Photosynthetic Electron Carriers at a Heptane-Water Interface

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Surface properties of monomolecular films of chlorophyll (Chl), plastocyanin (Pc), cytochrome c (Cyt) and ferridoxin (Fd) were measured at a heptane-water interface. Mixed films of Chl and the other components were examined in darkness and in light. The area/molecule, *A*, for Fd (from *Cl. pasteurianum*) is much larger at a heptane-water than Fd (from spinach) at an air-water interface. This difference in *A* may be the source of the Fd or the extent of denaturation at the different interfaces. There appears to be a photoreaction between Chl and Fd in the presence of ascorbate. The *A* for Cyt is much larger at a heptane-water than at an air-water interface. In mixed films there is a strong interaction between Chl and reduced Cyt in the dark. No such interaction is observed between Chl and oxidized Cyt. With mixed films of Chl and reduced Pc there is a significant, reversible, light induced change in ΔV.

Introduction

Monomolecular films may be used as a model system to simulate aspects of molecular orientation, composition and interfacial reactions that obtain in biological membranes. Monolayers of biological pigments at an air or nitrogen-water interface have been reported in the literature by several investigators. Such studies include chlorophyll[1], cytochrome[2,3], ferridoxin[4] and plastocyanin[5,6]. Chlorophyll (Chl) films on an alkaline buffer are stable in air provided they are kept in the dark[7], upon irradiation there is a pronounced decrease in area. Photoreactions have been reported in mixed monomolecular films containing Chl[3,4,6]. A preliminary report of Chl and cytochrome c (Cyt) at a heptane-water interface was reported by Karan and Brody[8].

The present work is directed at determining the surface properties of photosynthetic materials at a heptane-water interface. These results will be compared to those obtained previously at a gas-water interface.

Materials and Methods

The oil-water Langmuir surface balance and the technique followed in this work were described previously[9,10]. Surface pressure, π, was measured with a Beckman microbalance (Model LM 600) and a sand blasted Pt blade. The accuracy of measuring π is ± 0.02 dyn/cm. Surface potential, ΔV, was measured using the AC method of a vibrating plate electrometer[11]. The apparatus to measure ΔV was constructed and described by Mingins and Taylor[12]. The accuracy of the measurements of ΔV is ± 10 mV for the system described in this work.

Crystalline Chl a was prepared as described previously[2]. The final purification steps include crystallizing the Chl out of acetone and out of hexane. The Chl crystals were stored on phosphate buffer pH 7.8. Freshly chromatographed plastocyanin (Pc) was the generous gift of Dr. R. Gregory (Univ. Manchester). Cytochrome c (Cyt), acid modified, type XII from horse heart was supplied by Sigma Chem. Co. (St. Louis, Mo.). Ferridoxin (Fd) type I from *Cl. pasteurianum* was supplied by Sigma Chem. Co (previous studies of Fd monolayers used Fd from spinach[4]). Heptane was purified by passing it through aluminium oxide and then fractional distillation. Water was distilled from sodium hydroxide and permanganate. Sodium chloride (ultra pure grade from Merck) was roasted at 720 °C. Phosphate buffers (Sigma Chem. Co.) and *iso*-ascorbic acid, sodium salt (BDH reagent grade, 98% pure) were used without further purification. Potassium ferricyanide (ferri) was recrystallized twice from hot distilled water.

Concentration of Pc, Chl and Cyt were determined spectrophotometrically from the optical density of the absorption maximum at longest wavelength; the concentration given by Sigma was used for Fd. The molar absorption coefficients used for

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calculating concentrations for Pc, Chl, reduced Cyt and oxidized Cyt are respectively, 4.5×10^3, 7.95×10^4, 2.48×10^4 and 0.93×10^4 l/M cm. The best absorption ratio for Pc published in the literature is E 278/E 597 = 1.1 – 1.2 [13]. The Pc used in this work had a E 278/E 597 ratio of only 5.4.

The spreading solvent for Chl was a mixture of chloroform and heptane (1:2), for all other materials aqueous buffer was used. Samples were delivered to the interface with a gas tight, 10 μl Hamilton syringe.

Cyt, Fd and Pc were studied in the presence of 10^{-3} M ascorbate or 10^{-3} ferri. Either of the latter two components would be present both in the aqueous subphase as well as in the sample delivered to the interface.

The area/molecule at a particular π is indicated A_π. Theoretical areas are calculated using the expression $A(\text{THEOR}) = A_1 n_1 + A_2 n_2$ where subscripts refer to each component in the film and n is the mole fraction of each component in the mixed film.

The subphase was maintained at an ionic strength of 0.6 and pH of 7.8 with phosphate buffer and NaCl. While the precision of measuring was better than 1% the reproducibility between experiments was 5 to 10%.

All experiments were carried out in total darkness, except for illumination periods. When pigments were added to the surface a green safelight was used (Kodak Wratten 61). To observe the effect of white light on the monolayers a 1000 W slide projector with a tungsten lamp irradiated an area of about 150 cm^2 with an intensity of 2×10^5 ergs/cm^2, sec. Temperature of the liquid system was maintained at 15 °C.

Results and Discussion

1. Chlorophyll: Monomolecular films of Chl give fairly stable, reproducible isotherms up to a π of 9 dyn/cm. At higher surface pressures, and constant area, there is considerable drifting of π to lower values. From the initial pressure reading (after compression) until a steady state value is reached (after 5 to 30 min) there could be as much as a 3% decrease in π. When Chl is spread with chloroform, which has a high specific gravity, small droplets fall through the interface and sink to the bottom of the water layer.

On the other hand, Chl spread from a heptane-chloroform solution (2:1) results in an $A_{10} =$ 139 Å^2. However, isotherms still can not be reproduced to better than 3%. It is possible that there is some desorption of the Chl film.

At a nitrogen-water interface A_{10} is about 100 Å^2 [1]. On the basis of area it is apparent that Chl at the heptane-water interface is oriented more horizontally than at the nitrogen-water interface. The low solubility of Chl in heptane probably contributes to this orientation. The different orientation of Chl might be related to the differences observed at the two interfaces reported in the following sections. After illumination there is a decrease in A of about 10 Å at a low π, accompanied by a 10 Å^2 increase in A at high π (at 10 dyn/cm there is little change in A). This increase in slope of the π-A isotherm might reflect pheophytinization, as it was shown by Bellamy *et al.* [1] that the isotherm for pheophytin is steeper than that for Chl.

In contrast to the present work where the area is observed to decrease in the dark Karen and Brody [8], showed an increase of A_6. In light they showed A_6 to decrease, which is similar to that reported here at π less than 10 dyn/cm. The main differences in the experimental conditions used in this work and those of Karan and Brody are spreading solvent (benzene) and temperature (20 °C). Furthermore, there might also be different surfactive impurities in the buffers and salts used in the two sets of experiments.

2. Ferridoxin: Reproducible, rather stable isotherms are obtained with Fd. (However, there is a small time dependent drift to smaller A's in the dark.) In the presence of ascorbate $A_{10} = 830$ Å^2 and $\Delta V_{10} = 170$ mV (Fig. 1). At a nitrogen-water interface using Fd from spinach, $A_{10} = 167$ Å^2, and $\Delta V_{10} = 170$ mV [4]. It would appear on the base of the larger A_{10} reported in this work that Fd, from *Clostridium* may be much larger than Fd from spinach or Fd denatures more readily at a heptane-water interface than at an air-water interface. In either case ΔV is about the same for Fd from the two sources at the two interfaces. If partial denaturation occurs at the heptane-water interface it would appear that the charge density of Fd remains unchanged.

Mixed films of Chl and Fd were examined. For mol ratios of (Fd)/(Chl) between 1 and 2, $A(\text{EXPER}) \cong A(\text{THEOR})$, *e. g.* (Fd)/(Chl) = 1.4, $A_{10}(\text{THEOR}) = 546$ Å^2 and $A_{10}(\text{EXPER}) =$ 530 Å^2. At a nitrogen-water interface, on the other hand, a rather large interaction was reported between Chl and Fd [*i. e.* $A(\text{EXPER}) > A(\text{THEOR})$] in the same rang of mole ratios [4]. The absence of

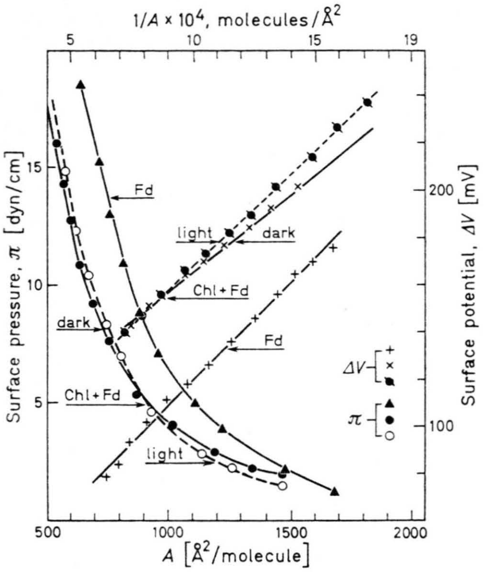

Fig. 1. Surface pressure (π) and potential (ΔV) isotherms as a function of area per molecule (A) for ferridoxin (Fd) and a mixed film of ferridoxin and chlorophyll (Chl) (mole ratio, 2:1). Monolayers were spread at a heptane water interface and illuminated for 15 min with white light intensity 2×10^5 ergs/cm² sec. The subphase contained 10^{-3} M ascorbate and phosphate buffer pH 7.8 at ionic strength 0.6 and was maintained at 15 °C. Notation Fd (—▲—) π-A, (—+—) ΔV-$1/A$; Chl-Fd (—●—) π-A dark, (—×—) ΔV-$1/A$ dark; Chl-Fd (——○——) π-A light, (——◐——) ΔV-$1/A$ light.

any evidence of an interaction between Fd and Chl in the dark at the heptane-water interface may be related to Fd being denatured or originating from a nonphotosynthetic organism. At a heptane-water interface addition of Chl to a Fd film results in a large increase of ΔV (see Fig. 1). At a N_2-water interface the addition of Chl to a Fd film gave rise to a much smaller increase of ΔV [4].

Irradiation of mixed films of Chl and Fd in the presence of ascorbate result in a small increase in A (at high π) and an increase in slope of the $\Delta V - 1/A$ isotherm (Fig. 1). The increase in A though small is opposite to the change observed with Fd and/or Chl in the dark. Consequently the increase in A appears to reflect a photoreaction between Chl and Fd in the presence of ascorbate. Further experiments are required to determine whether Chl is reduced or oxidized in the photoreaction, to what extent Fd is denatured and whether or not denatured Fd can participate.

3. *Cytochrome:* The π-A isotherm of Cyt c in the presence of ascorbate (reduced Cyt) changes slowly in the dark. At high values of π, A tends to increase while at low values of π, A tends to decrease. (In the neighborhood of $\pi = 12$ dyn/cm there is no change of A.) There is no sign of film breakage up to a surface pressure of 20 dyn/cm. The value of A for Cyt in the presence of ferri (oxidized Cyt) is larger than for reduced Cyt (see Table).

Table of results.

Film	A_{10} [Å²]	ΔV_{10}[mv]
Fd+ascorbate	830	170
Chl	139	—
Pc+ascorbate	5800	150
Pc+ferri	5600	117
Cyt+ascorbate	1020	—
Cyt+ferri	2400	240

The accuracy of A_{10} is ±2%.

In the dark A for oxidized Cyt tends to decrease while A for reduced Cyt tends to increase. The fact that the A's for oxidized and reduced Cyt change in opposite directions would argue against desorption (in which case the A's for both would decrease) or accumulation of impurities at the interface (in which case both A's would increase). However it is still possible that A changes differently because the composition of the subphase is not identical in the two systems. Reduced Cyt has ascorbate while oxidized Cyt has ferri in the subphase and film.

The A measured in this work for Cyt c (type XII, acid modified) at the lipid-water interface is much larger than the A's reported previously for other types of Cyt c at an air-water interface. Reduced and oxidized Cyt c (type II) were reported to have A_6's of 150 and 270 Å², respectively at pH 8.0 [3]. It appears there is an unfolding of Cyt at the lipid-water interface, a simple reorientation could not account for the order of magnitude increase in A over that measured at an air-water interface. Previous measurements of Cyt c (type III, free of ammonium sulfate and NaCl at a heptane-water interface) reported larger A_6's for reduced and oxidized Cyt, *i. e.* 5000 and 4100 Å², respectively [8]. Based upon the reports in the literature it appears the various types of Cyt c might assume different conformational states at an interface. This difference in surface properties might be related to their function *in vivo.*

Mixed films of Cyt and Chl were examined. With (oxidized Cyt)/(Chl) = 0.8 the A_{10}(THEOR) $\cong$ A_{10}(EXPER), *i. e.* 1220 and 1300 Å², respectively.

On the other hand, with reduced Cyt and Chl, A_{10} (THEOR) is less than A_{10}(EXPER), *e.g.* at (reduced Cyt)/(Chl) = 2.6, A_{10}(THEOR) = 775 Å^2 while A_{10}(EXPER) = 1000 Å^2. Apparently the presence of Chl promotes a further unfolding of reduced Cyt or an oxidation to the larger oxidized form. A similar result was reported by Karan and Brody [8] *i.e.* an interaction between Chl and reduced Cyt (type III) but no interaction between Chl and oxidized Cyt. The nature of the interaction reported previously is somewhat different since *A*(THEOR) was larger than A(EXPER). (Just opposite to the result reported above.) Again, this difference in results might arise from the different types of Cyt used.

After illuminating a mixed film of Chl and oxidized Cyt a decrease of *A* is observed; the maximum decrease observed was 18%. It remains to be established whether this decrease of *A* reflects a photoreaction or simply a continuation of the decrease of *A* observed for oxidized Cyt in the dark.

With mixed films of Chl and reduced Cyt the situation is different. After illuminating a film containing (reduced Cyt)/(Chl) = 0.4, there is a marked decrease (10%) of *A*. (In the dark *A* increases both for films of reduced Cyt and for mixed film containing reduced Cyt.) The complete reversal by light of the trend toward larger *A*'s would indicate some sort of light reaction. The observation of a light induced decrease of *A* in mixed films containing oxidized and reduced Cyt is highly suggestive that indeed a photo-reaction is occurring. The Cyt films probably have some oxidized and reduced forms present. Thus it is possible to observe the same photoreaction both with films containing either predominately oxidized or reduced Cyt. The fact that in light *A* decreases indicates that the much smaller reduced Cyt may be formed. If this is the case then the light reaction might be Chl + oxidized Cyt $\xrightarrow{h\nu}$ oxidized Chl + reduced Cyt.

This reaction is not analogous to the reaction between Chl and Cyt f as it seems to occur in photosynthesis. In the latter case oxidized Chl is reduced in the dark by reduced Cyt (perhaps via Pc). While no photoreaction is proposed between Chl and Cyt f *in vivo* one might take place in a mixed monomolecular film. At a nitrogen-water interface there is a specific interaction between Chl and reduced Cyt f but no interaction with oxidyed Cyt f [14].

4. Plastocyanin: Isotherms of Pc in the presence of 10^{-3} M ascorbate (reduced Pc) exhibit a small increase of *A* as successive isotherms are measured in the dark. Up to $\pi = 15$ dyn/cm no indication of film collapse or desorption is observed. The A_{10} and ΔV_{10} are given in the Table and shown in Fig. 2; the A_5 is 7800 Å^2. At a nitrogen-water interface reduced Pc has an $A_5 = 368$ Å^2 and a collapse or desorption pressure of $\pi = 6$ dyn/cm [6]. Since A_5 at a heptane-water interface is so much larger than at a nitrogen-water interface it would appear that reduced Pc may be at least partially denatured at the former interface.

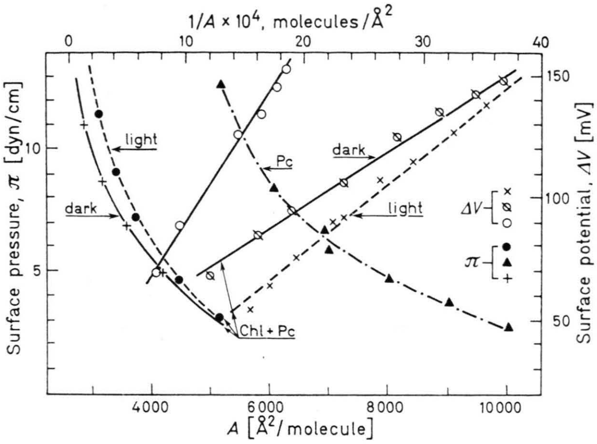

Fig. 2. Surface isotherms of reduced plastocyanin and mixed films of reduced plastocyanin (Pc) and chlorophyll (Chl) (mole ratio 1:1.1). Effects of light on π-*A* and Δ*V*-1/*A* isotherms. Subphase and illumination as in Fig. 1. Notation Pc (—▲—) π-*A*, (—○—) Δ*V*-1/*A*; Chl-Pc (— — ● — —) π-*A* light, (——+——) π-*A* dark; Chl-Pc (— — × — —) Δ*V*-1/*A* light, (——ϕ——) Δ*V*-1/*A* dark.

Mixed films of Chl and reduced Pc were examined. The A_{10}(THEOOR) is about the same as A_{10}(EXPER) in the dark, *e.g.* when (Pc)/(Chl) = 0.91 A_{10}(EXPER) = 3000 Å^2 and A_{10}(THEOR) = 3090 Å^2. Upon addition of Chl to a reduced Pc film there is a decrease of ΔV (Fig. 2). A similar result was observed at a nitrogen-water interface [6]. After illumination A_{10}(EXPER) = 3300 Å^2 which is significantly larger than A_{10}(THEOR). Thus light seems to induce a further unfolding of reduced Pc so that reduced Pc may not be completely denatured at the heptane-water interface.

At a nitrogen-water interface, the situation is reversed. *A*(THEOR) is distinctly larger than A(EXPER) indicating an interdigitation of Chl and reduced Pc [6]. Perhaps the denaturation of reduced

Pc at the heptane-water interface precludes interdigitation.

After illuminating films of Chl and reduced Pc there is invariably a small increase of A. The largest increase in A (of 8.7%) seems to occur at a mol ratio of (Pc)/(Chl) = 1.0. This may be contrasted with the findings at the nitrogen-water interface where a decrease in A is observed after illumination. At the latter interface oxidized Pc is smaller than reduced Pc, so the observed decrease in A is consistent with a photoreaction wherein reduced Pc is oxidized.

During illumination at constant area no change in π exceeding ± 0.2 dyn/cm could be observed. On the other hand, changes in ΔV are observed during irradiation (see Fig. 3). The light induced change in ΔV increases with irradiation time (see Fig. 3). In the dark between periods of illumination, there is a back reaction which tends to restore the change in ΔV (see Fig. 3). After 120 sec of irradiation a 10 mV increase in ΔV is observed. A subsequent dark period of 180 sec results in a 7 mV decrease of ΔV. The light induced change in ΔV (and perhaps the dark recovery) appears to be proportional to illumination time.

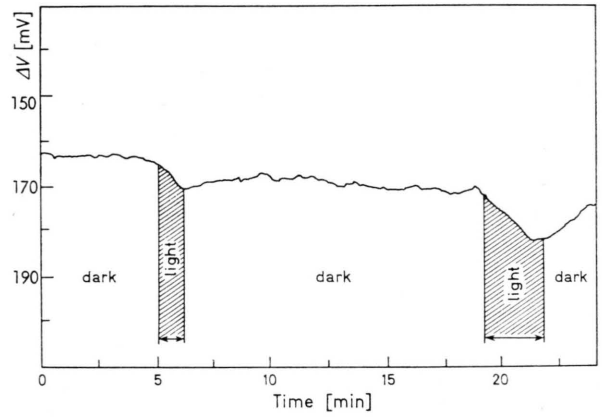

Fig. 3. Light-induced change in surface potential ΔV as a function of time for a mixed film of reduced plastocyanin and chlorophyll in mol ratio of 1 : 1.1. Subphase as in Fig. 1.

Though the effects of light on A and ΔV are small, nevertheless, it is apparent that Chl can sensitize light reactions at the lipid-water interface. The photoreaction between Chl and reduced Pc could be Chl + reduced Pc $\xrightarrow{h\nu}$ reduced Chl + oxidized Pc. This reaction may be quickly followed by an oxidation of Chl by oxygen. If oxidized Pc were formed one would expect ΔV to decrease rather than increase (Table), however, anomalous changes are often reported in monolayers[2]. While the direction of change is not what one expects, nevertheless, there is strong evidence indicating a photo-reaction.

The largest photoeffects with Chl monolayers were observed at a nitrogen-water interface. In a nitrogen environment the photoproducts may accumulate and not undergo the rapid oxidation which can occur in air. Such an oxidation could well account for the small photoeffects observed at the heptane-water interface.

Successive isotherms of Pc, in the presence of 10^{-3} M ferri cyanide, give increasingly larger values for A. This increase in A, might reflect conformational changes in Pc or simply, impurities from the subphase collecting at the interface. In Table are given A_{10} and ΔV_{10}. The oxidized and reduced forms of Pc have approximately the same value for A_{10}. However, ΔV_{10} for reduced Pc is considerably larger than for oxidized Pc showing that there is a distinct difference between the two species. It is of interest to note that at an air-water interface oxidized Pc has a smaller A than reduced Pc.

Mixed films of Chl and oxidized Pc were examined. In a film containing (oxidized Pc)/(Chl) = 0.85, A_{10}(THEOR) = 2662 Å^2 while A_{10}(EXPER) = 3200 Å^2. It would appear that Chl is promoting a conformational change in oxidized Pc so as to increase the projected area of the latter. Because A for oxidized Pc is so much larger than A for Chl the contribution of Chl to A(THEOR) is almost negligible. The above may be contrasted with the result at an air-water interface where A(THEOR) is larger than (EXPER)[5].

Illumination of a mixed film (oxidized Pc)/(Chl = 0.85, shows a small decrease in ΔV (8 mV) and a slight increase in A_{10} (5%). The latter increase may not be significant since there is also a slow increase of A in the dark. The main difference between dark and irradiated isotherms is that after illumination the slope of the π-A isotherm gets steeper (*i. e.* the film assumes a somewhat more solid character). The decrease may be accounted for if Pc was not completely oxidized so the reaction above between Chl and reduced Pc may take place.

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