Surface Properties of Monomolecular Films of Reduced Plastocyanin at a Nitrogen-Water Interface

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The area/molecule (A) and surface potential (ΔV) of reduced plastocyanin (pcyan) was measured as a function of pH. At pH 7.5 a maximum is observed for A while a minimum is observed for ΔV. At a surface pressure of 5 dyn/cm the maximum $A_5 = 378$ Å^2 and minimum $\Delta V_5 =$ 335 mV. There is an interaction between pcyan and chlorophyll (Chl) in mixed films. As shown by a decrease of A and V compared to theoretically calculated volues. The largest decrease occurs at a mol ratio of about 2 pcyan : 1 Chl.

When mixed films are irradiated in air no changes in surface properties are observed.

Irradiation of mixed films of reduced pcyan and Chl in nitrogen results in a decrease in A and an increase of ΔV. The maximum decrease occurs at a mol ratio of pcyan/Chl between 1.5 and 2.0. The direction of change of A is consistant with a conversion of reduced pcyan to oxidized pcyan.

I. Introduction

Plastocyanin is a naturally occurring electron carrier between photosystem I and II. An electron is transferred from plastocyanin to chlorophyll by a light driven reaction [1]. The exact position and role of plastocyanin in the photosynthetic mechanism is still under investigation [2]. To investigate the interactions and photoreactions between the lipid soluble chlorophyll and the water soluble plastocyanin in an environment similar to the *in vivo* membrane, monomolecular films of these substances were constructed.

In a previous study were reported the properties of a monomolecular film of oxidized plastocyanin (ox. pcyan) at an air-water interface [3]. It was shown that there is an interaction, possibly a complex, between chlorophyll (Chl) and ox. pcyan in a mixed monomolecular film. The surface potential and area of the mixed Chl-pcyan films are changed significantly upon irradiation indicating a photoreaction between Chl and ox. pcyan in air. Since no special efforts were taken in the above work to insure that all the pcyan was in the oxidized state there could be some reduced plastocyanin present in the film.

The present study considers the properties of monomolecular films of reduced plastocyanin (red.-pcyan) and of mixed films of red.-pcyan and Chl.

II. Methods and Materials

The apparatus and techniques used to measure surface properties is similar to that described previously [2, 4]. The electrophoretically pure pcyan used for this study was the generous gift of Professor N. Bishop of the University of Oregon (Corvallis). Plastocyanin was reduced with sodium ascorbate and oxidized with potassium ferricyanide.

Chlorophyll a was prepared and stored as described previously [4]. Unless otherwise noted all experiments were carried out in a nitrogen atmosphere (99.995% pure nitrogen, Matheson Gas Co., Rutherford, N.J.). The subphase was phosphate buffer pH 7.8, ionic strength 0.6 and thermostatically maintained at 15 °C.

The theoretical areas, THEOR, were calculated using the following relationship: $\text{THEOR} = n_c A_c + n_p A_p$ where n is the mole fraction, A is the area/molecule, subscripts c and p are for Chl and plastocyanin, respectively. The difference between the THEOR and experimental area, EXP, are of interest.

When a solution of red. pcyan (40 μl) is spread on the surface the ascorbate (used to reduce pcyan) probably goes into the subphase since ascorbate is not surface active. The final concentration of ascorbate in the subphase is less than 10^{-6} M. Concentrations of ascorbate less than 10^{-4} M have been shown to have no effect on the area per molecule, A or ΔV surface potential of Chl [5].

Films are irradiated using a low pressure, 100 W, mercury lamp (GE 100 AH) without any filters. The light intensity on the film is $1.4 \pm 0.5 \times 10^3$ ergs/cm^2 sec.

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III. Results

A. Protein films

The surface isotherm and surface potential of red. pcyan on a subphase at pH 7.4 are shown in Fig. 1 and Fig. 2, respectively. When comparing the surface isotherms measured for a mixture of ox. and red. pcyan with and without 10^{-3} M ascorbate in the subphase (pH 7.5) there is a very slight decrease in A in the presence of ascorbate, indicating that there is almost no reaction between the ascorbate in the aqueous phase and the pcyan film. On the other hand, when 10^{-3} M ascorbate is mixed together with pcyan in solution before spreading the film then there is a large increase in A. Thus it is apparent that in the reduced form pcyan is larger than ox. pcyan (see Table I). For convenience, the surface properties of oxidized pcyan are reproduced in Fig. 1 and Fig. 2. The surface pressure is indicated by π.

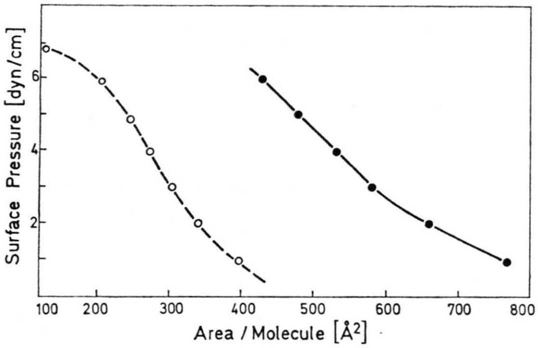


Fig. 1. Surface isotherms for oxidized plastocyanin (broken line) and reduced plastocyanin (solid line). The aqueous subphase contained phosphate buffer, pH 7.4, at a ionic strength of 0.6 and a temperature of 15 °C.

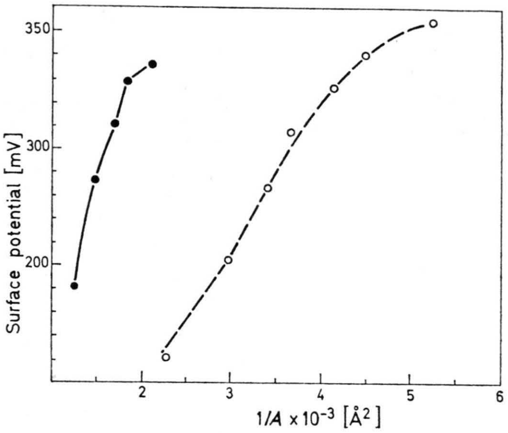


Fig. 2. Surface potential as a function of pigment concentration on the surface. The latter is given as the reciprocal of the area per molecule ($1/A$). Data for reduced plastocyanin is shown by a solid line; date for oxidized plastocyanin is shown by a broken line. Subphase the same as in Fig. 1.

Table I. Summary of surface properties of oxidized and reduced plastocyanin.

	Oxidized	Reduced
$^* A_1$	400 Å² (pH 7.2)	606 Å² (pH 7.4)
A_5	240 Å² (pH 7.2)	378 Å² (pH 7.4)
$^{**} \Delta V_1$	220 mV (pH 7.2)	250 mV (pH 7.4)
ΔV_5	312 mV (pH 7.2)	335 mV (pH 7.4)
max. A at pH	8.6	7.4

* Precision of measurements of A are ±1.0%.

** Accuracy of measurements of ΔV are ±20 mV.

The pH dependence of A and ΔV for red. pcyan is shown in Fig. 3. A maximum for A at $\pi = 5$ dyn/cm (A_5) equal to 378 Å² is obtained at about pH 7.5. This pH value is quite close to the physiological pH and to the pH used for the studies with Chl (see below). The dependence of A on pH for ox. pcyan determined previously at $\pi = 3$ dyn/cm was calculated for $\pi = 5$ dyn/cm using the original data[1]. These results are reproduced in Fig. 2 to facilitate comparisons between ox. and red. pcyan.

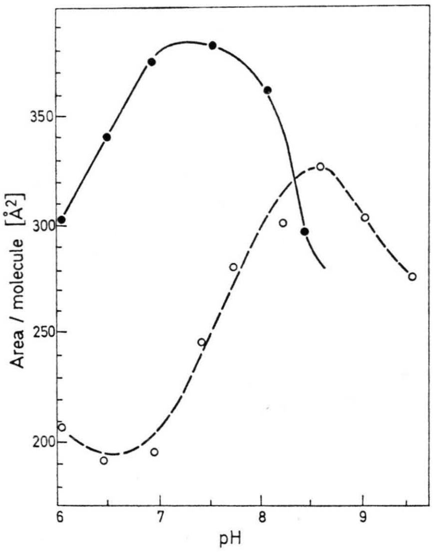


Fig. 3. Area per molecule measured at a surface pressure of 5 dyn/cm as a function of pH in the subphase. Data for reduced plastocyanin is shown by a solid line; data for oxidized plastocyanin is shown by a broken line. The aqueous subphase had an ionic strength of 0.6 and a temperature of 15 °C.

A minimum potential is obtained for ΔV at about pH 7.5 (Fig. 4). The minimum value for ΔV, at $\pi = 5$ dyn/cm (ΔV_5) is 335 ± 20 mV (pH 7.4). At $\pi = 1$ dyn/cm, ΔV_1 is about 250 mV. These film potentials were calculated using the potential measured for water in the absence of the film. Considerable variation was observed for the potential of water. This contributed markedly to the low precision given for the ΔV of the films. For comparison purposes, the dependence of ΔV on pH (determined previously) for ox. pcyan is also shown in Fig. 4.

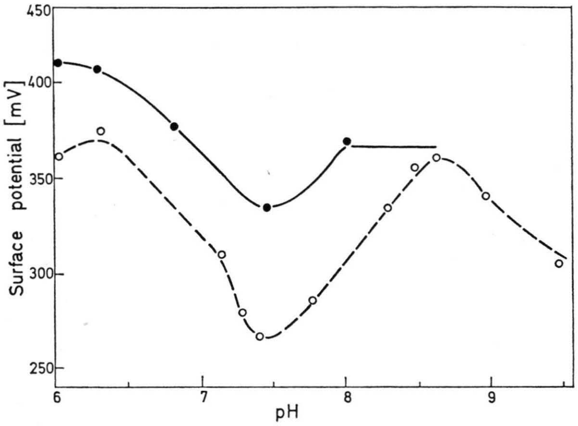


Fig. 4. Surface potential measured at a surface presure of 5 dyn/cm as a function of pH. Data for reduced plastocyanin is shown by a solid line; data for oxidized plastocyanin is shown by a broken line. Subphase same as Fig. 3.

Both ox. and red. pcyan have a maximum for A as a function of pH. With ox. pcyan the maximum value for A is observed at pH 8.6, while with red. pcyan it occurs at pH 7.4 (see Fig. 3). At pH below the oxidized form the pcyan molecule assumes an orientation or conformational state such that the area projected on the aqueous surface is smaller than in the reduced form.

Red. pcyan has a minimum value for ΔV while A exhibits a maximum. Ox. pcyan, on the other hand, has a maximum pH for both A and ΔV. The A's are ΔV's for ox. and red. pcyan and ΔV's for ox. and red. pcyan are summarized in Table I.

B. Mixed films

The miscibility of the mixed films of Chl and pcyan is shown by the change in collapse pressure, π_c, of the mixed film[6]. With ox. or red. pcyan, π_c is between 5.4 and 5.8 dyn/cm; in the presence of Chl π_c is between 12 and 24 dyn/cm depending on the ratio of Chl to pcyan.

With mixed films of red. pcyan and Chl a minimum value for EXP-THEOR is obtained at a mole ratio [pcyan]/[Chl] = 2 (at pH 7.8) (Fig. 5). It was shown previously that mixed films of ox. pcyan and Chl have a minimum at a mole ratio [pcyan]/[Chl] = 1 (at pH 7.8).

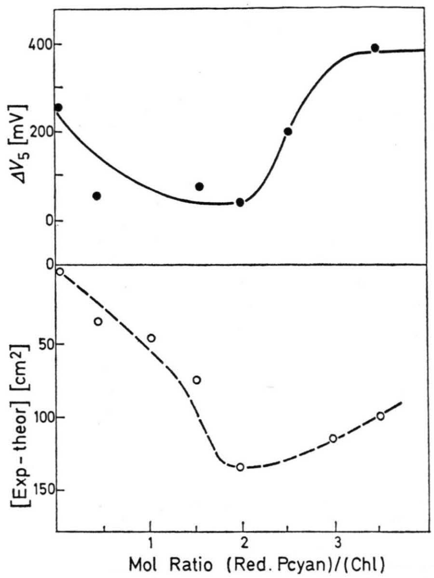


Fig. 5. Lower Curve: The difference between the experimentally measured area (Exp) and the theoretically calculated area (theor) at a surface pressure of 5 dyn/cm, as a function of the mole ratio of reduced plastocyanin to chlorophyll [(pcyan)/(Chl)]. Upper Curve: Surface potential of a mixed film of reduced plastocyanin and chlorophyll as a function of (pcyan)/Chl). The aqueous subphase contained phosphate buffer pH 7.8 at an ionic strength of 0.6 and temperature of 15 °C.

The value of EXP-THEOR is proportional to the free energy[7]. A positive value for EXP-THEOR shows a non-spontaneous reaction and *vice versa*. By referring to Fig. 5 it can be seen that the interactions between Chl and red. pcyan as well as between Chl and ox. pcyan are spontaneous. The surface potential for mixed films of red. pcyan and Chl as a function of the ratio pcyan/Chl may have a minimum at about a [pcyan]/[Chl] = 2 (Fig. 5). In the case of mixed films of ox. pcyan and Chl it was previously shown that a maximum value of ΔV (instead of a minimum) is obtained at about a [pcyan]/[Chl] = 1.

C. Light reaction

Irradiation of mixed films of red. pcyan and Chl in nitrogen results in a decrease in A. In Fig. 6 is shown A measured after irradiation minus A measured in the dark before irradiation (light-dark) as a function of the mole ratio of [red. pcyan]/[Chl] in the film. The maximum decrease of A occurs at a pcyan/Chl ratio of approximately 1.5 or 2. The direction of change of A is consistant with a conversion of red. pcyan to ox. pcyan; the area of reduced Chl is not known, but the photoreactions reported for Chl films with ascorbate and PMS indicate that A for Chl decrease upon reduction [5]. Such a decrease in A would be consistant with the light induced decrease of A in the mixed film reported here.

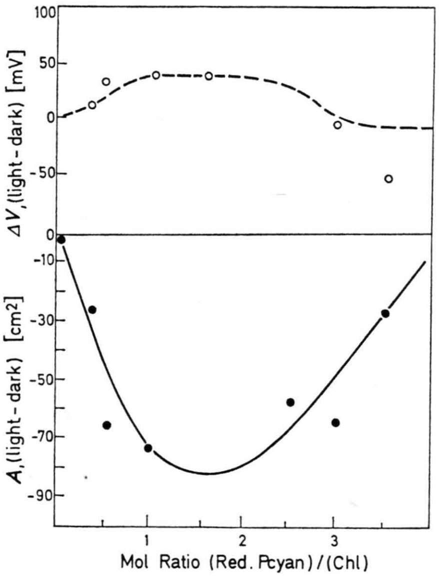


Fig. 6. The difference in surface area and surface potential before and after illumination (light-dark), as a function of the mole ratio of reduced plastocyanin to chlorophyll, (red. pcyan)/(Chl). Subphase same as in Fig. 5, irradiation carried out in nitrogen, with a mercury lamp, light intensity on the film surface was 1.4×10^3 ergs/cm^2 sec.

Irradiation also seems to induce an increase in ΔV, Fig. 6. Because of the low precision of the ΔV measurements only the qualitative aspects can be given for the (light-dark) of ΔV as a function of [red. pcyan]/[Chl]. For ΔV_5 no definite effect of light could be established since in some experiments decreases as well as increases in ΔV were observed at the same value of [red. pcyan]/[Chl].

Irradiation of mixed films of red. pcyan and Chl in air (instead of nitrogen) shows no change in surface properties indicating no photoreaction.

IV. Discussion

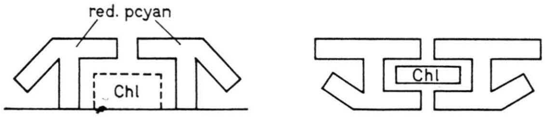
The fact that red. pcyan can undergo large pH dependent changes in area shows that the protein on the surface is still capable of undergoing conformational changes, so it is not (completely) denatured as is the case reported for other proteins [8].

The pH optimum for photooxidation of pcyan in air by digitonin treated chloroplasts shows a rather broad pH optimum around pH 8.0 – 8.5 [1, 9]. This pH value is similar to the pH which results in a maximum area for a film of ox. pcyan (Table I).

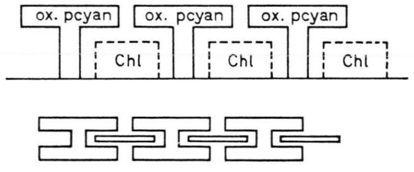
The pH optimum for photoreduction of pcyan by chloroplasts shows a pH optimum around pH 7.5 – 7.9 [1, 9]. This pH value is similar to the pH which results in a maximum area for a film of red. pcyan (Table I).

The interaction observed between Chl and pcyan in the oxidized and reduced state might involve a molecular complex between Chl and the copper or specific groups on the pcyan. Alternatively there may simply be interdigitation of the Chl and pcyan molecules.

Interdigitation accounts for the decreases in area of the Chl-pcyan films, as well as, the apparent absence of specificity of the oxidized and reduced forms of pcyan to interact with Chl. For red. pcyan maximum interaction occurs at a [red. pcyan]/[Chl] = 2, so that one Chl molecule may be partially interdigitated into two pcyan molecules. A simple pictorial arrangement is as follows:



For ox. pcyan, maximum interaction occurs at an [ox. pcyan]/[Chl] = 1 so that, on the average, one Chl molecule interacts with one molecule of ox. pcyan. In keeping with the picture used above for red. pcyan, the following model is proposed. It can be seen that a conformational change between ox. and red. pcyan allows for Chl to be interdigitated at two locations on the pcyan.



It is expected that some sort of specific complex exists if there is a photochemical reaction between Chl and pcyan in the mixed monolayer. Actually, Chl might simultaneously interdigitate and form a complex with pcyan.

In vivo it is proposed that the light reaction of System I is first a reduction of ferredoxin by Chl and followed by a dark reaction between oxidized Chl and red. pcyan (or a cytochrome), thereby restoring the original state of Chl[2]. The photoreaction between Chl and pcyan are not consistent with this model for the *in vivo* reaction. Perhaps alternate pathways are possible in photosynthesis in the event the "natural" substrate for the photoreaction is absent. In this sense the photoreactions observed between pcyan and Chl in monolayers may be deleterious reactions as regards photosynthesis *in vivo*. Alternatively, it is feasible that in photosynthesis red. pcyan may act to directly donate an electron to Chl of System I[10, 11] by a light sensitized reaction. If this is the case then the primary light reaction of System I would be a photoreduction of Chl rather than an oxidation of Chl. Reduced Chl would subsequently reduce ferredoxin in a dark reaction thereby restoring Chl to its original state.

Irradiation of mixed films of Chl and red. pcyan in N_2 results in a decrease of A. This is opposite to the increase of A previously observed with mixed films of ox. pcyan and Chl[3]. The decrease of A is consistant with the observed smaller area for ox. pcyan compared to reduced pcyan (at pH 7.8). At pH 7.8 red. pcyan has both a larger A and ΔV than ox. pcyan. If Chl were to undergo an oxidation as called for in the photoreaction with ox. pcyan, it should also contribute an increase to the film area; it was shown previously[4] that oxidation of Chl monolayers, in air, results in an increase in A from 122 to 203 Å^2. The overall reaction between ox. pcyan and Chl reported previously[3] appears to be Chl + ox. pcyan → ox. Chl + red. pcyan. The details of the reaction, however, are not yet resolved.

For the experiments reported in this paper, the overall reaction between Chl and red. pcyan in nitrogen appears to be Chl + red. pcyan → ox. pcyan + red. Chl.

The fact that the largest effects of illumination occure at the same ratio of [red. pcyan]/[Chl] that gives maximum interaction in the dark tends to support the validity of the observed light reaction. There is no apparent back reaction between the red. Chl and the ox. pcyan. Perhaps a change in conformation of the pcyan molecule upon oxidation may preclude any further interaction with red. Chl.

If red. Chl should be restored to its original state by an oxidation in air, then the photoreaction between Chl and ox. pcyan could result. The combination of these two reactions would tend to cancel out any changes in surface properties. This may explain the absence of any photoreaction between Chl and red. pcyan in air.

There are other possibilities to explain the reactions between Chl and pcyan in the mixed films. For example there may be a reaction on the surface between Chl and one or both redox forms of pcyan in the dark. The rather long times required for the films to stabilize might reflect such a dark reaction. In such a situation it will be necessary to reconsider whether we are dealing with two different photoreactions as suggested above or only one photoreaction.

In order to obtain a definitive description of the photoreaction in mixed films of Chl and pcyan, spectral measurements of the mixed monolayer before and after irradiation are planned using the apparatus described previously[12].

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