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Photoredox Reactions in Pigmented Bilayer Lipid Membranes

Paul K. Shieh and H. Ti Tien
BLM Research Laboratory, Biophysics Department
Michigan State University
East Lansing, Michigan 48824

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Abstract

Bilayer lipid membranes (BLM) formed from chloroplast extract and oxidized cholesterol in the presence of water-soluble dyes have been investigated. The two experimental systems described are: (1) the photoactive compound is incorporated into the BLM-forming solution, and (2) the photoactive substance is introduced to one side of the solution bathing the BLM. In the first system of Fe^{3+}/Chloroplast BLM/Ascorbic acid, for example, a photovoltage of nearly 300 mV has been observed when an external voltage is imposed. In the second system of Fe^{3+}, Fe^{2+}/oxidized cholesterol BLM/chlorophyllin, the photoresponse can exceed 350 mV under applied field conditions. The results of these experiments are interpreted in terms of redox reactions across the pigmented BLM where one side of the BLM biface is reducing and the other side oxidizing.

Introduction

The recognition of the importance of lamellar membrane structure in photosynthesis may be said to have begun with studies of chloroplasts under the light microscope. With the advent of the electron microscope the ultrastructure of the chloroplast has been revealed to consisting of highly organized membranes in the order of 100A thick [1, 2]. As a result of these investigations, several models of the photosynethic membrane structure have been proposed; all of which postulate an ultrathin layer comprising of pigments, lipids and proteins in an orderly array [1, 2, 3]. Katz [4] and others suggested that an organized structure containing chlorophylls and other pigments may possess photo-conductive properties resembling those of organic semiconductors. Arnold and Sherwood [5] reported semiconductive properties in dried chloroplast and chromatophore preparations. More recently, Tien [6] discovered photoelectric effects in artificially constituted bilayer lipid membranes (BLM) incorporating photosynthetic pigments.

As part of our continuous investigations on the photoelectrochemical properties of pigmented BLM, we have tested a variety of redox compounds, both inorganic and organic, in an attempt to uncover factors affecting the photoresponse. The present communication describes mainly our experimental results.

Mention should be made that light-induced photoeffects in pigmented BLM have been reported by a number of other workers [7, 8, 9, 10]. A detailed review on the interfacial photochemistry of pigmented BLM is available [11].

Experimental

Materials. All reagents were CP grade or best obtainable and were used without further purification. Some of the less common compounds used in this study were obtained from: p-Benzoquinone (Aldrich Chemical Co.); hydroquinone, quinhydrone, riboflavin, nicotinic acid, gallic acid, sodium azide, cobaltic hexamine chloride (Eastman Kodak Co.); ascorbic acid, thiamine, vitamin K_1, flavin mononucleotide, tannic acid, catechin, cytochrome-c (Sigma Chemical Co.); sodium arsenate, NaCl, NaBr, $SnCl_4$ iodine, ammonium molybdate (Mallinckrodt Chemical Co.); NaI, NaF, Na_2S, $FeCl_2$, KI, $CdSO_4$, $CrCl_3$, $K_3Fe(CN)_6$, $K_4Fe(CN)_6$ (Baker Chemical Co.); ceric ammonium sulfate (G. Frederick Smith Chemical Co.); $FeCl_3$ (Matheson, Coleman & Bell Co., Inc.); $Na_2S_2O_3 \cdot 5H_2O$ (Allied Chemical Co.). Fresh spinach leaves were purchased from local food markets.

Preparation of BLM-Forming Solutions. The procedure for preparing the lipid solution from spinach chloroplasts has been given in detail elsewhere [6, 12].

Apparatus and Procedure. Chloroplast bilayer lipid membranes (Chl-BLM) were formed and studied by techniques that have been described previously [6, 12]. Essentially, a BLM was formed in the aperture (≃ 2 mm diameter) of a Teflon cup (≃ 10 ml) separating two aqueous solutions. Electrical contact to each aqueous solution was made through a salt bridge (saturated KCl) attached to a calomel electrode. The potential across the BLM was measured with a Keithley electrometer (model 610B). The exciting light sources used were a 150-watt projection lamp (GE-DFG).

Experimentally, the following arrangement was used in gathering the data:

0.1 M acetate buffer (pH 5) + 10^{-3} M $FeCl_3$	Chl-BLM on Teflon support	0.1 M acetate buffer (pH 5) + modifier

where Chl stands for chloroplast pigments and the word modifier denotes the compound under investigation. Be it noted also that the left half of the arrangement is referred to as the outer solution and arbitrarily chosen as a standard "half-cell". Other pertinent experimental details are given in connection with the results.

Results and Discussion

In the absence of exciting light the d.c. resistance of a typical Chl-BLM (Chl denotes chloroplast) was $2\text{-}10 \times 10^5$ Ω-cm^2 and the dielectric breakdown voltage was generally much less than 200 mV. Current/voltage curves of Chl-BLM appeared to be linear up to 150 mV. As would be expected, only very small or no photopotentials were observed when the BLM was illuminated under *entirely* symmetrical conditions with respect to light intensity, bathing solution, electrical potential, etc. However, it was earlier observed that a very slight potential difference between the contacting electrodes was sufficient to give rise to a detectable photopotential in Chl-BLM separating two identical 0.1 M KCl. The presence of chemical asymmetry, such as by adding $FeCl_3$ to one side of the BLM bathing solutions, not only greatly enhanced the photopotential but generated two kinds of photoresponses: a very fast response followed by a slower one, the latter frequently of opposite sign. In view of this interesting observation, a series of experiments were carried out to characterize the Fe^{+3} /Chl-BLM system. The results are presented in the following paragraphs.

Effect of Applied Field

Owing to the ultra-thinness of the BLM ($\leqslant$ 100 Å) and its very high electrical resistance (many orders of magnitude higher than the bathing solution), a potential difference of even a few mV would result in a tremendous electric field across the BLM. Thus, the oppositely charged species whether generated directly by light or indirectly in the membrane should move in the direction of the external field and be collected at the opposite sides of the BLM (biface). Obviously, the field-assisted charge separation will be greater, the higher the applied voltage (see Fig. 1). The data obtained for a variety of compounds are given in Table I. It is interesting to note that: (a) The greater the applied voltage, positive on the side containing the electron acceptor, the larger is the observed potentials. (b) At zero dark potential, the strongest reducing agent appears to give the highest photopotential among the compounds tested.

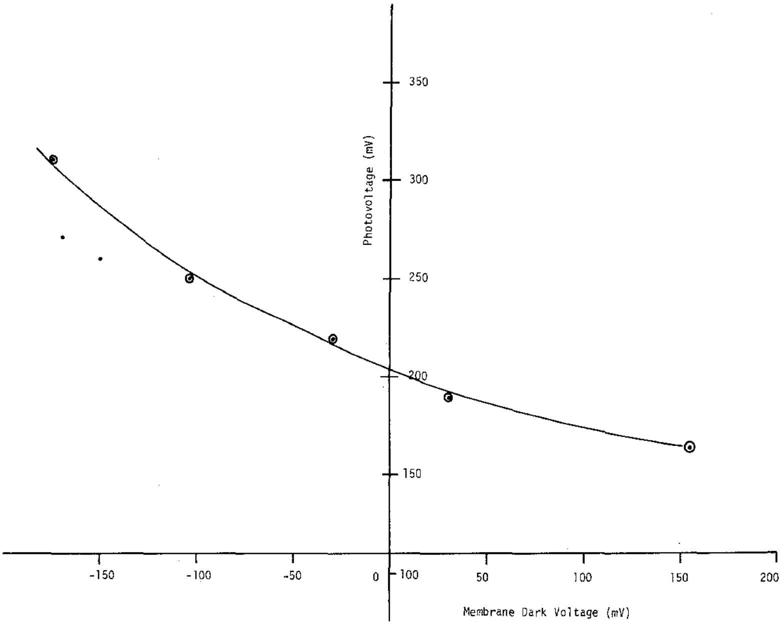

Figure 1. Effect of applied voltage on the photoresponse of a BLM. The cell arrangement used was as follows: Fe^{3+}, Fe^{2+}/oxidized cholesterol BLM/chlorophyllin. The bathing solution was 0.01 M KCl. Membrane voltages in the dark were provided by a battery through a 10^9 resistor.

TABLE I. Effect of Applied Field on the Chl-BLM Photoeffect in the Presence of Various Redox Compounds[a]

Inner Solution[b]	Photopotential (mV) at dark membrane voltage[c] -100	 0	 100
Sodium sulfide	18	-20	-75
Ammonium molybdate	100	65	-26
Flavin mononucleotide	145	92	-40
Catechin	175	98	-20
Tannic acid	185	105	-30
Potassium iodide[d]	158	110	25
Ascorbic acid	220	145	-62
Chlorophyllin[e]	250	203	173

[a] Outer solution was 0.1 M sodium acetate at pH 5 plus 10^{-3} M $FeCl_3$.

[b] Inner solution = 0.1 M sodium acetate at pH 5 plus the compound indicated.

[c] Voltage applied through a 10^9 Ω resistor.

[d] Dark membrane potential = 50 mV.

[e] In this particular case, the cell arrangement was as follows:
Fe^{+3}, Fe^{+2}/oxidized cholesterol BLM/chlorophyllin.

Effect of $FeCl_3$ Concentration

As already mentioned, the photoresponse can be greatly enhanced with $FeCl_3$ in one side of the BLM. In order to establish the optimal $FeCl_3$ concentration which would produce the maximum photo-emf (open-circuit photopotential) in the Chl-BLM, a concentration study was carried out. Experimentally, this was achieved by fixing the bathing solution (0.1 M acetate buffer) at pH 5 and increasing the $FeCl_3$ concentration in one side of the bathing solution. It was found that the presence of 4×10^{-3} M $FeCl_3$ on one side of the BLM gave a maximum enhancement of the photoresponse; the side containing $FeCl_3$ being electrically negative. This finding indicates that ferric ions, or their complexes, are adsorbed on the BLM surface, and that the number of adsorption sites on the Chl-BLM is finite. Besides enhancing the photoresponse, $FeCl_3$ also affected the membrane resistance. The maximal R_m was observed at a concentration of 2×10^{-3} M $FeCl_3$ at pH 5.0. The magnitude of the photo-emf is closely related to the membrane resistance. Very small or no photo-emfs could be detected in Chl-BLM with a resistance lower than 10^5 ohm-cm^2.

Effect of pH of the Bathing Solution

As far as the bathing solution was concerned, it was found that photo-emfs, at a given concentartion of $FeCl_3$ on one side of the BLM, were also pH dependent. This might be due to the effect of the H^+ ion concentration on the titratable groups contained in the Chl-BLM. It is interesting to note that a maximum photo-emf occurs at pH slightly around 5. The coincidence that both of the two maxima, resistance and photo-emf, fall near pH 5 strongly indicates that the photoresponse is closely related to the nature of the constituents and structure of BLM.

The Time-course of the Photoresponse

Owing to the high resistance of BLM, the observed voltage is due almost entirely to the potential drop or charge imbalance across the biface (which includes the electrical double layer adjacent to each of the two solution-BLM interfaces). In the usual measurement, the membrane potential is monitored as a function of time. The resulting time-course curves vary from a simple monotonic response to a complex tri-phasic waveform. The system, containing Fe^{+3} in both sides of the bathing solution, appears to be completely reversible and reproducible.

The photopotentials consisted of at least a fast phase followed by a slower phase, usually of opposite sign. The initial fast response is much more labile than the slow response. The fast response did not appear until sufficient chemical asymmetry was present to give an electrochemical potential of 25 mV or more. The slow response was always opposed to the electric field, and magnitude of which depended on the magnitude of the dark potential and could be reversed by applying a potential of opposite polarity. In order to reverse the sign of the fast response, however, the applied potential had to be equal to or greater than, the magnitude of the dark potential. For instance, the fast response of a Chl-BLM separating two solutions (pH 4 and pH 6, respectively) did not disappear until the applied voltage exceeded 112 mV. Furthermore, continuous illumination (> 30 sec) of the BLM destroyed the fast response but not the slow one when light excitation periods were 1 to 3 min apart. It was possible that, given sufficient time in the dark (e.g., 30 min), the fast response could be rejuvenated.

Effect of Inorganic Compounds

Table II lists inorganic substances that have been found to enhance the Chl-BLM photoeffect. A number of other compounds not listed in the Table were also tested and their ability to enhance the

TABLE II. Effect of Inorganic Compounds on the Chl-BLM Photo-emf
The Cell Arrangement:
Salt bridge | Outer solution[a] | Chl-BLM[b] | Inner solution[c] | Salt bridge

Inner solution (M)	Photo-emf (mV)	Remarks
NaI (5×10^{-3})	74-127	Biphasic response
$Na_2S_2O_4$	44-120	
$K_4Fe(CN)_6$	109-150	also observed with $K_3Fe(CN)_6$ in place of $FeCl_3$
$Na_2S_2O_3$	74-93	Dark red ppt. with Fe^{+3}
$FeCl_2$ (4×10^{-3})	80-86	
$(NH_4)_6Mo_7O_{24}$	64-81	Dark potential induced, white ppt. with Fe^{+3}
Sn^{4+}/Sn^{3+}	92	see footnote (d)
Na_2HAsO_4	44	
NaN_3 ($1{,}3 \times 10^{-2}$)	34-65	
TlCl (1.8×10^{-2})	38-67	
NO_3^-/NO_2^-	60	see footnote (d)
CuCl (2×10^{-2})	54-72	10-20 mV dark potential
Aqueous acetate buffer	20 ± 5	
$[Co(NH_3)_6]Cl_3$	-15	Better electron acceptor than Fe^{+3}
Na_2S (3×10^{-3})	-7 to -30	Better electron acceptor than Fe^{+3}
I_2 (6×10^{-3})	-8 to -31	Better electron acceptor than Fe^{+3}
Ce^{4+}/Ce^{3+}	-35	see footnote (d)

[a] Outer solution = 0.1 M acetate at pH 5 + 10^{-3} M $FeCl_3$.

[b] Chl-BLM forming solutions were prepared using procedure given in Rev. 12.

[c] Inner solution = 0.1 M acetate at pH 5 + the compound indicated.

[d] The following cell arrangement was used: Fe^{3+}, Fe^{2+}/Chl-BLM/Redox couple. The bathing solution was 0.1 M NaAc at pH 5.

photoresponse remains to be ascertained in further experiments. These compounds include alkali metal chlorides (Li, Na, K, Rb, Cs), Na_2HAsO_4, and $LaCl_3$. However, definite effects were obtained with KNO_3 and $NaNO_2$.

Effect of Organic Materials

A number of organic compounds, listed in Table III, have been found equally effective in increasing the Chl-BLM photoresponse. By far, not only the largest photovoltage was observed in the system of $FeCl_3$/Chl-BLM/Ascorbic acid, but appeared completely reversible to repeated illumination. When the reference electrode (the outer solution) is poised at Fe^{3+}/Fe^{2+} equal to unity and the inner solution contains 1.5×10^{-3} M of ascorbic acid, a photovoltage on the order of 175 ± 15 mV is usually observed. The mid-point potentials for the couples, under similar experimental conditions, are, 340 mV for Fe^{3+}/Fe^{2+} and 127 mV for dehydroascorbic/ascorbic acid, respectively. It is seen that the difference of the two redox potentials

TABLE III. Effect of Organic Compounds on the BLM Photo-emf[a]

Inner solution (M)	Photo-emf (mV)	Remarks
Ascorbic acid (4×10^{-3})	125-188	240 mV with an applied field
Flavin mononucleotide (FMN)	138-167	White ppt. disappearing with time
NQ-OH (10^{-2})[b]	140	see footnote (d) Table II
Oolong tea (4×10^{-3} g/ml)	132	
Tannic acid (4.5×10^{-7})	113-133	Also observed with Oolong tea
Catechin (3.5×10^{-3})	92-121	
Cystine/cysteine	108	see footnote (d) Table II
Riboflavin (8×10^{-5})	79-99	
Hydroquinone (7×10^{-4})	79-96	
$NQ\text{-}2\text{-}SO_3(K)$ (10^{-2})[c]	80	see footnote (d) Table II
Gallic acid (10^{-2})	72-84	
Methyl viologen (4×10^{-2})	73	$K_4Fe(CN)_6$ used in place of $FeCl_3$
Ferrozine	85	A complexing agent for Fe^{+2}
β-NAD (6×10^{-4} g/ml)	67	Gave a negative dark membrane potential
Cytochrome c (2×10^{-5} g/ml)	48	Generated a dark membrane potential
Quinhydrone (7×10^{-4})	45-64	
Vitamin K (2.4×10^{-2} g/ml)	37	
Benzoquinone (2.8×10^{-4})	−4 to 16	10-15 mV dark potential
Eosine + KI (1 mM)	225-245	see footnote (d) below

[a] See Table II for other experimental details.

[b] NQ-OH = 2-hydroxyl-1,4 naphthoquinone.

[c] $NQ\text{-}2\text{-}SO_3$ (K) = potassium salt of 1,4-naphthoquinone-2-sulfonic acid.

[d] The cell arrangement was as follows:
Tetraphenylboron/oxidized cholesterol BLM/Eosine, KI. The bathing solution was 0.1 mM Na acetate at pH 4.

(340 – 127 = 213) is in good agreement with the observed photovoltage, thereby providing support to the idea that the Chl-BLM functions as two coupled redox electrodes with one side reducing and the other side oxidizing [6, 11].

Photosensitization of Oxidized Cholesterol BLM by Dyes

Certain BLM, such as those formed from oxidized cholesterol or phospholipids which are otherwise not photoactive, can be sensitized by certain inorganic ions [7] and organic dyes [11] dissolved in the aqueous solution. Experimentally, the BLM was formed in the usual manner, on a hole in the side of a Teflon cup separating two aqueous solutions. A standard BLM-forming solution of oxidized cholesterol in *n*-octane was used [12]. After the membrane had reached the black state, a known volume of the bathing solution on one side of the BLM was withdrawn and an equal volume of solution, containing the dye was added. Fig. 2 illustrates typical photo-voltage vs. time curves of a

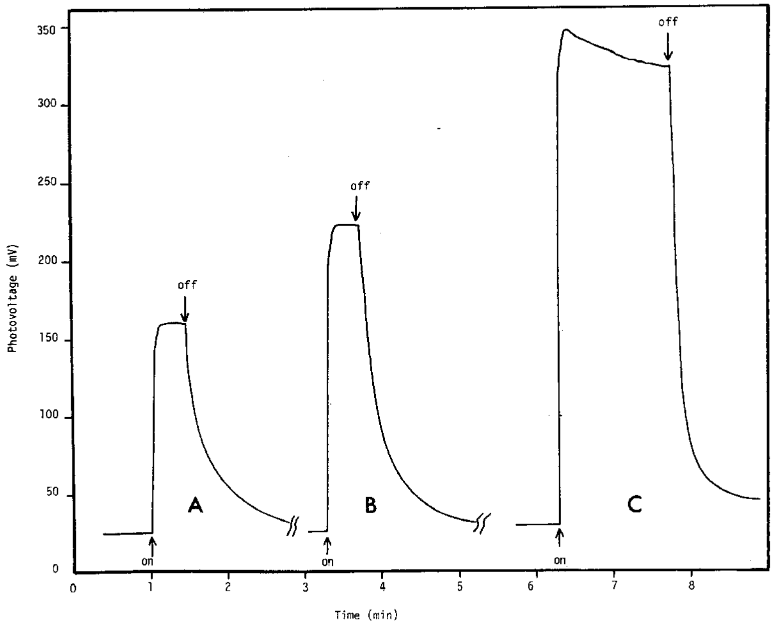

Figure 2. Photovoltage vs. time curves of an oxidized cholesterol BLM. Other experimental conditions are given in Fig. 1. A. Open-circuit response. B. Photovoltage measured with a 10^9 ohm shunt resistor but without externally applied voltage (membrane dark voltage = 30 mV, the chlorophyllin side positive). C. Photovoltage measured with –175 mV applied through a 10^9 ohm shunt resistor with the chlorophyllin side negative. Membrane resistance = 10^9 ohms.

oxidized cholesterol BLM sensitized by water-soluble chlorophyll (Chlorophyllin). This system is of particular interest in that the photoresponse has been found to be much larger than that of Chl-BLM. It was noted also that the presence of the dye lowered the BLM resistance by an order of magnitude. Evidently the dye not only interacts with the membrane but serves a dual function that of acting as light absorbing pigment in the membrane and as an electron donor (or acceptor as the case may be) in the bathing solution. Similar photoresponses were observed with eosine and other dyes in oxidized cholesterol BLM (see Table III).

General Discussion and Conclusions

The usual picture of a BLM separating two electrolyte solutions consists of a liquid hydrocarbon core ($\sim$50 Å) interposed between two polar regions which contain the sites for ion building. These sites, if charged, determine the interfacial potentials, and constitute one of the two major factors governing the ion transport; the other factor being the hydrophobic interior of the BLM. As previously suggested [6], pigmented BLM is considered as a photovoltaic device and the transport of charges across the membrane can take place by means of either electronic or ionic processes or both [11]. Conduction by ions, however, is governed by the nature of surface-active species in the BLM system owing to the existence of electrical double layers at the two co-existing aqueous-solution/BLM interfaces (biface). The observed photoeffects have been explained as follows. The photon energy of exciting light absorbed by the pigment in or/and near the BLM, causes charge carriers in the form of electrons and hole (ion-radicals) to be generated, presumably *via* either direct electron ejection or exciton dissociation. To sustain steady state photoeffects, coupled redox reactions (e.g. oxidation of water and reduction of electron acceptor) across the BLM must occur. Strong evidence supporting electronic processes apart from the photovoltaic effect, is that the action spectrum of a photoactive BLM resembles the absorption spectrum of the photoactive species in solution [11]. The results of the present investigations further reveal that the photoeffects in pigmented-BLM are strongly influenced by externally applied voltage and dark membrane potential as well as by the nature of modifiers present in the bathing solution.

The observation of photoeffects in pigmented BLM affords an opportunity to speculate on possible mechanisms of energy conversion and storage *via* ultrathin lipid membranes as depicted in Fig. 3. The energy levels of the electron acceptor and donor must lie, respectively, below the ground state and above the excited state of the photoactive species in the BLM. The energy of the absorbed photon is stored, therefore, in terms of the redox products, since the transferred electrons are at a higher energy level than that of the donor (Fig. 3B). In the second case, shown in Fig. 3A, a thermodynamically favoured reaction is able to proceed as a result of the lowering of energy barrier (e.g., the hydrocarbon portion of the BLM). It is quite analogous to certain classical photosensitized reactions where the chlorophyll acts as the photosensitizer. Although photosensitized reactions of the second type are of interest, for example, in the elucidation of reaction mechanisms, they are not energy-conserving processes. From the viewpoint of conversion of light into chemical energy, research effort with BLM are being centered on reactions where energy storage is effected; the ultimate source of electrons desired should originate from water.

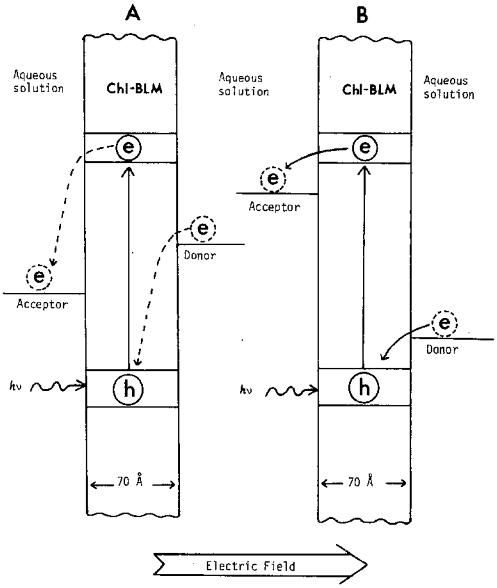

Figure 3. Mechanisms of light-elicited phenomena in pigmented BLM. The BLM is considered as an ultrathin layer of liquid crystalline phase separating two highly conducting aqueous solutions. (A) Photosensitized redox reactions where light mainly lowers the activation energy for thermodynamically favoured processes to proceed. (B) Energy conversion via energy transducing membrane where light is the driving force. The energy of the absorbed photon is stored in terms of redox products.

In this investigation, we have examined the BLM photoeffects of a large variety of modifiers (electron acceptors and donors). The compounds tested include simple inorganic electrolytes (e.g., $FeCl_3$ and

KI) and complex organic molecules (e.g. tannic acid and flavin mononucleotide). The magnitude of the observed photopotentials appeared to be related to the standard redox potentials of the compounds. The effect of ligands in the case of transition elements (Fe^{+3} and Co^{+3}) is noted.

At present, we are attempting to correlate the observed photo-emfs with redox potentials, electron affinity, and ionization potentials of these compounds. If this can be done, a useful table may be constructed listing the relative oxidizing or reducing power of these compounds which may aid us in the understanding of membrane redox processes in nature.

Acknowledgements

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