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# Chlorophyll–water interaction during oxygen photoevolution at the octane–water interface

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**The interaction between water and chlorophyll *a* dissolved in octane has been studied. Factors governing the formation of the hydrated chlorophyll oligomer with the absorption maximum at 742 nm have been identified. It has been revealed that the water photooxidation reaction sensitized by chlorophyll absorbed at the interface requires the presence of water in the octane phase during self-assemblage of the catalytic complex responsible for the reaction.**

## Introduction

Photooxidation of water by chlorophyll under exposure to visible light occurred when chlorophyll was immobilized on a metal electrode [1], on a bilayer lipid membrane [2,3], on aerosil [4], and at the interface between two immiscible liquids [5–12,32,33]. Though several papers have been written on the state of aggregation of immobilized chlorophyll and its interaction with the solvent [1,12–16,33], the question of how chlorophyll participates in the reaction proceeding at the interface between octane and water is still unresolved.

It was discovered previously that oxygen evolution takes place [5–9] if the octane–water interface is exposed to visible light in the presence of chlorophyll, a proton acceptor, dinitrophenol or pentachlorophenol, in octane and an electron acceptor ($K_3Fe(CN)_6$, $NADP^+$, $NAD^+$) in water. The quantum yield at the wavelength 660 nm is about 0.6% of the number of incident light quanta and 10% of that of absorbed quanta. With a 1 h exposure per molecule of chlorophyll *a* adsorbed at the octane–water interface $10^4$ molecules of oxygen evolve. The abrupt change in potential that occurs in this case at the octane–water interface is proportional to the amount of adsorbed chlorophyll and the oxygen-evolution rate, which correlates with the phenomenological theory of catalytic reaction that take place at the interface between two immiscible liquids [17]. The action spectrum of the oxygen-evolution rate is similar to the absorption spectrum of chlorophyll *a* in the octane–water system.

## Materials and Methods

Absorption spectra were recorded on spectrophotometers Specord M-40 (G.D.R.) and Beck-

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man (Model 35, U.S.A.), and circular dichroism spectra on the Japanese spectrophotometer Jasco-40AS. Fluorescence spectra were registered by a Hitachi MPF-4 spectrophotometer (Japan).

Circular-dichroism spectra were registered by a dichrograph in a 1 cm quartz cell. The dichrograph was calibrated using d-10-camphorsulphonic acid. Circular-dichroism spectra are represented as the dichroic density $\Delta A = A_l - A_r$ ($A_l$ is the absorbance of a sample for light with left circular polarization, $A_r$ the absorbance for light with right circular polarization). The Volta potential was measured by the vibrating electrode method [18–20] in the chain:

| | | | | | |
|---|---|---|---|---|---|
| Au | air | octane | water | water | $Hg_2Cl_2Hg$ |
| | | | electron | KCl- | |
| | | chlorophyll | acceptor | saturated | |
| | | proton acceptor | buffer | | |

(1)

and registered automatically by a KSP-4 recorder. The block diagram of the unit is described in Refs. 18 and 19. The measuring cell was filled with 10 ml of the aqueous phase upon which 1 ml of the octane fraction was superposed. The interface area was 3 $cm^2$.

The power of light incident on the interface for all the wavelengths used was measurement with a thermocouple ,and also with a ferrioxalate actinometer at the wavelength 429 nm. The quantum yield was estimated from the energy of the incident light expressed in terms of the number of quanta.

Oxygen concentration was measured polarographically with a Clark-type electrode [6]. The electrode temperature coefficient did not exceed 1.5% per ° C, which virtually excluded the effect of temperature during measurement of the action spectrum. This was confirmed by control experiments as well. The illumination of the Clark electrode in the octane–water system, with a light intensity two orders greater than that used conventionally, did not induce an electrical response ascribed to oxygen evolution, if the system contained neither chlorophyll nor charge acceptors. The electrode measured oxygen concentration down to $10^{-8}$ M. The diameter of the platinum cathode was 20 $\mu$m, the polypropylene film (Radiometer, Denmark) was 25 $\mu$m thick.

The experiments were conducted at 20 ° C. The interface was illuminated with a QGM-300W iodine incandescent lamp. In all the experiments in which oxygen was determined, the cell was initially flushed with argon to remove all atmospheric oxygen and then checked for air-tightness. The interface area in the polarographic cell was 21 $cm^2$. The action spectrum was measured using interference light-filters. The bandwidth of the filter with $\lambda_{0.5} = 680$ nm was 8 nm. Before entering the cel, the light passed through a water filter.

The system of two immiscible liquids containing dinitrophenol or pentachlorophenol was equilibrated in a separate vessel for 24 h. Chlorophyll and a water-soluble electron acceptor were introduced just before the experiment. Chlorophyll *a* was isolated from nettle using the usual method [21]. An aliquot of the concentrated chlorophyll ($10^{-2}$–$10^{-3}$ M) was added to dry or 'wet' octane, up to a concentration of $10^{-6}$–$10^{-5}$ M. All the solutions were prepared with double-distilled water. The salts were recrystallized twice. Dinitrophenol, pentachlorophenol and potassium ferricyanide were recrystallized three times by conventional methods. Tris-HCl was recrystallized three times. Chemically pure *n*-octane was additionally refined.

## Results and Discussion

If chlorophyll *a* and $K_3Fe(CN)_6$ are added to the octane–water system preliminarily equilibrated and containing 2,4-dinitrophenol, then illumination of the interface sets up a photopotential (Fig. 1) which depends on the wavelength and intensity of the incident light as well as on the concentration of all the reaction components. The photopotential did not arise unless octane and water were preliminarily brought to an equilibrium and mutually saturated. An analogous effect was observed in other reactions in which chlorophyll participated as a photocatalyst [22,23].

Fig. 2 shows the relationship between the Volta-potential variation in the chain (1) and the time when 'dry' octane came into contact with the water phase containing dinitrophenol and ferricyanide. The contact time is the time between the

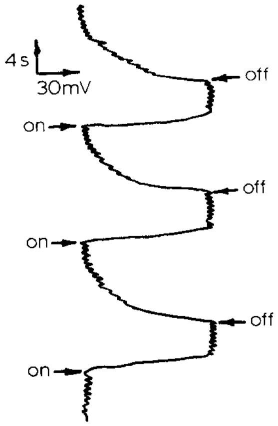


Fig. 1. Kinetics of Volta-potential generation, registered in chain (1). Medium: octane-water, $10^{-3}$ M dinitrophenol, $10^{-2}$ M $K_3Fe(CN)_6$, $10^{-5}$ M chlorophyll *a*, $10^{-2}$ M Tris-HCl (pH 7.7).

moment when dry octane is superposed on the water phase to that when chlorophyll is added. If chlorophyll was added immediately, the photopotential did not arise for 1.5 h (curve 1, Fig. 2). If chlorophyll was added 5 min after the formation of the octane–water interface, the photopotential did not appear during 25 min, then it slowly increased with time, and in 1 h, it reached 35–40 mV. If chlorophyll was added 10 min after the contact between dry octane and water was established, the photopotential arose under illumination after only 10 min, then it gradually increased and reached the maximum value of 100 mV after 1 h (curve 2, Fig. 2). For comparison, the same figure shows (curve 3, Fig. 2) that if chlorophyll *a* was added 24 h after the contact between the aqueous phase and octane, containing a proton acceptor dinitrophenol, the photopotential appeared immediately and reached 100 mV, provided 10 mM $K_3Fe(CN)_6$ was added to the water phase as an electron acceptor. The photopotential registered in the chain (1) is due to the reaction taking place at the interface:

$$2\,H_2O + 4[Fe(CN)_6]^{3-} \xrightarrow[Chl]{4h\nu} O_2 + 4H^+ + 4[Fe(CN)_6]^{4-} \qquad (2)$$

which is accompanied by the capture of protons released during reaction (2) by proton acceptors:

$$(\text{dinitrophenol})^-_{ads} + H^+ \rightleftarrows \text{dinitrophenol}\cdot H \qquad (3)$$

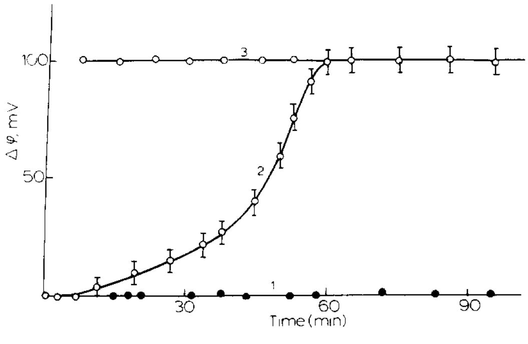


Fig. 2. Dependence of Volta-potential registered in chain (1) on the illumination time with different contact times of aqueous phase with dry octane. Medium: $10^{-3}$ M dinitrophenol, $10^{-2}$ M $K_3Fe(CN)_6$, $10^{-2}$ M Tris-HCl (pH 7.7). Chlorophyll in concentration $10^{-5}$ M was added after 1–0 min, 2–10 min, 3–24 h.

Dinitrophenol or pentachlorophenol adsorbed at the interface between two immiscible liquids dissociates, charging octane negatively in relation to water. The value of the Volta-potential in the chain (1), following the addition of dinitrophenol to the octane–water system was −0.2 V. When photoreaction (2) starts, the potential shifts abruptly in the positive direction by 0.1 V (Figs. 1 and 2). The stoichiometry of reaction (2) was previously varified polarographically [9].

It is difficult to understand why the penetration of water into the octane layer adjacent to the interface is essential for the formation of hydrated chlorophyll oligomers capable of catalyzing the reaction of water photooxidation, if one assumes that the reaction involves a monolayer rather than a structured layer of hydrated chlorophyll oligomers.

The difference in the kinetics of photopotential generation as a function of the contact time between the aqueous and octane phases can be explained by the absorption spectra of chlorophyll in dry and wet octane.

Chlorophyll *a*, dissolved in dry octane, has the distinctive spectrum shown in Fig, 3. If a chlorophyll solution in dry octane is brought into a short time (1–3 min) contact with water, the chlorophyll absorption spectrum is not affected (Fig. 3, curve 2). The picture is different when chlorophyll is dissolved in water-saturated octane (‘wet’ octane).

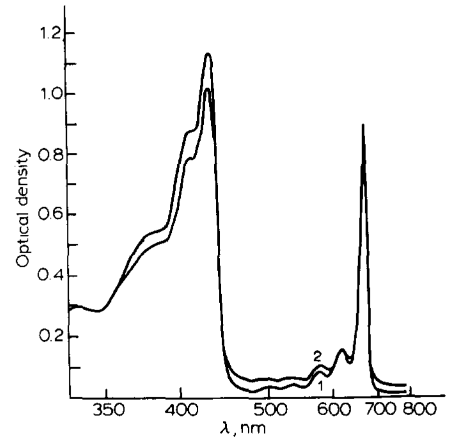


Fig. 3. Absorption spectrum of chlorophyll *a* in dry octane (1) and in octane after 3 min contact with water (2).

In this case an additional absorption maximum is invariably observed at 742 nm [12,33], which corresponds to the absorption maximum of the hydrated chlorophyll oligomer [13–16,24,25]. If chlorophyll is dissolved in dry octane, then, with prolonged contact with water under intensive shaking of the octane-water system for approximately 1 h, a maximum at 742 nm appears as well. The appearance of a maximum at 742 nm is accompanied by the formation of a shoulder on the absorption spectrum of the hydrated chlorophyll oligomer near 460 nm [12,33].

It is known that chlorophyll *a* dissolved in non-polar hydrocarbons aggregates in the range of fairly high concentrations ($10^{-2}$–$10^{-3}$ M), forming oligomers [13–16]. Chlorophyll is mainly present in octane in the form of tetramers and dimers [13] if the concentration of it is high enough. In the investigated range of chlorophyll concentrations ($2 \cdot 10^{-6}$–$2 \cdot 10^{-5}$ M), it is present in wet octane as a mixture of monomers, hydrated monomers and hydrated oligomers, which is evidenced by the fluorescence and circular dichroism spectra.

Fig. 4 shows a circular dichroism spectrum of chlorophyll in wet octane. As can be seen from Fig. 4, there are negative bands at 760 nm, 748 nm, 740 nm and 732 nm in the region of $Q_y$ transition and a positive band near 715 nm. This testifies that the oligomer consists of at least 5–6 chlorophyll molecules.

It should be noted that the aggregate has a very strong rotation force. The Kuhn anisotropy factor

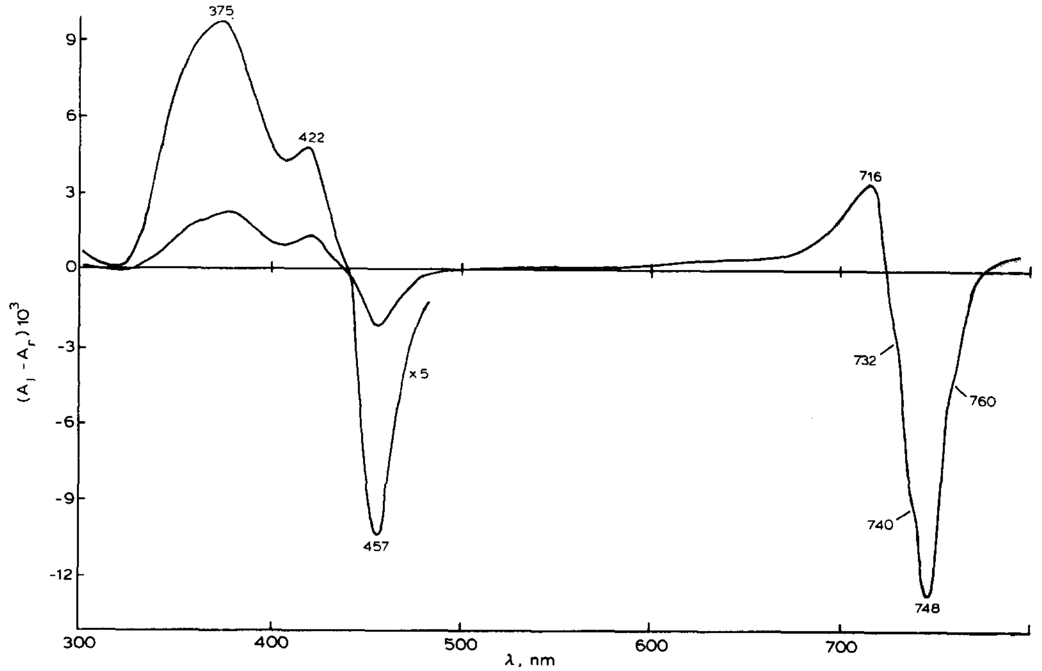
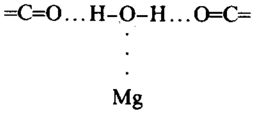


Fig. 4. Circular dichroism spectrum of chlorophyll *a* in 'wet' octane. Chlorophyll *a* concentration is $10^{-5}$ M. is Absorbance $A_{742} = 0.27$.

at 748 nm is $g = \Delta A/A = 5 \cdot 10^{-2}$, which is nearly 500-times more than with monomer chlorophyll.

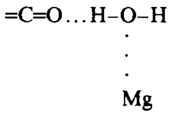
Naturally, comparison of chlorophyll properties in the bulk of the 'wet' octane phase and at the oil–water interface can only be compared with certain reservations.

Chlorophyll molecules adsorbed at the octane–water interface are aggregated as a result of a strong attaction between pigment molecules [6,8] and close monolayer packing. In the case of close packing (i.e., in monolayers or films) molecular vibrations result in intermolecular damping of luminescence owing to the overlapping of electron clouds of porphyrin rings, when molecules come into contact during vibrations [12,13,26,27,33]. When chlorophyll oligomers are hydrated, the coordination interaction C–O...Mg is completely disturbed and water molecules are coordinated by the central atom of chlorophyll magnesium:



$$=\mathrm{C{=}O\ldots H{-}\underset{\underset{Mg}{\vdots}}{O}{-}H\ldots O{=}C{=}}$$

with the absorption maximum at 720 nm or



$$=\mathrm{C{=}O\ldots H{-}\underset{\underset{Mg}{\vdots}}{O}{-}H}$$

with the absorption maximum at 740–745 nm [13–16,26]. Chlorophyll oligomers are hydrated during interaction with water; the process, is a slow one, however, since the former coordination bonds =C=O...Mg must break up and the new ones are formed.

The absence of photopotential in the dry octane–water system and the dependence of its amplitude in water-saturated octane on the chlorophyll incubation time prove that photopotential generation requires a certain structural organization of the interface layer. A high surface concentration of the adsorbed chlorophyll favours its aggregation, whereas contact between the porphyrin ring and the aqueous phase ensures the high dielectric permeability of the surroundings necessary for the formation of chlorophyll cation radicals. Since it can be hardly assumed that hydration of monomer chlorophyll in the octane phase is essential for the pigment organization at the interface where the porphyrin ring is in the aqueous medium, the observed dependence of the photopotential on the presence of water in octane is more likely to be explicable in terms of the effect of water on the aggregation of chlorophyll in octane and the subsequent adsorption of aggregates at the interface.

Indeed, in dry octane in the studied range of chlorophyll concentrations ($10^{-5}$ M), chlorophyll is mainly present in the monomer state (Fig. 3). According to the absorption spectra, with a short time (2–3 min) contact of chlorophyll with water there is no significant formation of aggregates as yet and an adsorption layers at the interface is formed mainly of monomer chlorophyll.

The picture is quite different if chlorophyll is dissolved in water-saturated octane. In this case an additional absorption maximum at 742 nm (Fig. 5) belonging to the hydrated chlorophyll oligomer [12–16,24,25,33] appears immediately in the freshly prepared solution. Thus it may be assumed that when chlorophyll is dissolved in wet octane, the hydrated chlorophyll oligomers formed in the octane phase take part in the formation of the adsorption layer at the octane–water interface. This hydrated oligomer shows itself in the action spectra of oxygen evolution (Fig. 5).

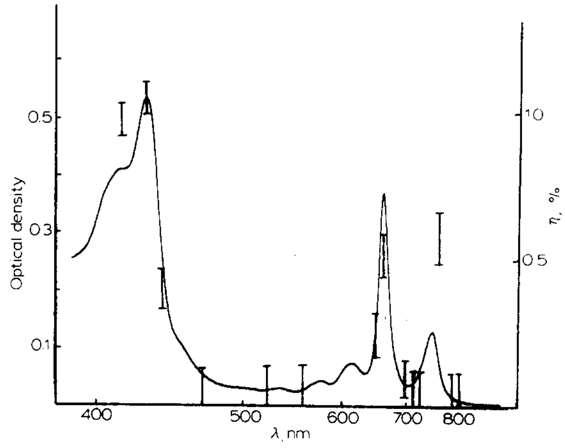


Fig. 5. Action spectrum (1) of water photooxidation reaction in the presence of chlorophyll. Medium: 1 mM pentachlorophenol, $10^{-2}$ M Tris-HCl (pH 7.7), $10^{-5}$ M chlorophyll *a*, $10^{-3}$ M NAD$^{+}$, octane-water. For comparison the figure shows the absorption spectrum of chlorophyll solution in octane equilibrium with water.

Thus, the photopotential generation in the system containing 'wet' octane and, correspondingly, the fact that the mentioned effect does not appear in the system with dry octane may be attributed to the presence and absence, respectively, of hydrated chlorophyll oligomer at the interface. It was shown previously [6] that the action spectrum of the water photooxidation reaction repeats the chlorophyll absorption spectrum. Fig. 5 depicts the absorption spectrum of hydrated chlorophyll oligomer in 'wet' octane and the action spectrum of the water photooxidation reaction recorded in a wider band of wavelengths than before in order to find out whether oxygen is evolved in the region specific to the absorption of hydrated chlorophyll oligomer. As can be seen from Fig. 5, the action spectrum of the reaction indicates that dry monomer chlorophyll and hydrated chlorophyll oligomer participate in the reaction. This supports the molecular mechanism of the water photooxidation reaction in model systems proceeding through an oxidized form of hydrated chlorophyll oligomer, as suggested in Refs. 10, 12, 28, 30, 31, 32 and 33. The estimation of the quantum yield of fluorescence of the aggregate shows, however, that it is three orders 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 (e.g., chlorophyll dimers, trimers and monomers).

Such a large decrease of the quantum yield of fluorescence is most likely caused by the increase of the internal conversion constant and not by that of the interconversion constant, which must lead to a decrease of the quantum yield of triplets of the $\mathrm{Chl}_{742}$ set. As the photochemical reactions of chlorophyll in solutions known today proceed exclusively through the triplet state, the water photooxidation reaction seems unlikely to be caused directly by a hydrated chlorophyll oligomer. Most probably, this set sensitized the reaction by nonemissive energy transfer to some undetected catalytic sites and this results in its appearance in the action spectrum (Fig. 5).

If, however, the reaction proceeds through a singlet state, then the hydrated chlorophyll oligomer will act as a photocatalyst of the water oxidation reaction.

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