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A VOLTAMMETRIC INVESTIGATION OF TCNQ-CONTAINING BILAYER LIPID MEMBRANES

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SUMMARY: Cyclic voltammetry (CV) is a useful tool for investigating electrochemical redox reactions. A number of bilayer lipid membranes containing TCNQ (7,7,8,8-tetracyanoquinodimethane) have been studied by the CV technique. The results indicate electron movement across the lipid bilayer and redox reactions at the membrane/solution interfaces.

The mechanisms by which oxidation-reduction (redox) reaction takes place in, on and/or across biomembranes are not well understood owing mainly to the complexity of living structures. Numerous attempts however have been made in investigating simpler, better defined artificial lipid bilayers (planar bilayer lipid membranes or BLM for short and spherical bilayer lipid membranes or liposomes). The results obtained to date, especially on pigmented BLMs and liposomes under illumination, provide support for light-induced charge separation and eventual redox reactions (1-4). These electronic processes appear to be the principal mechanisms for energy transduction. In the absence of light evidence for electronic processes in these BLMs and liposomes is somewhat equivocal, although certain experimental data are supportive (4-6). One reason for the lack of a clean-cut demonstration of redox reactions in the dark in these lipid bilayers stems from the fact that a suitable experimental technique with well-established theoretical basis has not been tried. To remedy this situation, we have had occasion to use the method of cyclic voltammetry (CV), which is eminently suited for investigating redox reactions (7). The method of CV was used in the course of our studies of photoeffects in dye-sensitized BLM (8). In this paper we describe briefly the essential aspects of cyclic voltammetry (CV) as applied to the BLM system and report a new type of BLMs containing TCNQ (7,7,8,8-tetracyanoquinodimethane) whose

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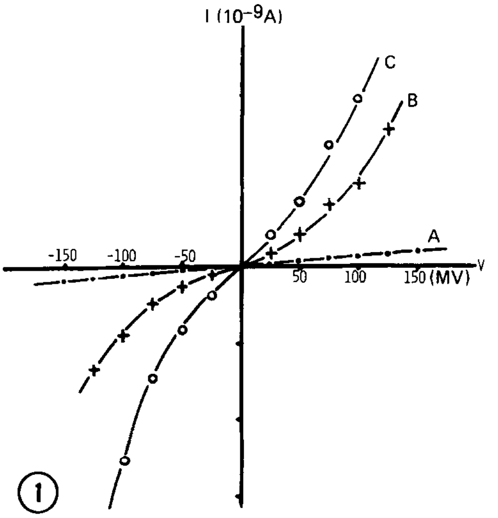
electrical properties are such as to leave little doubt that transmembrane redox reactions are occurring in the dark, with electrons being transferred from one side of the BLM to the other.

MATERIALS AND METHODS: All reagents and special organic compounds such as 7,7,8,8-tetracyanoquinodimethane (TCNQ) and crystal violet (gentian violet) were obtained commercially and were of the highest quality available. The bilayer lipid membranes (BLMs) were formed at room temperature according to the technique described elsewhere (1). The hole of 0.15 cm diameter in the Teflon cup was usually pretreated with the BLM-forming solution to be used later. The BLM-forming solution consisted of a mixture of phosphatidylethanolamine, PE (3.3%) and phosphatidylserine, PS (1.3%) in n-decane. To form TCNQ-containing BLM, aliquots of a saturated solution of TCNQ in $CHCl_3$ were added to the lipid solution. The bathing solution usually contained 0.1M KCl and 0.1M sodium acetate buffer (pH 5.5). Unless otherwise noted, the outer solution also contained 0.5mM $K_3Fe(CN)_6$ and $K_4Fe(CN)_6$.

The electrical properties of TCNQ-containing and other BLM were investigated by a voltammetric method known as cyclic voltammetry (CV). The basics of cyclic voltammetry consist of cycling the potential of a working electrode in an unstirred solution and measuring the resulting current. The potential of the working electrode is controlled relative to a reference electrode, which is provided by a triangular potential waveform generator. The current/voltage (I/V) response patterns obtained during the potential scan by measuring the current at the working electrode is known as a voltammogram. Interpreting such voltammograms based on electron transfer and subsequent redox reactions at the working electrode/solution interface has been well-established and widely employed (7,9). Typical working electrodes used in CV are either Pt, glassy carbon or carbon paste electrode. It is realized of course that an unmodified BLM behaves essentially as an excellent insulator (specific resistivity $>10^{14}\ \Omega$) and does not function as a working electrode. It is necessary therefore to incorporate certain compounds into the BLM endowing it with the desired characteristics. For such purposes, we have tried a number of compounds such as TPP (tetraphenylporphyrin) and organic conductor TCNQ. The other experimental details are given elsewhere (8).

RESULTS AND DISCUSSION: In an earlier study (8) it was observed that certain dyes, when incorporated into BLM, exhibit unusually large open-circuit voltage (examples: Chlorozol Black E, 86mV; Methyl violet 2B, -82mV; crystal violet or gentian violet, -110mV). Of these dyes we decided to investigate gentian violet in more detail. Fig. 1 shows clearly that the resistance of BLM is greatly altered as the dye concentration is increased. This observation is consistent with the findings of Sudo et al. (10), who have found that certain dyes in spherical bilayer lipid membranes (liposomes) can move in the lipid phase and penetrate the bilayer easily, because most of them are amphipathic. In our study such dyes (crystal violet, malachite green and Janus green) act as electron acceptors (8). Although only Fe^{3+} was added to one side of the bathing solution, a minute amount of Fe^{2+} was always present. Therefore, the redox reaction $Fe^{2+} - e \rightleftharpoons Fe^{3+}$ takes place at the membrane/solution interface



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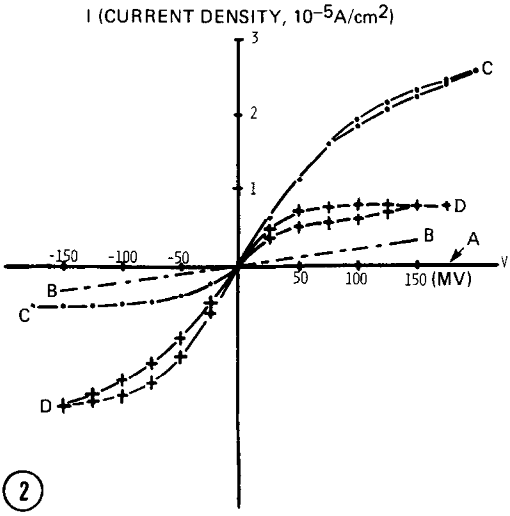

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Fig. 1. Effect of gentian violet (crystal violet) on PE/PC BLM voltammograms. BLMs were formed in 0.1M KCl and 0.1M sodium acetate at pH 5.6. The bathing solution on both sides of the membrane also contained gentian violet at three different concentrations: (a) 7.8 X 10^{-7}M (b) 1.25 X 10^{-5}M (c) 5 X 10^{-5}M.

Fig. 2. Typical voltammograms for TCNQ-containing BLMs except (a). All BLMs were formed from the PE/PC lipid solution in 0.1M KCl. Other conditions were: (a) 5 X 10^{-4}M $K_3Fe(CN)_6$ + $K_4Fe(CN)_6$ // BLM // 2.4 X 10^{-3}M ascorbic acid. (b) 0.1M KCl // TCNQ-BLM // 0.1M KCl. (c) 5 X 10^{-4}M $K_3Fe(CN)_6$ + $K_4Fe(CN)_6$ // TCNQ-BLM // 2.4 X 10^{-5}M gentian violet. (d) 5 X 10^{-4}M $K_3Fe(CN)_6$ + $K_4Fe(CN)_6$ // TCNQ-BLM // 9.4 X 10^{-4}M ascorbic acid.

mediated by the dye-containing BLM. Such a behavior of the dye (crystal violet) is corroborated by a negative dark membrane potential as already noted.

An unmodified BLM behaves essentially as an excellent insulator and does not function as a working electrode. We have found that upon incorporation of certain organic semiconductors such as TCNQ (11) into BLM, very exciting results are obtained. TCNQ belongs to the class of molecular conductors, which have shown remarkably large conductivity at room temperature ($\sim 10^2 - 10^3\ \Omega^{-1}cm^{-1}$). It is a planar, symmetric, "electron-poor" molecule that can easily gain an electron to become an anion radical (11). Fig. 2 shows the voltammograms of TCNQ-containing BLMs (curves b-d). With equal molar concentration of $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ on one side of the TCNQ-BLM, the membrane can play the role of a working electrode. In the absence of TCNQ in BLM with other experimental conditions the same, the I/V is linear; it practically coincides with the x-axis on the scale used (Curve A, Fig. 2). In the absence of redox couples in the bathing solution, but with TCNQ in the BLM, the I/V curve remains linear (Curve B). Dramatic changes in voltammograms are seen when redox or membrane-active compounds are introduced into the bathing solutions. Curve C of Fig. 2 shows the effect of crystal violet on one side of the TCNQ-BLM, the other side contained the $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ couple. The polarity of E_m is negative on the side of the dye, which is caused by the reduction of crystal violet via transmembrane electron movement from the $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ side. When ascorbic acid is present instead of crystal violet, just the opposite results are obtained as shown in Curve D, Fig. 2. Here ascorbic acid is believed to be oxidized to dehydroascorbic acid with electrons moving towards the $Fe(CN)_6^{-3}/Fe(CN)_6^{4-}$ side via TCNQ-BLM.

In conclusion, we have applied the cyclic voltammetric technique to a new type of BLM containing TCNQ, whose electrical properties are such as to leave little doubt that transmembrane redox reactions are occurring in the dark, with electrons being transferred from one side of the BLM to the other side.

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