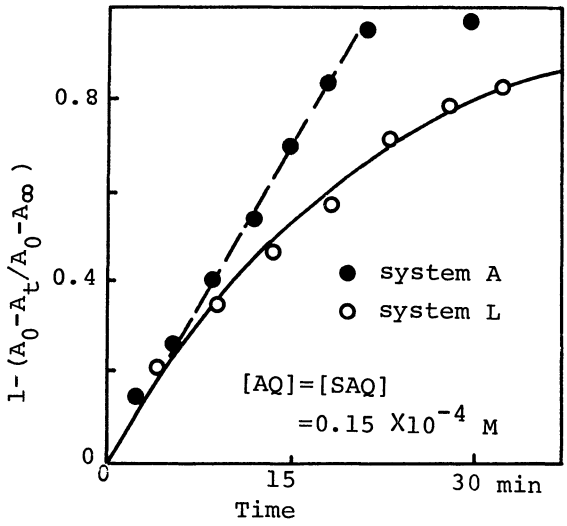


Fig.1 The change in absorption at 500nm with time of irradiation. (A=absorption)

These results can be explained as follows. In the system A, neither AQH nor AQ* is stable, and the rate-determining step is the reaction (3). The reaction rate was independent of the concentration of FRA.

On the other hand, in the system L, SAQ* and SAQH were stabilized in the membrane probably due to the effect of the hydrophobic environment, and the reverse reaction, reaction (4), was inhibited because SAQH in the lipid phase was separated from Dehydro ASC in aqueous phase. So the rate was not determined by the reaction (3), and stabilized SAQH was found by fluorescence technique only in the photolyzed solution of the system L.

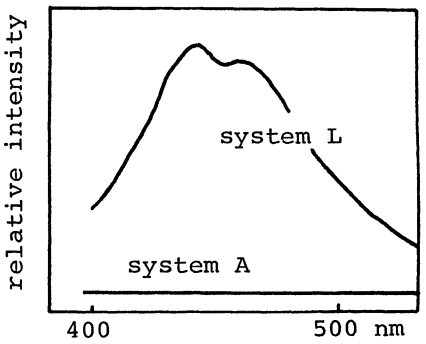


Fig.2 Fluorescence spectra of photolyzed solution of the system L. (Excitation wavelength=400nm)

As shown in Fig.3, a linear relationship exists between AQ and the initial reaction rate of fading of FRA (V_0), and no saturation of V_0 was observed in the system A. In addition, a concentration quenching was not observed under this condition.

On the other hand, approximate linear relationship was found between V_0 and [SAQ] while [SAQ] was low in the system L. and V_0 was four times as great as V_0 in the sys-

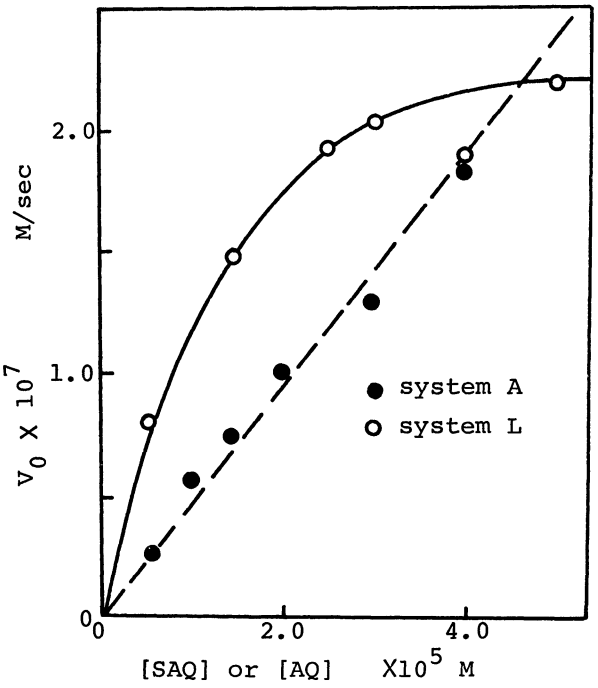


Fig.3 The dependence of initial rates (V_0) on [SAQ] or [AQ].

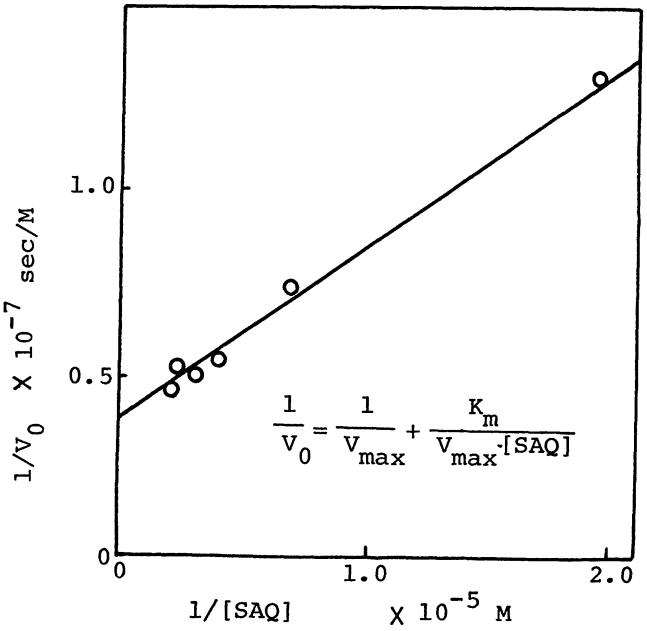


Fig.4 The Lineweaver-Burk plot of the system L.

tem A. In the initial stage of the reaction, the reaction was accelerated in the lower concentration of SAQ. But the initial reaction rate (V_0) appears to level off in the higher concentration of SAQ. This saturation was also found in the relation between k_1 and SAQ.

Figure 4 shows the Lineweaver-Burk plot of the system L, and a good linear relationship was found (correlation factor = 0.993). From this plot and the saturation curve (descrived above), it is concluded that the reaction proceeds through the Michaelis-complex in the system L.

Further investigation of the mechanism of the stabilization of SAQH and SAQ* are now undertaken.

References

1) A. A. Kranovskii, Dokl. Acad. Nauk. SSSR, 60, 421 (1948).
2) Leo P. Vernon, Acta. Chem. Scand., 15, 1639 (1961). Arch. Biochem. Biophys., 109, 92 (1965).
3) G. R. Seely, J. Phys. Chem., 69, 821 (1965).
4) S. M. B. Costa, Proc. Roy. Soc. London A, 341, 167 (1974).
5) M. Mangel, Biochim. Biophys. Acta., 430, 459 (1976).
6) Y. Toyoshima, M. Morino, and M.Sukigara, Nature, 265, 187 (1977).
7) K. P. Clank and H. I. Stonehill, J. Chem. Soc. Farad. Trans. 1, 68, 187 (1977).
8) S. A. Carlen and D. M. Hercules, Photochem. Photobiol., 17, 123 (1973).
9) K. Kano and T.Matsuo, Bull. Chem. Soc. Jpn., 47, 2836 (1974).
10) C. H. Lea, D. H. Rhodes, and R. D. Stoll, Biochem., 60, 353 (1955).

(Received June 19, 1978)