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A CAROTENOPORPHYRIN - QUINONE TRIAD DESIGNED FROM A BASKET-HANDLE PORPHYRIN : SYNTHESIS AND PHOTO ELECTROCHEMICAL PROPERTIES.

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ABSTRACT : The synthesis of a carotenoporphyrin-quinone triad in which electron donor and acceptor moieties are covalently linked to superstructures of a photochemically active basket-handle porphyrin is described and its characterization by ^{1}H NMR and UV - visible spectroscopy is reported. Under light excitation of this compound, when incorporated into a phospholipid bilayer membrane and in the presence of redox compounds in the aqueous phases bathing the membrane, a steady-state photocurrent is observed, which implies a charge separation.

INTRODUCTION

The mechanism of the primary processes of photosynthesis at the molecular level has been successfully pursued in recent years. Most of the membrane components and chromophores implied in these processes have now been identified. A realistic view of their location and their orientation in the membrane has been attained.

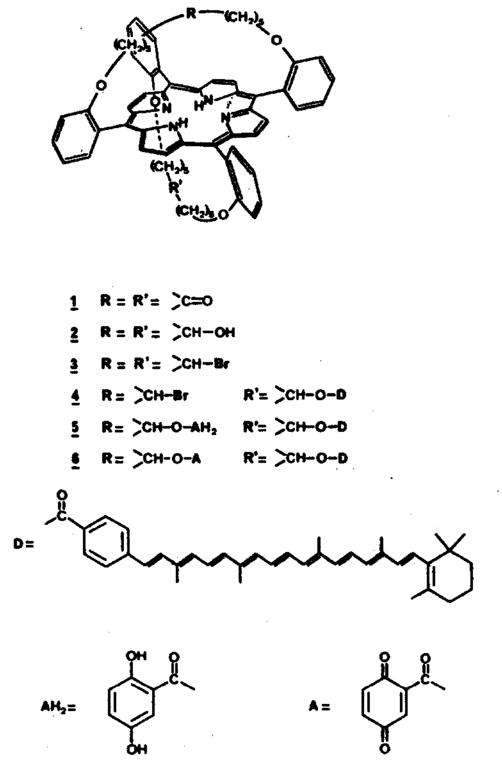
The structural knowledge required for a more subtle understanding of the mechanisms has recently been given by the magnificent work which has just won Deisenhoffer, Huber and Michel the Nobel prize in Chemistry. They crystallized bacterial photosynthetic reaction centers and determined their three-dimensional geometry (1). However, despite this considerable advance in the structural knowledge of the energy converting assemblies, the physical mechanisms of the long-range (> a few Å) electron transfers are still uncertain. These take place in quasi rigid structures where diffusion- collision processes are not physically possible . A flurry of theoretical activity in the field (2-5) attempts to clarify how these critical long-range electron transfers occur in simple chemical systems as well as in protein-based systems. Similarly, the synthesis of model reaction centers, from the simplest chromophores to the recently synthesized tetrades (6) can be of much help. These molecules are designed in order to mimick the electron phototransfers and the multiple steps which lead to the stabilization of the photoseparated charges. The chemical nature of the electron exchanging centers and of the links between them, their distances, their mutual orientation as well as the kind of environment where the transfers take place are factors which condition the mechanism and define the yield, the lifetime and the kinetics of the charge separation.

In this paper we report the synthesis of a new reaction center model, a molecular assembly made up of a carotenoid, a basket-handle porphyrin and a quinone. The originality of this species arises from the nature of the links between the three centers. The electron acceptor quinone is attached through an ester junction to one of the two handles of the porphyrin; the donor carotenoid group is similarly fixed to the second handle. Such a structure fixes the distance between the porphyrin and the adjacent groups, which cannot turn back towards the porphyrin. It will be possible to modulate this distance according to the number of carbon atoms in the basket-handle. In addition, the handles ensure an enhancement of the hydrophobic character of the chromophore, and favor its location inside the lipid core of the membrane where the triad has been incorporated.

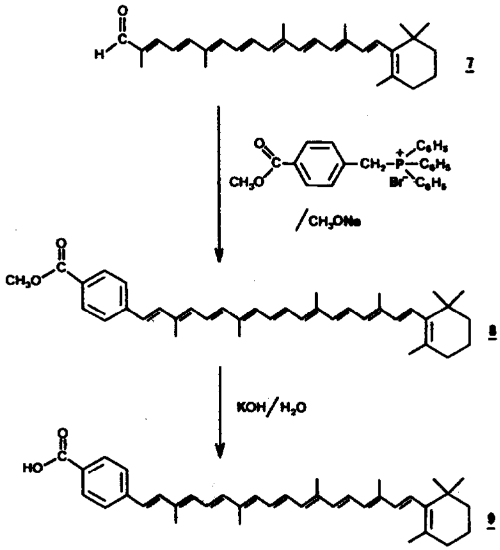
Electron transfer under illumination is generally investigated spectroscopically, providing data on the nature and lifetimes of charged species present. Warman et al (7) used an elegant time-resolved conductivity method. Here, in contrast, we incorporate these molecular assemblies into bilayer lipid membranes and measure the electrical photoconduction properties of the system with aqueous redox couples on both sides of the membrane. The photocurrents thus observed prove that a transmembrane charge transfer takes place. The appearance of a photocurrent confirms the coupling between the light absorption and the electrical conduction; it reveals the feasibility of secondary interfacial electron transfers which allow the storage of the converted energy (8).

RESULTS

Synthesis. Contrary to a large variety of molecular assemblies designed to study intramolecular electron transfers which consist of carotenoid polyenes and quinones covalently linked to meso-phenyl groups of the porphyrin ring(9-16), we have synthesized a molecule in which the donor and acceptor moieties are attached to superstructures of the so-called basket-handle porphyrins. These compounds derived from 5, 10, 15, 20 tetraphenylporphyrin are sterically protected on each side of the macrocycle by a polymethylenic chain which is anchored in the ortho position of the two opposite phenyl groups so as to provide a cross-trans arrangement, an ether being the anchoring group(17). In order to obtain a convenient attachment position for the carotenoid and quinone moieties, the two handles are functionalized at the central carbon atom. Among such symmetrical difunctionalized porphyrins, which have been previously reported(18), the dibromoporphyrin 3 appears to be a good compound. Furthermore the latter was obtained from the diketoporphyrin 1, the key intermediate for this new series of compounds, in an almost quantitative yield by sodium borohydride reduction and subsequent bromination with CBr_4- Ph_3P of the dihydroxyporphyrin 2. The coupling reaction between 3 and 7'-apo-7' (4-carboxyphenyl)β-carotene which was prepared by a slightly modified version of a procedure described by D. Gust et al(11), in dimethylformamide in the presence of hydrogen carbonate at 100°C gave the diad 4 in 29 % yield after purification by chromatography on silica gel. Triad 5 was then prepared in an analogous fashion by the coupling reaction between 4 and commercially available 2,5 - dihydrobenzoic acid in a 45 % yield. Conversion of the hydroquinone portion of 5 to the corresponding quinone was achieved by treatment with lead dioxide. However, after the usual work up, analytical t.l.c. on silica gel showed that the quinone moiety was unstable. On standing, it slowly reverted to the hydroquinone form. This transformation has also been observed by Ho et al in a series of linked quinone-porphyrin molecules (10).



Scheme 1



Scheme 2

Characterization of compound 5. The proposed structural formula of the new compound 5 is consistent with data obtained by ^1H NMR spectroscopy. The spectrum in $CDCl_3$ recorded at 400 MHz is shown in figure 1. Despite the complexity of the molecule, all the resonances can be assigned to individual protons by integration and selective homonuclear decoupling experiments. Considering the porphyrin, the single proton borne by the central carbon of each functionalized handle appears at 3.87 and 3.55 ppm respectively for the chains bearing carotenoid and hydroquinone substituents. They are shifted upfield ($\Delta\delta$ = 1.3 ppm) relative to the same proton in free isopropylbenzoate taken as the reference product. These data strongly indicate that they are affected by the ring current of the porphyrin due to their pointing toward the porphyrin centre. The other protons of the macrocycle appear to have the same chemical shifts as those of ether basket-handle porphyrin in a cross configuration for which the proton chemical shifts may be regarded as being dominated by the porphyrin anisotropy.

The spectrum of compound **5** also indicates that the protons of the carotenoid and dihydroquinone linked to the protected handles were not significantly shifted from their original positions in their unlinked acid forms (15). However, the resonances for the aromatic protons (Ar H_D) are shifted by 0.5-0.6 ppm to high field as compared with the same protons in the extended triads synthesized by Gust and Moore (15). This is in agreement with the point of attachment of the carotenoïd on the basket handle which locates these protons in the shielding region of the porphyrin ring current.

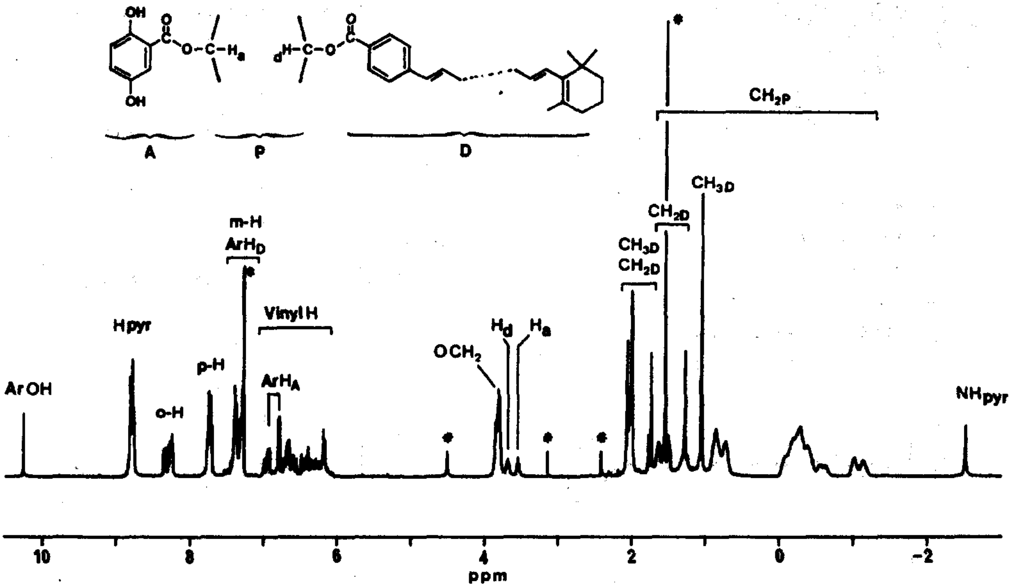


Figure 1. 400 MHz Proton NMR spectrum of compound **5** recorded in $CDCl_3$. The asterisks denote impurities

Table 1. Absorbance maxima (in nm) and molecular extinction coefficients (in parentheses, in l $mmol^{-1}$ cm^{-1}) of compounds in methylene dichloride.

3		419.5 (428)		514 (18.5)	547.5 (6.2)	591 (5.9)	647 (2.6)
9			482 (86)	511 (72.3)			
4		419.5	480	511.5	shoulder	590	646
5		419.5 (405)	479 (73)	512 (70)	shoulder	591 (5.4)	646 (2.2)
6	246	419.2	479	511.5	shoulder	591	646
3 + **9**		419.5 (445)	482 (89.6)	513 (90)	shoulder	590 (6.6)	647 (2.8)

Absorption maxima and molecular extinction coefficients of compound 5 are given in Table 1 and compared to those of precusor compounds. This spectrum recorded in CH_2Cl_2 shows typical absorption bands of the porphyrin ring. Superimposed on these bands are carotenoid absorptions. This spectrum is essentially identical to that obtained by mixing equivalent amounts of carotenoid and porphyrin without broadening or red-shifting. This observation provides good evidence for the absence of ground-state interactions between the porphyrin and both carotenoid and hydroquinone moieties. The oxidation of the hydroquinone compound 5 to the corresponding quinone 6 is characterized by a slight increase in absorption at 246 mn, whereas the Q bands in the visible region and the Soret band of the porphyrin remain identical, indicating that the macrocycle is unaffected during the reaction.

Photo-electrochemical properties

a) Monolayer experiments. The fact that the triad behaves as a monolayer at the air-water interface proves its amphipathic nature. The quinone head possesses a polar character, the basket-handle porphyrin and the carotenoid tail a hydrophobic one. It is thus expected that the quinone lies at the interface whereas the tail is perpendicular to it (Fig. 2). This orientation is corroborated by the value of 160 Å^2 for the area occupied per molecule when there are no intermolecular interactions (intersection of the straight part of the surface pressure vs molecular density curve in Fig. 3 and the zero-pressure axis). The 160 Å ^{2} value cannot accomodate the three pigment centers lying at the interface. It has precisely the calculated value for the cross-section of the basket-handle porphyrin sphere, based on structural data for tetraphenylporphyrins (19). The collapse region, around 100 Å^2, is poorly defined; the pigment forms a solid condensed phase rather than a liquid condensed one.

b) Light intensity. When the light intensity is enhanced, the photocurrent amplitude increases (Fig. 4). This increase is not linear vs the light intensity but tends to slow down as can be expected from the saturation generally observed with such systems (20).

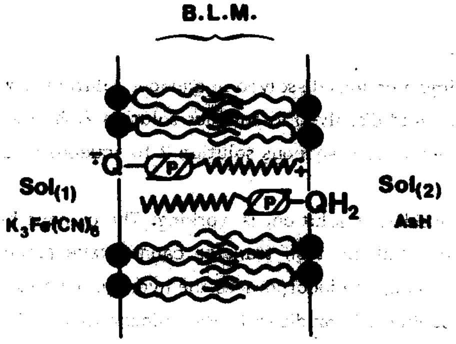


Figure 2. Postulated arrangement of triads in the phospholipid bilayer (B.L.M.)

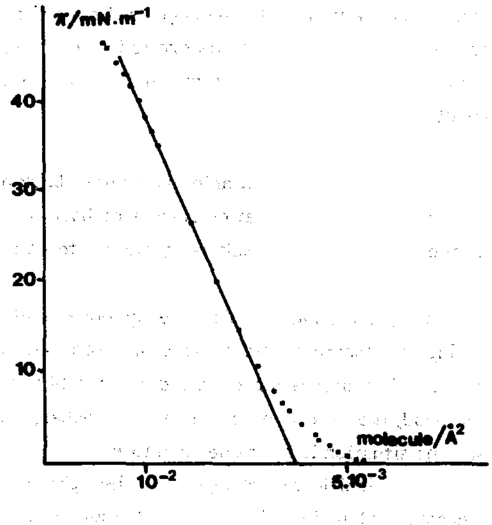


Figure 3. Surface pressure-molecular density curve for the caroteno-basket-handle porphyrin-quinone triad

c) Membrane potential. The variation of the photocurrent as a function of the external voltage applied between the two Ag/AgCl electrodes is linear (Fig. 4).

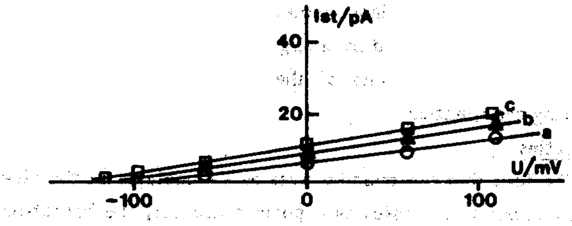


Figure 4. Photocurrent amplitude versus external applied voltage for various light intensities.
a) 10 mW; b) 20 mW; c) 40 mW. Standard conditions (see text).

DISCUSSION

The monolayer experiments show the amphipathic character of the basket-handle triad and its orientation perpendicular to the air-water interface. There is a high probability for the pigment to retain the same orientation when inserted into a phospholipid planar bilayer membrane. It has been shown to be the case for the caroteno-porphyrin-quinone triad synthesized by Moore et al (21). Both triads exhibit linear photocurrent versus external voltage plots (22). This behavior observed in intramolecular and transmembrane electron photo-transfers can be contrasted with the exponential curves obtained when the pigment, such as a simple porphyrin (20), diffuses across the membrane.Thus, similar electron transfer mechanisms are expected to work for both triads. In its excited state, the porphyrin gives an electron to the quinone and takes it back from the carotenoid end of the molecule. This intramolecular charge separation is followed by interfacial reactions.

The quinone radical anion is oxidized back by the ferricyanide ions dissolved in aqueous solution 1, while the carotenoid radical cation is reduced back by the ascorbic acid dissolved in aqueous solution 2. At the end of the cycle, the net result is equivalent to an electron transfer from aqueous solution 2 to aqueous solution 1.

Only the triad in which the quinone head faces the oxidizing solution, is working. The quinone heads facing the ascorbic acid solution are reduced and hence internal electron transfer cannot take place. If, instead of incorporating the triad into the bilayer lipid membrane, one incorporates the hydroquinone form of the triad, the experimental results are unchanged. The hydroquinone is oxidized to the quinone at interface 1 and the situation is the same as before.

No significant change in the photocurrent amplitudes was observed going from neutral (phosphatidylcholine, phosphatidylethanolamine) to negatively charged (phosphatidylserine, cardiolipin) lipids. This means that neither the location of the triad molecules nor the interfacial electron transfer are sharply affected by the electrical charge of the interfaces.

The photocurrent amplitudes in the standard conditions are about 10 pA, more than one order of magnitude lower than those observed for the triad studied by Moore et al (8). At the moment, it is not possible to assign a definite reason for this decrease in the efficiency of the whole process. The more hydrophobic environment of the chromophore in the basket-handle triad, as well as the differences in nature and length of the links between the three centers, certainly affect the lifetime of the charge separated state, and/or the intramolecular and the interfacial electron transfer rates.

A knowledge of the photophysical and photochemical properties of the new caroteno-basket-handle porphyrin–quinone triad is needed for a better understanding of the results at the membrane level.

EXPERIMENTAL SECTION

Chemicals. Except for the β apo 8'- carotenal (trans) purchased from Fluka, all chemicals used were of reagent grade and were purchased from Aldrich. Reaction solvents (Prolabo) for the synthesis were purified before use. Dimethylformamide and dimethylacetamide were distilled and kept over a 4 Å molecular sieve. Merck silica gel 60 (40-60 μm) was used for column chromatography. Merck precoated preparative plates (silica gel 60, 2mn) were used for t.l.c. Proton N.M.R. spectra in deuteriochloroform ($CDCl_3$) (CEA, France) were measured using a Brucker AM- 400 instrument. Optical spectra in the UV and visible region were recorded using a Varian DMS-200 spectrometer.

Synthesis.

α-5, 15 : β - 10, 20 - Bis 2,2'- (6- Oxoundecamethylenedioxy) - diphenyl porphyrin 1. A solution of 1,11 - dibromoundecan - 6 -one (18) (3.608 g, 11 mmol) in dry dimethylformamide (200 ml) was added dropwise during 5 h to a mixture of 5,10,15,20 -tetrakis (o - hydroxyphenyl) porphyrin (17) (3.39 g, 5 mmol) and an excess of potassium carbonate (10 g) in the same solvent (750 ml) at 100°C under argon. Once the addition was complete, stirring was continued for 3 h at the same temperature and the mixture was then filtered and the filtrate evaporated to dryness. The residue was dissolved in toluene, filtered again and subjected to column chromatography (silica gel, 4 x 30 cm). Elution with methylene dichloride afforded a first fraction which was indentified as the title compound. It was crystallized from methylene dichloride-methanol (0.872 g, 17.3 %). Found : C, 77.9; H, 6.5; N, 5.7. $C_{66}H_{66}N_4O_6$ requires C, 78.4; H, 6.6; N, 5.5 %.

α - 5,15 : β - 10,20-Bis 2,2'- (6-Hydroxyundecamethylenedioxy) - diphenyl porphyrin 2. This compound was prepared from porphyrin 1 by Na BH_4 reduction (85 %) (18).

α - 5,15 : β - 10,20-Bis 2,2'- (6-Bromoundecamethylenedioxy) - diphenyl porphyrin 3. This compound was obtained by reduction with BH_3-THF of dihydroxyporphyrin 2 (18). (90 %).

7'- apo-7'- (4 - carbomethoxyphenyl) - β - carotene. 8. This compound was prepared by the reported procedure (11) with slight modifications. To 50 ml of Toluene containing 320 mg (8 mmol) of NaH, 405 μl (10 mmol) of methanol was added with stirring, at room temperature. After the end of hydrogen evolution, 2.46 g (5 mmol) of 4 -carbomethoxybenzyltriphenylphosphonium bromide and 1.25 g (3 mmol) of β- apo 8'-carotenal 7 were successively added, and the resulting solution was refluxed under argon. The reaction was monitored by t.l.c. on analytical silica gel plates and shown to be complete after 1 h. After addition of 200 ml of chloroform, the solution was washed with water, dried (Na_2SO_4) and then evaporated to dryness under reduced pressure. The solid residue was dissolved in toluene and chromatographed on a silica gel column (4.5 x 30 cm). Elution with toluene gave a first fraction which was identified as the title compound which was crystallized in methylene dichloride-methanol (1.54 g, 94 %).

7 - Apo- 7'- (4 - carboxyphenyl) β- carotene 9. This compound was prepared using the previously described method (11).

Carotenoporphyrin 4. To a solution of dibromoporphyrin 3 (226 mg, 0.2 mmol) in dimethylformamide (10 ml), 7'-apo-7'- (4-carboxyphenyl) βcarotene (200 mg, 0.38 mmol) and sodium hydrogen carbonate (32 mg, 0.38 mmol) were added under argon. The resulting solution was heated at 100°C for 12 h in the dark with stirring. After cooling, chloroform (50 ml) was added and the resulting solution was washed with dilute hydrochloric acid, water, dried (Na_2SO_4) and then evaporated to dryness. The residue was dissolved in toluene and the solution loaded onto a silica gel column (2 x 30 cm). Elution with the same solvent gave a main fraction (Rf = 0.54) which was thought to be the title compound. (91.6 mg, 29 %). ^{1}H NMR; δ(ppm) 8.81 (8H, m, Hpyr), 8.26 (4H, m, o-meso Ph), 7.7 (4H, m, p-meso Ph), 7.37 - 7.26 (8H, m, m-meso Ph + 4H, ArH_D), 7-6.11 (14H, m, -CH=CH-), 3.83 (8H, m, O-CH_2), 3.68 (m, -CH-handle D), 2.15-1.67 (15H, -CH_{3D} + 2H, $>CH_{2D}$), 1.63-1.28 (4H, $>CH_{2D}$), 1.04 (6H, -CH_{3D}), 1.67 to -1.22 (32H, methylenic- CH_2- + 1H, - CHBr-), - 2.56 (2H, s, NHpyr).

Carotenoporphyrin-hydroquinone Triad 5. To a solution of the carotenoporphyrin 4 (60 mg, 0.038 mmol) in dimethylacetamide (3ml) 2,5-dihydroxybenzoic acid (77 mg, 0.5 mmol) and sodium hydrogeno-carbonate (42 mg, 0.5 mmol) were added. The reaction mixture was stirred at 100°C overnight under argon, and was then diluted with 25 ml of toluene. The solution was washed with water, 10 % aqueous hydrogeno carbonate, water, dried (Na_2SO_4), and then evaporated to dryness. The solid residue was dissolved in methylene dichloride and was directly subjected to column chromatography (silica gel, 2 x 20 cm). Elution with the same solvent gave a first minor fraction (Rf = 0.85). A more polar fraction (Rf = 0.49) was thought to be the desired triad compound 5 which was crystallized from methylene dichloride-hexane (28.5 mg, 45 %), ^{1}HNMR, δ(ppm) 10.26 (2H, s, ArOH), 8.78 (8H, m, Hpyr), 8.27 (4H, m, o-meso Ph), 7.7 (4H, m, p-meso Ph), 7.4 - 7.27 (8H,m, m-meso Ph + 4H, ArH_D), 7-6.11 (14H, m, vinyl H), 6.94 and 6.8 (3H, ArH_A), 3.87 (8H, m, O-CH_2), 3.72 (m, -CH-handle D), 3.55 (m, - CH-handle A), 2.15-1.7 (15H, - CH_{3D} + 2H, $>CH_{2D}$); 1.63-1.27 (4H, $>CH_{2D}$), 1.04 (6H, -CH_{3D}), 1.67 to - 1.22 (32H, methylenic CH_2), - 2.55 (2H, s, NHpyr).

Carotenoporphyrin-quinone Triad. 6 The carotenoporphyrin – hydroquinone 5 (6 mg) in methylene dichloride (3 ml) was stirred with an excess of lead dioxide (30 mg) at room temperature. The reaction was monitored by analytical t.l.c. on silica gel using methylene dichloride as eluant. The quinone derivative (Rf = 0.75) which moved faster than the starting material was quantitatively obtained after 2h. The lead dioxide was filtered off and washed with CH_2Cl_2. Attempts at purification were unsuccessful because a slow reversal reduction took place.

Methods

A membrane was formed from a solution made of phospholipids, pigment and decane on the 1 mm^2 orifice of a teflon septum. The lipid was phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine or cardiolipin. The pigment was either the quinone or the hydroquinone form of the triad. The septum separates the two chambers of the cell, which contain aqueous solutions. The current flowing across the membrane was measured in the external circuit connecting Ag/AgCl electrodes dipped in each aqueous solution. The experimental set-up for bilayer lipid membrane experiments was the same as already described (23) and the chemicals used had the same origin. The sign of the external membrane potentials was referred to the side of the aqueous phase containing the electron donor. The photocurrents were always measured with respect to the steady state value of the dark current . A positive current corresponds to positive charges flowing from the oxidizing compartment to the reducing one. The standard working conditions are the following : membrane forming solution triad/phosphatidylethanolamine/n decane : 0.06/2/100; buffer 10^{-1}M NaCl, 5.10^{-3}M KH_2PO_4; oxidant compartment 10^{-2}M $K_3Fe(CN)_6$; reductant compartment 10^{-2}M ascorbic acid; excitation warelength 514 nm; light intensity 10 mW. mm^{-2}.The monolayer experiments were performed as previously described (24). A known quantity of a solution of the pigment in dichloromethane was spread at the air water interface. The interfacial amphiphilic layer was slowly compressed and the resulting change in the surface pressure recorded. The aqueous phase was the above mentioned buffer.

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