REVIEW

ENERGY CONVERSION AT LIQUID/LIQUID INTERFACES: ARTIFICIAL PHOTOSYNTHETIC SYSTEMS

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Abstract—This chapter focuses on multielectron reactions in organized assemblies of molecules at the liquid/liquid interface. We describe the thermodynamic and kinetic parameters of such reactions, including the structure of the reaction centers, charge movement along the electron transfer pathways, and the role of electric double layers in artificial photosynthesis. Some examples of artificial photosynthesis at the oil/water interface are considered, including water photooxidation to the molecular oxygen, oxygen photoreduction, photosynthesis of amphiphilic compounds and proton evolution by photochemical processes.

Key words: multielectron reaction, artificial photosynthesis, liquid/liquid interface, amphiphilic molecules, monolayers, photoelectrochemistry, ITIES.

INTRODUCTION

Light energy is harvested for the biosphere by photosynthetic pigment systems in which the electronic structure of excited-state chlorophyll donates an electron to a primary acceptor pheophytin, the first component of an electron transport chain. The electron carries with it the energy of the original photon of light that was absorbed, and in the process of electron transport the energy is captured in two ways. The first involves coupling a proton pump mechanism to the sequential redox reactions in one part of the electron transport chain, so that a proton gradient is established across the membrane. The electrochemical energy of the proton gradient is then used to drive ATP synthesis in the ATP synthase. The second energy capture occurs when an acceptor molecule such as NADP is reduced to NADPH, which in turn is used to reduce carbon dioxide in the Calvin cycle. It follows that systems modeling photosynthesis should have the capability of carrying out relatively simple versions of these fundamental reactions.

Understanding photosynthetic mechanisms is essential for successful design of artificial solar energy utilization systems. The redox potential scale is thereby altered, making it possible to carry out reactions that cannot be performed in a homogeneous phase.

The interface of two immiscible liquids is a widely used example of a heterogeneous system[1-33]. For instance, fundamental processes of photosynthesis[2-9, 22, 34-37], biocatalysis[10, 13, 15-17, 37-40], membrane fusion[24], ion pumping[20, 25, 41, 42] and electron transport[6, 13, 15, 16, 43, 44] have all been investigated in interfacial systems. Extraction of the reaction products and adsorption of the reaction components can establish catalytic properties at interfaces, recognized recently as interfacial catalysis[4, 10-12, 38]. The interface between two immiscible liquids with immobilized photosynthetic pigments can serve as a convenient model for investigating photoprocesses that are accompanied by spatial separation of charges. The present review focuses on electrochemical mechanisms of photocatalytic systems at the oil/water interface. In order to provide a biological perspective, we will first describe the function of photosystem II and the thermodynamics and kinetics of the multielectron reaction:

\[ 2H_2O \rightleftharpoons O_2 + 4H^+ + 4e^- \]  

(1)

STRUCTURE AND COMPOSITION OF THE OXYGEN-EVOLVING COMPLEX IN VIVO

The redox map of photosynthesis in green plants can be described in terms of the well-known Z-scheme proposed by Hill and Bendall[45]. The main
advantage of the currently accepted Z-scheme depicted in Fig. 1 lies in the specific mechanism of charge transfer at the stage of water oxidation, which is a multielectron reaction mechanism involving no unknown intermediates.

The molecular organization of a thylakoid membrane is shown in Fig. 2. The spectral characteristics of photosystem II indicate that the primary electron donor is the dimer of chlorophyll P680 with absorption maxima near 680 and 430 nm. Water can be oxidized by an oxygen evolving center (OEC) composed of several chlorophyll molecules, two molecules of pheophytin, plastoquinol, several plastoquinone molecules and a manganese-protein complex containing four manganese ions. The oxygen-evolving complex is a highly ordered structure in which a number of polypeptides interact with one another to provide the appropriate environment for cofactors such as manganese, chloride and calcium, as well as for electron transfer within the complex.

Manganese binding centers were first revealed in thylakoid membranes by EPR methods, and it is now understood that four manganese ions are necessary for oxygen evolution during water photooxidation[3, 4, 7, 46–55]. Plastoquinone (PQ) acts as a transmembrane carrier of electrons and protons between reaction centres of two photosystems in the case of noncyclic electron transfer and may also serve as a molecular “tumbler” that switches between one-electron reactions and two-electron reactions[36]. Pheophytin is an intermediate acceptor in photosystem II. Direct formation of P680 pheophytin ion radical pairs was revealed by experiments on magnetic interactions between Pheo and PQ-reflected in the EPR spectra.

THERMODYNAMICS OF WATER OXIDATION

Water oxidation to molecular oxygen is a multielectron process that proceeds with a surprisingly high quantum efficiency[34]. The pathways of water oxidation to molecular oxygen are schematically represented in Fig. 3. The water oxidation reaction can proceed upon illumination at 680 nm, a wavelength of light that excludes any process following one-electron mechanisms via hydroxyl and oxygen rad
Fig. 2. A schematic model of the electron transport chain with most of the light-harvesting pigment-protein complexes omitted. The depiction of PS II is adapted from[50, 51] and the organization of the plastoquinol-plastocyanin oxidoreductase or cytochrome b$_{6}$/f complex is based on[52] and[53]. D is a component similar to Z (same EPR signal but different kinetics) which does not seem to be involved in electron transfer from water to P680; D has recently been shown to be a tyrosine radical[54]. The $F_{0}, F_{1}$, and $F_{n}$ are thought to be 4Fe-4S centers. The organization of PS I is adapted from recent overviews given in Refs. [53, 55]. The organization of the H⁺-ATPase ($cF_{1}-cF_{0}$) is highly schematic. The hydrophobic $cF_{o}$ appears to contain 4-6 copies of DCCD (N,N'-dicyclohexylcarbodiimide) binding protein or subunit III.

Fig. 3. Energy scheme for water oxidation to molecular oxygen. For a three-electron reaction a stronger oxidant than the cation-radical P680$^{+}$ is needed. A 2 : 2-electron pathway of the reaction is thermodynamically possible if the standard free energy of binding of the two-electron intermediate is about $-40$ kJ mol$^{-1}$. This value corresponds to the energy of two hydrogen bonds which forming between H$_{2}$O$_{2}$ and the catalytic center. For this case a molecular mechanism can be suggested to the reaction shown in Fig. 4 and will be discussed below.

Synchronous four-electron oxidation of water to molecular oxygen (Fig. 5) is one thermodynamically possible pathway. One-electron mechanisms of water oxidation are likely to be operative in some model systems with a low quantum efficiency, but provide no opportunity for two- or four-electron reactions to occur due to kinetic limitations. Nevertheless, the
intermediates formed in these systems are highly reactive and can readily enter into side reactions of hydroxylation, oxidation and destruction of chlorophyll and other components of the reaction center.

**KINETIC ASPECTS OF MULTIELECTRON REACTIONS**

An important parameter in the quantum theory of charge transfer in polar media is the medium reorganization energy $E_r$ that determines activation energy. In the harmonic approximation, the activation energy of charge transfer is given by

$$E^* = E_{comp} + (E_s + AG)^2/4E_s$$

where $E_{comp}$ is the energy of catalytic complex formation from individual reagents, and $G$ is the configurational Gibbs energy. In a homogeneous medium, the medium reorganization energy for transfer of a charge $n$ between two reaction centers can be evaluated in terms of the formula:

$$E_r = n^2e^2(1/e_{opt} - 1/e_n)(1/2a_1 + 1/2a_2 - 1/h_{12})$$

where $n$ is the number of charges transferred in a single elementary events, $z_i$ is the radius of the $i$th component, and $h_{12}$ is the charge transfer distance[56].

It follows from equation (3) that $E_r$ is proportional to the square of the number of charges transferred. This factor makes multielectron processes impossible in the majority of homogeneous redox reactions due to the high activation energy resulting from a sharp rise in the energy of solvent reorganization. For multielectron reactions the exchange currents of $n$-electron processes are small compared to those of one-electron processes, which makes the stage-by-stage reaction mechanism more advantageous. Therefore, multielectron processes can proceed only if the formation of an intermediate is energetically disadvantageous[36, 57]. However, it is clear from equation (3) that conditions can be chosen which reduce $E_r$, during transfer of several charges, to the level of the reorganization energy of ordinary one-electron reactions. These conditions primarily are systems with a low dielectric constant, large reagent radii and optimized correlation of their sizes, inclusion of the substrate into the coordination sphere of the charge acceptor, simultaneous use of several charge donors or acceptors bound into a multicentre complex[36, 58]. Several recent papers[10–12, 38] presented theoretical studies on the kinetics of heterogeneous multielectron reactions at the interface between two immiscible liquid media: water/oil, and water/biomembrane. The interface proved to be capable of catalyzing multielectron reactions and sharply reducing the activation energy.

The energy of solvent reorganization in systems with complicated charge distribution was calculated by Kharkats[59, 60]. Reagents and products can be represented by a set of $N$ spherical centers arbitrarily distributed in a polar medium. The charges of each of the reaction centers in the initial and final state and $z'_1$ and $z'_2$, respectively. Taking $R_k$ to represent coordinates of the centers and $e_i$ for dielectric constants of the reagents it follows that:

$$E_s = 0.8 \left( \frac{1}{e_{opt}} - \frac{1}{e_n} \right) \times \left\{ \sum_{p=1}^{N} \left( \frac{\delta z_i}{2R_{pk}} \right)^2 + \frac{N}{2} \sum_{k-p}^{N} \frac{\delta z_k \delta z_k}{2R_{pk}} \right. \right.$$  

$$+ \left\{ \sum_{k=1}^{N} \sum_{i=1}^{N} \frac{\delta z_k \delta z_i}{2R_{pk} R_{ki}} + \sum_{k-p}^{N} \sum_{i-p}^{N} \frac{\delta z_k \delta z_i}{2R_{pk} R_{ki}} \right\} \left( \frac{3e_i^2}{(2e_i + e_k)^2} \right)$$

(4)

where $(\delta z_k) = z'_k - z'_1$, $R_{pk} = R_p - R_k$, $z'_1$, $z'_2$ are charge numbers of particle $k$ in the initial and final states, respectively, $a_k$ is the radius of particle $p$, $R_{ki}$ is the coordinate of $k$-particle centre, and $e_i$ is the dielectric constant of reactant. Reactions with synchronous transfer of several charges present a particular case of equation (4)[59]. As a particular case, equation (4) gives medium reorganization energy for several simultaneous charge transfer reactions.

The most effective coupling of ATP synthesis and the electron transport chain can be obtained if the activation energy of the coupled process is lower than that of the charge transfer in the electron transport chain. It is obvious from equation (4) that in a general case such a situation can be attained by a simultaneous transfer of opposite charges, so that the second and the third terms of equation (4) should be negative. An optimal geometry between the centers of charges of donors and acceptors must also be chosen.

Consider two instances of multielectron reactions: simultaneous transfer of $n$ charges from one donor to an acceptor and simultaneous transfer of several charges (one from each of the centres) to $m$ acceptors ($m \leq n$). In the former case $E_r$ is proportional to $n^2$, while in the later it may be significantly lower (depending on the sign of the charge being transferred and the reciprocal positions of reagents). A multicentre process with $E_r \approx n$ is also possible. The concerted multicentre mechanism of multielectron reactions is likely to contribute to a marked reduction of $E_r$, and hence of the activation energy, as compared with two-centre multielectron process. Electrostatic interactions between reagents may constitute an additional factor favoring the activation energy reduction of multielectron processes at the membrane/solution interface. Unlike the electron transfer process in a homogeneous dielectric
medium, where the work of the mutual approach of reagents or reaction products becomes zero if one of the reagents or products is electroneutral, the energies of electrostatic interactions in the heterogeneous process in question can never be equal to zero due to interactions with image charges[60]. With an appropriate arrangement of the reagents the electron transfer activation energy in such heterogeneous multielectron reactions occurring on the surface of thylakoid membranes may be many times lower than the energy of medium reorganization[10–12].

The term “synchronous multielectron” reaction does not mean that all n electrons started synchronously, since this is impossible according to quantum mechanics. Instead, each electron is transferred from donor to acceptors individually, but the time required for “intermediate” formation is much less than the time of the reorganization of the medium, so that “intermediates” as individual chemical compounds do not exist.

Synchronous multielectron reactions in membranes have recently drawn the attention of both chemists and biologists. In a synchronous multielectron reaction the energy will be used very economically[22, 57]. Furthermore, the biotechnological application of multielectron reactions makes it possible to drive redox reactions in relatively mild conditions under the action of weak oxidants or reductants. Synchronous multielectron reactions may proceed without formation of intermediate radicals, which are highly reactive and can readily enter a side reaction of hydroxylation and destruction of the catalytic complex. Since multielectron reactions do not produce significant toxic intermediates, they can be used by living organisms for biochemical energy conversion in respiration and photosynthesis. In multielectron reactions that occur as consecutive one-electron stages, the Gibbs energy necessary per single electron transfer obviously cannot be completely uniformly distributed over the stages. The energy needs for various stages will be different and the excess energy in the lower energy stages will be converted into heat.

MOLECULAR MECHANISM OF OXYGEN EVOLUTION IN VIVO

Membrane-bound P680 enters an excited state upon illumination. In dimers and other aggregated forms of chlorophyll the quantum efficiency of triplet states is low, and it is the singlet excited states that undergo photochemical transformations. In several picoseconds, an electron is first transferred to pheophytin, then to plastoquinone Q1, and from plastoquinone Q1 to another polypeptide-bound plastoquinone Qb in thylakoid membrane (Fig. 1) resulting in an oxidized pigment and a reduced acceptor. The cation radical P680* successively oxidizes four manganese ions, which in turn drives the production of molecular oxygen. Formation of cation radical of chlorophyll or oxidation of manganese ions is accompanied by dissociation of water bound to the reaction center and ejection of protons. A synchronous multielectron process that describes all four oxidizing states of the oxygen evolving complex was proposed in Ref. [36]. The transfer of electrons in a 1:1:1:1 series from a manganese cluster to the electron-transport chain is accompanied by the ejection of 1:0:1:2 protons and the evolution of molecular oxygen.

Protons are released from reaction centers either by regulators of proton distribution or by hydrogen bond transfer (analogous to a Grotthus mechanism) through the hydration shell of manganese ions. The hydration sphere of manganese is known to contain water molecules that rapidly exchange protons with bulk water[61]. The presence of bivalent cations at the interface between two immiscible electrolyte solutions facilitates strong adsorption of water molecules belonging to the second hydration shell of ions. Thus, a portion of coordinatively bound water enters the compact part of the electric double layer, which results in a change of its differential capacity at the interface. In the case of multivalent ions with small radii, the electric field near a cation is large. This can disturb the microstructure of the adjacent intrathylakoid space and bring about dielectric saturation effects.

Manganese ions play a particularly important role in the evolution of dioxygen during photosynthesis[34]. Although there are several manganese pools in chloroplasts, only one of them is involved in water oxidation. The manganese ions associated with chloroplast OEC can perform a number of functions:

1. Mn-polypeptide complex is a redox intermediate that protects the reaction center from redox and radical destruction.
2. Mn-clusters are redox buffers facilitating accumulation of four holes in the reaction center of photosystem II, which are needed to ensure water photooxidation.
3. Hydrated multivalent manganese cations bring water to the reaction center so that rapid proton exchange and transport through the hydration shell of manganese ions in the zone of water oxidation.
4. Multivalent manganese ions induce dielectric saturation effects in the polar region of the reaction center of photosystem II, which reduces the reorganization energy of the medium during charge transfer.

PHOTOINDUCED CHARGE TRANSFER ACROSS A LIQUID/LIQUID INTERFACE

Although many photochemical reactions of artificial photosynthesis have been studied in homogeneous systems, they are characterized by low quantum yield and low efficiency. Mathai and Rabinovich[62], and Frankowiak and Rabinovich[63] suggested that only heterogeneous systems such as liquid/liquid interfaces can increase yields, due to the separation of photoproducts in different phases. A classic example is the photochemical reaction at the ether/water interface:

\[
T + nFe^{2+} \xrightarrow{\text{Light}} (S + I) + nFe^{3+} \quad (5)
\]
where $T$ is the purple colored cation of thionine (or methylene blue), $S$ and $L$ its reduced forms semithionine (or semiquinone) and leukothionine (leucomethylene blue), respectively. Potential differences ranging from 234 to 358 mV could be measured in the cell, depending on the concentration of the reaction products[62].

From thermodynamic and kinetic principles[7, 10–12, 36, 38] the interface between two immiscible liquids can have catalytic properties for interfacial charge transfer reactions.

For example, at the oil/water interface the following redox reaction can occur:

$$\text{Red} \leftrightarrow \text{Ox} + n\text{e}^- + n\text{H}^+ \quad (6)$$

The electrons which are the products of reaction (6) can be accepted at the interface by another substance if it is dissolved in one of the two phases.

The standard Gibbs energies of the reaction (6) for each phase, $\alpha$ and $\beta$, are:

$$\Delta G^\circ_\alpha = \mu_{\text{red}}^\circ - \mu_{\text{Ox}}^\circ - n\mu_e^0 - M_{\alpha\text{H}^+} \quad (7)$$

$$\Delta G^\circ_\beta = \mu_{\text{red}}^\circ - \mu_{\text{Ox}}^\circ - n\mu_e^0 - m_{\beta\text{H}^+} \quad (8)$$

Subtraction of equation (7) from equation (8) gives the change of the standard Gibbs energy at the interface if the electron acceptor is located in one phase only, or localized at the phase boundary:

$$\Delta G^\circ_\beta - \Delta G^\circ_\alpha = (\mu_{\text{red}}^\circ - \mu_{\text{Ox}}^\circ) - (\mu_{\text{red}}^\circ - \mu_{\text{Ox}}^\circ) - m_{\beta\text{H}^+} + \alpha_{\text{H}^+} \quad (9)$$

or

$$\Delta G^\circ = RT \ln \frac{P_{\text{red}}}{P_{\text{Ox}}(P_{\text{H}^+})^m} \quad (10)$$

where $P_i$ is the distribution coefficient of the $i$th ion:

$$RT \ln P_i = \mu_{\text{red}}^i - \mu_{\text{Ox}}^0 \quad (11)$$

In the case of a $n$-electron reaction, the standard redox potential $AE^\circ$ at the interface is determined by:

$$AE^\circ = \frac{-RT}{nF} \ln \frac{P_{\text{red}}}{P_{\text{Ox}}(P_{\text{H}^+})^m} \quad (12)$$

A thermodynamic analysis of redox and mixed potentials at the liquid/liquid interface was published by Markin and Volkov[64].

It is possible to shift the redox potential scale in a desired direction by selecting appropriate solvents, thereby permitting reactions to occur that are highly unfavorable in a homogeneous phase. If the resolution energies of substrates and products are very different, the interface between two immiscible liquids may act as a catalyst. The kinetic mechanism underlying the catalytic properties of the liquid/liquid interface was discussed in[10–12, 36].

**ARTIFICIAL PHOTOSYNTHESIS AT OIL/WATER INTERFACE IN THE PRESENCE OF CHLOROPHYLL**

Three types of artificial photosynthetic reactions at the liquid/liquid interface can occur:

1. Photoredox reactions at the interface between two immiscible liquids[5, 7, 9, 13].
3. Photochemical reactions in one phase following extraction of products in two different phases[62, 63, 66–76].

In this section we will discuss artificial photosynthesis at the oil/water interface in the presence of chlorophyll $a$.

**P745: Hydrated oligomer of chlorophyll $a$.**

The photoelectrochemical properties of chlorophyll $a$ in monolayers, multilayers and thin films at oil/water, gas/water and solid/water interfaces have been studied extensively to obtain a thorough understanding of its role in the primary events in plant photosynthesis, i.e., light harvesting, energy transfer and charge separation in the photosynthetic unit[5–10, 13, 15, 16, 29, 32, 37, 39, 40, 77–90]. The state of chlorophyll molecules in the photosynthetic apparatus, believed to be in various aggregated forms, is still under active investigation[91–96].

Several forms of chlorophyll are involved in the primary events of photosynthesis. Absorbance peaks at longer wavelengths than those characteristic of chlorophyll in solution have been observed in vivo and attributed to varying degrees of chlorophyll aggregation. Chlorophyll $a$ dissolved in anhydrous liquid hydrocarbons (hexane, octane, decane) gives the characteristic absorption spectrum shown in Fig. 6 (curve 1). This spectrum does not change if the solution is allowed to contact water for a short time (5 min). However, when chlorophyll $a$ is dissolved in water-saturated hydrocarbon an additional band appears in the absorption spectrum with a maximum between 740 and 745 nm, which reflects the hydrated oligomer of chlorophyll $a$ (Fig. 6, curve 2). The electronic absorption spectrum of anhydrous chlorophyll $a$ solution shows two bands at 428 and 660 nm similar to the absorption spectra of chlorophyll $a$ in non-polar solvents[7]. The hydrated oligomer of chlorophyll $a$ is characterized by shifts of its absorption spectrum to lower energy wavelengths.
Energy conversion at liquid/liquid interfaces

with maxima at 448 and 745 nm[7, 90]. It is worth noting that the intensity ratio of the blue to red adsorption bands is 1.3 for the dry sample and 0.6 after hydration[90]. The differences in the absorption spectra of dry and wet solutions, coupled with marked decreases in energy of the lowest electronic transition of hydrated oligomer of chlorophyll a \((\Delta \nu = 1700 \text{ cm}^{-1})\) show that in water saturated hydrocarbon chlorophyll a exists as organized aggregates. Such self-organized molecular assemblies of hydrated chlorophyll a molecules are characterized by lower energy electronic transitions with an absorption band between 740 and 745 nm and a fluorescence band at around 755 nm. The lifetime of the emission is 0.1 and 0.2 ns[90].

Chlorophyll a dissolved in anhydrous hydrocarbon solvents at relatively high concentrations (1–10 mM) also undergoes aggregation to form oligomers. For instance, in hexane at higher concentration ranges, chlorophyll a is present as tetramers and dimers, provided its concentration is sufficiently high. In the range of chlorophyll a concentrations examined in water saturated hexane \((<10^{-5} \text{ M})\), it is present mostly as a mixture of its hydrated oligomers and monomers, as can be inferred from the fluorescence and circular dichroism spectra.

Circular dichroism spectra of chlorophyll a in water saturated hexane (Fig. 7) have negative bands at 765 nm in the region of Q\(_{\text{y}}\) transition[27, 37, 82]. This suggests that the oligomer consists of at least four or six chlorophyll molecules. The aggregate exhibits a relatively strong optical rotation, with a Kuhn anisotropy factor 0.03 at 748 nm, 500 times that of monomeric chlorophyll[87].

Hydrated oligomers of chlorophyll a in n-octane have an intense narrow dark ESR signal (Fig. 8) with a line width of 4.7 gauss and a g-value of 2.0025 ± 0.0003. Hydrated oligomers of chlorophyll a in the form of solid colloidal particles have been investigated as well[87, 89, 97–100] and it was found that colloidal particles of dry and hydrated chlorophyll a have different EPR signals. The line width for dry chlorophyll a was about 12 G[97], while the line width for colloidal particles of hydrated chlorophyll a was 1.6 G[98], 6 G[97], 2.3 G[99] or 1–14 G[100] due to different aggregation states of hydrated chlorophyll in solid films[97–99] or colloidal suspension[100]. The number of chlorophyll molecules \((N)\) in the cluster of hydrated oligomer of chlorophyll a can be found by equation[101]:

\[
N = \left( \frac{\Delta H_m}{\Delta H_a} \right)^2
\]

where \(\Delta H_m\) is the linewidth of chlorophyll a monomer and \(\Delta H_a\) is the linewidth when the unpaired spin is delocalized over \(N\) molecules. It follows from equation (13), that hydrated oligomer of chlorophyll a in hexane solution consists of six molecules of chlorophyll a CD-spectra and surface tension measurements[82] show the same number of molecules in a cluster of hydrated chlorophyll.

Chlorophyll molecules in monolayers, multilayers and thin films also form aggregates due to the strong attraction between the pigment molecules in the densely packed monolayer. Results of NMR- and ir-spectroscopic studies revealed formation of coordination linkage between the carbonyl group of a chlorophyll molecule and the magnesium atom of another molecule[96]. In densely packed structures such as monolayers or films, thermal vibrations lead to attenuation of luminescence due to overlap of the porphyrin ring electron clouds during molecular collisions[7, 96].

Hydration of chlorophyll oligomers inhibits the expected coordination interaction between carbonyl oxygen and magnesium in chlorine rings:

\[
\text{C} = \text{O} \cdots \text{Mg}
\]

Instead, water molecules are coordinated by the central magnesium atom of chlorophyll:

\[
\text{C} = \text{O} - \cdots \text{H} - \text{O} - \cdots \text{H} - \cdots \text{Mg}
\]

with an adsorption maximum at 720 nm or:

\[
\text{C} = \text{O} - \cdots \text{H} - \text{O} - \text{H} - \cdots \text{Mg}
\]

with an adsorption maximum at 740–745 nm.
Adsorption of chlorophyll at the liquid/liquid interface

Chlorophyll molecules readily form monolayers which considerably reduce interfacial tension during adsorption at the interface. This property can be used to determine the Gibbs surface excess of chlorophyll. Figure 9 shows the dependence of interfacial tension $\gamma$ on the chlorophyll $a$ concentration in dry (curve 1) and water-saturated (curve 2) octane. A gradual reduction of $\gamma$ was observed in chlorophyll $a$ concentration ranges from $10^{-6}$ to $5 \times 10^{-6}$ M for wet chlorophyll and from $10^{-6}$ to $1.5 \times 10^{-5}$ M for anhydrous chlorophyll.

Using this interfacial tension data and the Gibbs adsorption equation

$$\Gamma = -\frac{1}{RT} \frac{d\gamma}{d\ln C} = -\frac{C}{RT} \frac{d\gamma}{dC}$$  \hspace{1cm} (14)$$

the adsorption chlorophyll $a$ isotherms at the octane/water interface can be plotted (Fig. 10). It is clear that adsorption isotherms of dry and wet chlorophyll $a$ are quite different. The packed monolayer areas of the monolayer at the n-octane/water interface correspond to surface areas of 29 Å$^2$ per molecule for anhydrous chlorophyll $a$ and 101 Å$^2$ for adsorbed cluster of hydrated chlorophyll oligomer.

Figure 11 shows the dependence of surface pressure of anhydrous and hydrated chlorophyll $a$ at the octane/water interface. Using the isotherm of adsorption of amphiphilic compounds at the liquid/liquid interface\cite{21, 30} it is possible to calculate the parameters of adsorption. The standard Gibbs free energy of adsorption at the n-octane/water interface is equal to $-27.4$ kJ/mol for dry chlorophyll $a$ and $-35.2$ kJ/mol for hydrated oligomer of chlorophyll $a$. The positive sign of the attraction constant $a = 0.59$ for dry chlorophyll $a$ shows attractive forces between adsorbed molecules of chlorophyll. The negative constant $a = -0.215$ shows repulsion between clusters of the hydrated chlorophyll $a$ oligomer\cite{21, 30}.

To our knowledge, there are no other examples in colloid chemistry in which a surface active compound can be in two different adsorbed states such as dry chlorophyll $a$ molecules and clusters of wet chlorophyll $a$ self organized molecular assemblies.

Water photooxidation

The ability of light-excited chlorophyll molecules to be involved in reversible redox reactions underlies the primary process by which chloroplasts convert solar energy conversion into electrochemical energy. A few years ago, hydrated oligomers of chlorophyll immobilized on the interface between two immiscible liquids\cite{2, 5, 7-10, 13, 18, 22, 27, 29, 32, 34, 36-40, 102}, on a bilayer lipid membrane\cite{103, 104}, on an electrode\cite{78, 91, 105, 106}, in lipid crystals\cite{107}, in lipid gel\cite{108}, Aerosil\cite{109} or present in non-aqueous solvents containing traces of water\cite{92, 93, 95} were found to catalyze oxygen evolution during photooxidation of water. The quantum efficiency in the model oil-water system in terms of oxygen production amounted to 10–20% in the absence of transition metal ions\cite{2, 5, 7-10, 13, 18, 22, 27, 29, 32, 34, 36-40, 102}. Oxygen production was measured both polarographically and by mass-spectra analysis\cite{2, 5, 7-10, 13, 18, 22, 27, 29, 32, 34, 36-40, 102, 110}. Investigations with the Clark oxygen electrode\cite{27} and mass spectrometry\cite{110} conclusively proved that the presence of oxygen resulting from the reac-

![Fig. 9](image-url)  \hspace{1cm} Fig. 9. The dependence of surface tension at octane/water interface on concentration of chlorophyll $a$: (1) dry monomer of chlorophyll $a$; (2) hydrated oligomer of chlorophyll $a$; pH = 7.4[21].

![Fig. 10](image-url)  \hspace{1cm} Fig. 10. Concentration dependence of adsorption of dry chlorophyll $a$ (1) and hydrated oligomer of chlorophyll (2) at octane/water interface[21].

![Fig. 11](image-url)  \hspace{1cm} Fig. 11. Concentration dependence of surface pressure at octane/water interface for dry chlorophyll $a$ (1) and hydrated oligomer of chlorophyll (2)[21].
tion (1) was not due to thermal effects of illumination on the Clark electrode, as suggested by Tien[11] in discussing the results of illumination on the Clark electrode, as suggested by Tien[11] in discussing the results of103. Visible light absorption spectra (Fig. 6, curve 2) indicated that hydrated oligomers of chlorophyll a were present. If chlorophyll a was replaced by chlorophyll b, no oxygen evolution occurred, presumably due to different supramolecular structures of chlorophyll a and b aggregates.

Several authors[8, 22, 92, 93] have proposed a molecular mechanism for water photooxidation in model systems containing chlorophyll. It is likely that chlorophyll was directly involved in photooxidation of water to molecular oxygen early in evolution of light harvesting pathways. However, because hydrated chlorophyll aggregates can operate only for a few hours[2, 7, 9] the structure and composition of the oxygen-evolving complex must have undergone significant evolutionary changes to increase stability.

The interface between two immiscible liquids with immobilized photosynthetic pigments can serve as a simple model convenient for studying photo-processes accompanied by spatial separation of charges, as in biological membranes. Upon illumination of the octane/water interface in the presence of P745, proton acceptors in octane, and electron acceptors (nicotinamide adenine dinucleotide NAD, nicotinamide adenine dinucleotide phosphate NADP or K,Fe(CN)6) oxygen evolution from water takes place at the oil/water interface. Oxygen production was proportional to the area of the interface and did not depend on the volume of the oil or water phases.

Illumination also induces an interfacial photopotential if chlorophyll a and K,Fe(CN)6 are added to the previously equilibrated octane/water system containing 2,4-dinitrophenol (Fig. 12). The magnitude of this potential depends on the wavelength and the intensity of incident light as well as on the concentrations of all reagents. Photopotentials did not occur if octane and water had not been mutually saturated and equilibrated. Similar effects were observed in other experiments in which chlorophyll was used to catalyze redox reactions[6, 15, 16].

The photopotential results from the following reaction taking place at the interface:

\[
2H_2O + 4A + 4PCP^- \rightarrow O_2 + 4PCPH + 4A^- \tag{16}
\]

The reaction is accompanied by the capture of protons released by proton acceptors. We also found that dinitrophenol (DNP) or pentachlorophenol (PCP -) are adsorbed at the interface between two immiscible liquids such as octane and water. Such compounds dissociate to produce a net negative charge on the octane phase relative to water. The magnitude of the measured potential was \(-0.2\) V[7], following addition of dinitrophenol to the octane-water system. When the photoreaction (16) was initiated, the potential shifted abruptly in the positive direction by 0.1 V. The stoichiometry of reaction (16) was previously verified polarographically[13].

Figure 13 shows an absorption spectrum of hydrated chlorophyll oligomer in hydrated octane and an action spectrum of the water photooxidation reaction. The action spectrum in Fig. 13 suggests that hydrated chlorophyll oligomers, P745, and dry chlorophyll monomers are involved in the reaction. Monomeric forms of chlorophyll a may serve as sensitizers, or antennae in this system. These results support a molecular mechanism of water photooxidation in model systems involving oxidized form of hydrated chlorophyll oligomers, as suggested in[7].

![Fig. 12. Dependence of photopotential, measured in the chain (7), on the time of illumination with different contact times of aqueous phase with dry n-octane. Medium: 1 mM dinitrophenol, 10 mM K3Fe(CN)6, 10 mM Tris-HCl (pH 7.7). Chlorophyll in concentration 10 mM was added after 1–10 min, 2–0 min[29].](image1)

![Fig. 13. Action spectrum of water photooxidation in the presence of chlorophyll. Medium: 1 mM pentachlorophenol, 10 mM tris-HCl, pH 7.7, \(10^{-5}\) M chlorophyll a, 1 mM NAD, octane/water. For comparison, absorption spectrum of chlorophyll solution in octane equilibrated with water is presented.](image2)
The hydrated chlorophyll oligomer, Chl₆(H₂O)ₙ (n > 2), adsorbed on the surface and closely packed so that the electron clouds of porphyrin rings may overlap[7] enters an excited state upon illumination, which leads to pigment oxidation and acceptor reduction (Fig. 14). In the reaction center water is coordinated to the magnesium atom of one chlorophyll molecule and linked by hydrogen bonds to the carbonyl group of a second chlorophyll molecule and a pentachlorophenol anion, which is also adsorbed at the interface and forms part of the catalytic reaction center. The PCP⁻ anion is required for water binding at the reaction center and protection of chlorophyll against pheophytinization during the course of the reaction. The redox potential of hydrated chlorophyll oligomer is about 0.92[9,1], so only one possible pathway is available for water oxidation—a direct four-electron oxidation which produces molecular oxygen. It should be mentioned that one- and two-electron reactions shown in Fig. 3 are formally possible if their intermediates are absorbed at the interface and their binding energy is high enough. However, the radicals formed in the course of multistage processes would inevitably oxidize and destroy the catalytic complex. The quantum yield of fluorescence of the aggregate shows that it is 10³ less than the quantum yield of monomer fluorescence. This indicates that the molecular ensemble responsible for the reaction of water oxidation to molecular oxygen, in addition to hydrated oligomer, involves other catalytic sites (eg, chlorophyll dimers, trimers and monomers).

In the reaction described above, P745 acts both as a photosensitizing agent and a catalyst. It is possible to uncouple these functions by adding a dye (β-carotene) that can also sensitize the photooxidation of water[5, 9]. Carotene not only protects chlorophyll against photodynamic destruction, but also is capable of absorbing light and imparting the excitation energy to chlorophyll a. The Van-der-Waals interaction known to exist between the tetrapyrole ring of chlorophyll and β-carotene apparently stabilizes a weak complex[5, 9]. The complex can then participate in water photooxidation at wavelengths where β-carotene rather than chlorophyll absorbs light (Fig. 15)[5,9].

Figure 16 illustrates the relationships between oxygen evolved, the length of the illumination period, and different sequences of reagent addition to the octane/water system. Addition of a proton acceptor (PCP), an electron acceptor (NAD⁺) and chlorophyll results in light-induced evolution of oxygen. No oxygen was evolved during illumination at 462 nm (Figure 16a) but addition of β-carotene caused oxygen evolution at this wavelength. The process stops completely in the dark and returns at the initial rate upon reillumination. In the presence of β-carotene the quantum yield at the incident light wavelength 462 nm is approximately 0.5% (calculated on the basis of incident light quanta). Figure 16 shows that oxygen evolution in the complete system is independent of the order of chlorophyll and β-carotene addition. However, no oxygen evolution in the absence of chlorophyll (Fig. 16b) was found.

Carotenoids do not appear to be directly involved in water photooxidation during photosynthesis, but perform several other functions. Due to their optical properties, they absorb short-wave blue light and excite chlorophyll by resonance energy transfer, thus increasing the efficiency of light energy utilization. As antioxidants carotenoids protect chlorophyll from oxidation by singlet oxygen produced during photosynthesis. Carotene can also participate in electron transfer through its system of conjugated bonds.

We also carried out experiments to establish the duration of the oxygen evolution[9]. The results show that the oxygen evolved without β-carotene was proportional to the illumination time during the

Fig. 14. Proposed mechanism of water photooxidation by hydrated chlorophyll oligomer at the octane/water interface.
Energy conversion at liquid/liquid interfaces

Fig. 16. Dependence of the amount of oxygen evolved on the incubation time and the sequence of addition of reagents. Composition: water/octane, 1 mM NAD⁺, 20 mM tris-HCl, pH 7.4, 1 mM pentachlorophenol. Chlorophyll and β-carotene were added to the octane fraction in concentrations of 4 μM and 10 μM, respectively. The interface was illuminated with monochromatic light at the wavelength of 462 nm.

first 2 h, and that the absorption spectrum of the octane phase was coincident with the initial spectrum. The rates of oxygen evolution began to decrease after 3 h, and entirely ceased between the 4th and the 5th hours of illumination. At that time chlorophyll in the octane phase was discolored as a result of pheophytinization and photobleaching, even though the pH of the aqueous phase remained neutral (buffered by 20 mM tris-HCl at pH 7.4). Addition of β-carotene (10⁻⁵ M) did not significantly increase either duration of molecular oxygen evolution or the stability of chlorophyll a.

Oxygen photoreduction by monolayers of hydrated chlorophyll a oligomer

Optically transparent electrodes, modified with monolayers of photosynthetic pigments using Langmuir-Blodgett technique, are suitable for the investigation of chemical models of photosynthesis[81-90]. During photocatalytic oxidation of hydroquinone by chlorophyll a or pheophytin a in monolayers, the standard Gibbs free energy of electrochemical reaction is negative, so that light energy can be used to overcome the activation barrier. In the absence of hydroquinone, it is possible to use water molecules as electron donor and NADP⁺ or methyl-viologen as an electron acceptor. In this case light energy can be used to perform the work.

The phenomenon of photorespiration by hydrated chlorophyll a monolayer deposited on optically transparent electrodes was studied in[86, 87, 89]. An optically transparent SnO₂ electrode doped with antimony is a quasi-metallic conductor. The SnO₂ electrode is transparent in the visible region of the optical spectrum (Fig. 17) and has a high reflectivity for infrared radiation. The SnO₂-OTE is often used as a working electrode in photoelectrochemistry due to its large potential window and its optical transparency in the visible region. Using such an electrode, the electronic absorption spectrum of wet chlorophyll a monolayer on SnO₂-OTE was measured (Fig. 17). The oligomer possess a Soret band at 425 nm and a low energy band at 664 and 742 nm, while the glass side of the OTE gave absorption maxima at 434, 676 and 744 nm.

During illumination of the hydrated oligomer of chlorophyll a monolayer at the SnO₂-OTE in the presence of an artificial electron donor hydroquinone (QH₂), anodic photocurrents can be measured at voltages between +200 and +50 mV (Fig. 18 curves 1-4). The action spectra under these conditions coincide with the absorption spectrum of a dry chlorophyll a monolayer. At more negative polarization of the electrode in the presence of oxygen, cathodic photocurrents are observed. The action spectra of these cathodic photocurrents correspond to absorption spectra of hydrated oligomers of chlorophyll a. Under polarization in the range of -50 to

Fig. 17. Electronic absorption spectra of SnO₂-OTE (1) and hydrated chlorophyll a monolayer on SnO₂-OTE (2)[89].
I I, I I I

400 600 800

WAVELENGTH (nm)

Fig. 18. Action spectra of hydrated chlorophyll a monolayer on SnO$_2$-OTE in the presence of hydroquinone. Medium: 0.1 M KCl, 0.05 M QH$_2$, 0.025 M NaH$_2$PO$_4$, pH 6.9. Electrode potential against saturated calomel electrode: (1) +200 mV, (2) +150 mV, (3) +100 mV, (4) +50 mV, (5) -50 mV, (6) -100 mV, (7) -150 mV, (8) -200 mV, (9) -250 mV. The aqueous solution was preliminarily deaerated by argon[89].

-150 mV, both cathodic and anodic photocurrents caused by hydrated and dry chlorophyll a appear in the action spectra (Fig. 18 curves 5-9). In the chlorophyll a monolayer two types of particles exist: molecules of dry chlorophyll a and clusters of hydrated oligomer of chlorophyll a. Dry chlorophyll a is capable of phototransfer of electrons from QH$_2$ to SnO$_2$-OTE while the hydrated oligomer of chlorophyll a can transfer electrons from the SnO$_2$ electrode to molecules of oxygen in an aqueous solution of the electrolyte. These processes of phototransfer of electrons in opposite directions are caused by the difference of redox-potentials of dry and wet chlorophyll a and by the capability of clusters of wet chlorophyll a to undergo synchronous multielectron reactions[89]. If oxygen is carefully removed from the aqueous solution, the cathodic photocurrent disappears.

When QH$_2$ is absent from the aqueous solution initially saturated with O$_2$ before illumination, only a cathodic photocurrent appears (Fig. 19). This results from oxygen reduction by the hydrated oligomer of the chlorophyll a monolayer. The cathodic photocurrent is proportional to O$_2$ concentration and strongly depends on the electrode potential and wavelength of the incident light. The action spectrum of the cathodic photocurrent coincides with the absorption spectrum of the hydrated oligomer of chlorophyll. We have monitored this event for 5 h in the presence of oxygen and found that the photocurrent is stable for the entire time. The number of electrons transferred through the electrode interface is thousands of times higher than the number of chlorophyll molecules in the monolayer. The absorption spectra of wet chlorophyll before and after the photoreaction coincides. The monolayer of hydrated oligomer of chlorophyll a is the photocatalyst of oxygen reduction. The cathodic photocurrent depends on the concentration of oxygen in the aqueous solution (Fig. 20).

Quantum efficiency, $\Theta_4$ for photocurrent generation at wavelength $\lambda$ was calculated by the following equation:

$$\Theta_4 = \frac{I_\lambda}{qn(1-10^{-A_\lambda})} \quad (17)$$

where $I_\lambda$ is the photocurrent density at wavelength $\lambda$, q is the elementary charge, n is the number of incident photons and $A_\lambda$ is the absorbance of the chlorophyll a monolayer in contact with the aqueous solution. Using this relation, the maximum quantum yield of oxygen photoreduction at pH 6.9 and an electrode potential of -50 mV was found to be 0.45 ± 0.05%. If three or five monolayers are deposited on SnO$_2$-OTE, instead of one monolayer, the cathodic photocurrent increases, but the quantum yield of oxygen reduction slightly decreases[89].

EMULSION PHOTOBIOELECTROCHEMISTRY

The mechanism of active ion transport by biological membranes is a central problem of bio-
Energy conversion at liquid/liquid interfaces

electrochemistry, and can be investigated in model systems of microemulsions. We note that although emulsions are attractive experimental systems, earlier investigators sometimes have not defined their properties, which can lead to conflicting results. We will therefore briefly outline the relevant parameters.

A system of two immiscible liquids can form 6 types of structures, shown in Fig. 21:

(a) two individual immiscible liquids;
(b) water-in-oil emulsion/aqueous phase;
(c) oil/oil-in-water emulsion;
(d) two immiscible liquids and emulsion upon the interface;
(e) oil-in-water emulsion or water-in-oil emulsion;
(f) water-in-oil emulsion/oil-in-water emulsion.

When studying reactions at the oil/water interface, one of the above structures should be specified. It should also be noted that transitions from one structure to another can occur if concentrations of surface active compounds or electrolyte are varied, or if components of the system are altered by chemical reactions.

**Bacteriorhodopsin in emulsions**

Bacteriorhodopsin functions in the photovoltaic transformation of light energy, and is one of the most extensively studied ion pumps. Bacteriorhodopsin sheets from *Halobacterium halobium* were one of the first membrane proteins to be investigated at the octane/water interface [14, 20, 23, 25, 112, 113]. These studies were carried out in several laboratories, with differing results and interpretation. Post et al. [25] established a method to immobilize bacteriorhodopsin sheets in octane-in-water emulsions. This considerably simplified measurements, so that proton pumping in aqueous emulsions could be carried out using conventional pH meters. The emulsion system also facilitates quantitative measurements of other ion transport processes through the water/lipid interface, using ion selective electrodes. The large interfacial area makes it possible to obtain the products of heterogeneous reactions in macroscopic quantities, and the low dielectric constant of the non-aqueous phase can decrease of activation energy of transport reactions [10-12]. Emulsion enzymology thus allows study of naturally immobilized membrane enzymes in conditions close to native states.

Earlier papers [14, 20, 23, 25, 112] showed that bacteriorhodopsin immobilized at the water/lipid-in-octane interface is capable of phototransfer of protons from water into octane. The photoeffect was not inhibited by uncouplers of oxidative phosphorylation and depended upon the ionic strength of the solution. However, the molecular mechanism by which immobilized bacteriorhodopsin carried out the pumping remained unclear. Some authors [113] claimed that bacteriorhodopsin is denaturated at the octane/water interface and, in presence of phospholipids, forms closed structures with a "third water" at the octane/water interface. Upon illumination the bacteriorhodopsin was thought to pump protons from the aqueous phase into the "third water" rather than transferring protons directly from water into octane. In order to measure the photoeffect Voltapotential measurements were used [14, 23, 112]. This method yielded information on the variation of the potential difference and the charge upon the interface but did not specify whether transfer of charges from one phase into another occurred, since both the orientation of dipoles at the interface and transfer of charges could affect the value of Voltapotential.

The capacity of bacteriorhodopsin to phototransfer protons through the water/lipid interface in octane was investigated by Gugeshashvili et al. [20] who analyzed the mechanism of fusion of purple membranes with monolayers of lipids at the octane/water interface and proposed a theoretical model of the photoeffect in the emulsion. The measurements were carried out in octane-in-water emulsions, the weight ratio being 1 (octane)/8 (water) [20, 25]. 2 M NaNO₃ or 2 M NaCl was initially dissolved in the water phase, and sonication resulted in octane-in-water emulsions covering the layer of aqueous electrolyte solution.

Upon illumination the emulsions with white light, pH shifts were observed in the aqueous phase of the emulsion. Fig. 22 shows the reversible pH shift in

![Fig. 22. The reversible light-dependent pH shift in octane-in-water emulsion containing bacteriorhodopsin sheets; pH of emulsion in dark condition being 6.0. The aqueous phase contained 2 M NaNO₃ [20].](image)
octane-in-water emulsion. The pH of the aqueous phase markedly increased in the light (Fig. 22). If one of the components (either bacteriorhodopsin or inorganic salt) was excluded from emulsion, the pH shift was not observed. Upon illumination the photoreponse rose very quickly and attained steady state in approximately 2–3 s. In the dark, the pH returned to the initial level more slowly. The photoeffect was reversible and could be repeated many times. If the prepared emulsion did not contain phospholipids, denaturation of bacteriorhodopsin was observed. Introducing the pentachlorophenol (PCP) or tetrachloro-2-trifluoromethylbenzimidazole (TTFB) into the emulsion did not inhibit proton transport.

Sonicating the octane + water + NaNO3 + bacteriorhodopsin system without asolectin leads to denaturation of bacteriorhodopsin. Substitution of a more polar solvent, 1,2-dichloroethane, for octane also causes some denaturation. However, dichloroethane is the most extensively used non-aqueous solvent in electrochemical studies of two immiscible electrolyte solutions[26]. Therefore we could use classic electrochemical methods (e.g., cyclic voltammetry)[1] to quantitatively estimate the photo-transfer of protons through the dichloroethane interface.

Figure 23 shows the reversible light-induced pH shift in the aqueous phase during illumination of emulsion containing bacteriorhodopsin and inorganic salt by light of different wavelengths. The magnitude of the photoeffect clearly depends upon the wavelength of the incident light. The absorption spectra of bacteriorhodopsin sheets in water and action spectrum are shown in Fig. 24, which indicates that the action spectrum correspond to the absorption spectrum. A mathematical model for the photoeffect in emulsions was developed by Markin and Portnov[20]. The theoretical curve in Fig. 25 is quite similar to the experimental curve drawn upon the same figure.

Two types of incorporation of bacteriorhodopsin into the water/octane + lipid interface are possible (Fig. 26): (a) fusion with lipid monolayer or (b) creation of the Plateau border with an aqueous cavity between the bacteriorhodopsin and octane (the so-called “third” water). The proton transport in the systems with “third” water may be inhibited using uncouplers of oxidative phosphorylation, such as dinitrophenol, pentachlorophenol, or TTFB. Because the introduction of uncouplers does not decrease the photoeffect, we conclude that protonophores are not involved in the proton transfer in closed membrane structures with aqueous cavities, but rather become
acceptors of protons at the interface. Pentachlorophenol is a weak acid dissociating into PCP$^-$ and H$^+$. PCP$^-$ is adsorbed upon the octane/water interface, thereby charging the octane phase negative with respect to water[7]. It is possible to estimate the maximum value of the possible photoeffect if PCP operates as a surface acceptor of protons. The radius of the emulsion droplet is about 1 μm, and the volume of octane is 0.8 ml. The number of droplets is $2 \times 10^{11}$ with a total surface of about 2 m$^2$. Assuming the surface concentration of PCP$^-$ is $10^{-11}$ mol/cm, the pH shift from an initial value of pH 6.1 should not exceed 0.1 pH units. It follows the photoeffect observed by experiment[20] reflects the functioning of PCP$^-$ as a surface proton acceptor.

Illumination increases transfer of protons from water in the octane the passive anionic co-transport. According to Bell[114] the partition coefficient of acid between water and octane is such that inorganic acids can be present in octane in tangible quantities. For example, HCl dissolves in octane in concentrations up to 0.21 g/l,$^1$, and HNO$_3$ has an even greater solubility[114]. As shown in[20] larger anion and smaller cation radii permit higher pH during illumination of the emulsion. This fact can be explained by the theory of resolvation of ions between two immiscible liquids[115-120], which shows that larger ionic radii increase the negative free energy of transport from water into oil.

PHOTOCHEMISTRY IN THE PREBIOTIC ENVIRONMENT

Carbonaceous meteorites, interplanetary dust particles and apparently comets all contain polycyclic aromatic hydrocarbons (PAH) as the most abundant organic components. There is a developing consensus that late extraterrestrial infall was a significant source of organic compounds on the early Earth[19], and we have proposed that such reactions may have been involved in primitive photo-synthetic reactions related to chemical evolution[31, 33, 121]. The discussion to follow addresses the question of how PAH could have contributed to the chemical evolution leading up to the appearance of life nearly 4 billion years ago.

Photochemical synthesis of amphiphiles

Because light is the most abundant energy source in the contemporary biosphere, and presumably was equally abundant on the early Earth, we are investigating whether photochemical synthesis of amphiphiles could occur under simulated prebiotic conditions.

Polycyclic aromatic hydrocarbons absorb near-uv and far blue light energy. If PAH are partitioned into liquid/liquid interfaces or lipid bilayer membranes, they can capture light energy by donating electrons to produce molecules with higher chemical potential. They may also release protons during photochemical reactions, and photosensitize the photooxidation of alkanes.

In a simple demonstration of alkane photooxidation, a 49:1 hexadecane-2-ethyl-anthracene mixture can be deposited as a droplet on an aqueous subphase[122]. Upon illumination, the hydrocarbon droplet immediately begin to spread, a process that can readily be followed by the fluorescence of the 2-ethyl-anthracene. When the spreading film fills the surface of the Petri dish, marked changes in the surface tension occur, finally reaching a surface pressure in the range of 15 mN m$^{-1}$, at which point a solid film is present. The reaction ceases immediately when illumination was stopped, and resumes upon reillumination. We have extended this result to microemulsions in order to better understand the reaction. In these experiments, 2-ethyl-anthracene was dissolved in hexadecane and dispersed by sonication in anaerobic 1 M LiCl or 1 M NaCl. Measurements of pH changes and O$_3$ concentration were carried out in a reaction chamber during interfacial photochemical reactions mounted on a vibration-stabilized table (Fig. 27). Upon illumination, this system released protons, producing acidification of the aqueous phase (Fig. 28). Analysis of the hexane extract from emulsion after illumination by gc-ms indicated that 1-hexadecanol and 2-hexadecanone were major products of the reactions (Fig. 29). These amphiphilic compounds would account for the surface activity observed during the course of the reaction.

Klein and Pilpel[123] were among the first to demonstrate that amphiphiles can be synthesized by a light-dependent reaction using polycyclic aromatic hydrocarbons as photosensitizers. A general reaction scheme for such processes was proposed by Gesser et al[124] and is outlined below:

$$X + h\nu \rightarrow X^* \quad (18)$$

$$X^* + RH \rightarrow XH + R^* \quad (19)$$

$$XH + O_2 \rightarrow X + H_2O_2 \quad (20)$$

$$R^* + O_2 \rightarrow RO^*_2 \quad (21)$$

$$RO^*_2 + RH \rightarrow ROOH + R^* \quad (22)$$

$$RO^*_2 + XH \rightarrow ROOH + X^* \quad (23)$$

$$ROOH \rightarrow RO^- + OH^- \quad (24)$$

$$RO^- + RH \rightarrow ROH + R^* \quad (25)$$

$$ROOH + R^* \rightarrow RO^- + ROH \quad (26)$$

where X is an aromatic compound such as xanthone and RH is an aliphatic hydrocarbon. Note that the primary products are alcohols (ROH).

Although PAH clearly act as photosensitizers in such systems[31, 33, 121], they do not appear to be absolute requirements, particularly under conditions of long-term illumination in air. For example, Folsome and Morowitz[125] illuminated hexadecane in air over concentrated solutions of phosphate and found that aggregates of phosphate-containing compounds were produced. Seleznev et al.[126] illuminated hexadecane-sea water systems in air, and reported that amphiphilic compounds were produced, some of which took the form of multilamellar liquid crystals that could be visualized by polarization light microscopy. Both of these studies related their observations to the possible synthesis of membrane-forming compounds...
that might be able to serve as primitive lipid-like molecules on the prebiotic Earth.

The present study confirms that u.v. illumination can drive oxidation of hexadecane to more polar compounds, particularly long chain alcohols, which are surface active. 2-Ethyl anthracene, a model for polycyclic aromatic hydrocarbons present in carbonaceous meteorites, is able to act as photosensitizer. The reaction at air-water interfaces required trace molecular oxygen to yield significant amphiphilic products within 1-2 h of illumination. However, if the hydrocarbons were dispersed by sonication before illumination, we found that significant amounts of polar products were produced even under relatively stringent anaerobic conditions in which the samples were continuously purged with high purity deoxygenated argon. The rate limiting step does not depend on oxygen concentration, because carrying out the reaction under aerobic conditions did not increase the rate or yield.

**Photoproduction of protons**

Upon illumination of the anaerobic alkane/water system, marked acid pH shifts were readily observed. For instance, 2-ethyl-anthracene dissolved in dodecane and dispersed by sonication in 1 M LiCl, produced pH shifts corresponding to the release of 4 mM protons upon illumination with a Zeiss 75 watt filtered mercury arc lamp (Fig. 28). The production of protons in the anaerobic aqueous system of dispersed hydrocarbons described above was unexpected, and we do not yet understand the underlying mechanism. Nonetheless, it is clear that PAH dissolved in hydrocarbon environments can absorb light energy, then react to release protons. Proton production in this case must reflect a more basic photochemical process, most likely involving oxidation-reduction reactions in the components of the system. Since molecular oxygen is not present at the start of the reaction, it is possible that a source of the electrons is water itself.

The quantity of protons produced during illumination of the emulsion depends on concentration of inorganic electrolyte and initial pH of the emulsion. The reaction is favored by high electrolyte concentrations. The nature of an inorganic cation also
had a modest effect, in the sequence: Li⁺ > Na⁺ > K⁺ > Rb⁺.

Photoreduction of carbon dioxide

We have also studied the possibility that carbon dioxide can act as an electron acceptor in such reactions. When oxygen-free carbon dioxide was gently flushed through the above system, we found that a soluble acid was produced with the Ω (Fig. 30) and FTIR (Fig. 31) spectral characteristics of saturated organic acid such as, for example, formic acid. The Ω spectrum is virtually identical to that of short-chain organic acid under the same conditions.

We obtained the Ω spectrum of the water soluble product, which also resembled a carboxylic acid (Fig.

Fig. 30. Gas chromatography—mass spectrum of polar products after illumination of emulsion (4 ml of aqueous solution of 1 M LiCl and 0.2 ml of 2% solution of 2-ethyl-anthracene in hexadecane. Products of photoreaction were extracted in 4 ml of hexane. pH of the emulsion before illumination was 3.7. Photoreaction was carried out in CO₂ atmosphere. The gas chromatogram of underivatized products showed unreacted hexadecane (No. 476) together with 1-hexadecanol and 2-hexadecanone peaks (No. 605 and 620, respectively).

Fig. 29. Absorption spectrum of the emulsion after 10 min illumination. Medium: 4 ml 1 M LiCl, pH = 3.7, 0.2 ml 2% 2-ethyl-anthracene in hexadecane. Reference: emulsion before illumination, 1 cm pathlength.
The absorption maximum at 206 nm was not observed if argon was substituted for CO₂. It is highly dependent on lower pH ranges. For instance, the absorption at 206 nm was 3.7.

31). The absorption maximum at 206 nm was not observed if argon was substituted for CO₂. It is highly dependent on lower pH ranges. For instance, no products were observed if the reaction was carried out at pH 7 in 1 M NaHCO₃ atmosphere. (2) control sample without illumination. Emulsion consists of 4 ml 1 M LiCl, 0.2 ml 2% 2-ethylanthracene in hexadecane. pH before illumination was 3.7.

Taking this reaction as a working hypothesis, we are now exploring possible ways to test the hypothesis and directly demonstrate water soluble organic acid synthesis from carbon dioxide.

CONCLUSIONS

The interface between two immiscible liquids has unexpected properties that follow from thermodynamic and kinetic principles controlling reactions in biphasic environments. We have shown in this chapter that photochemical reactions in particular have remarkable characteristics when carried out at interfaces. Biological reaction pathways such as oxygen evolution by chlorophyll a, and proton transfer by bacteriorhodopsin, can be investigated in systems involving liquid/liquid interfaces. Early photochemical processes that may have permitted light harvesting by primitive cells can also be studied at liquid/liquid interfaces of emulsified alcanes and polynuclear aromatic hydrocarbons. An important general insight is that multielectron reaction centers in biological membranes can be understood as self-organized catalytic interfaces. In the future, such molecular ensembles will likely be central features of systems that capture solar energy by artificial photosynthesis.

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