

## Surface Properties of Plastocyanin at an Air-water Interface

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The area/molecule,  $A$ , and surface potential,  $\Delta V$ , of plastocyanin (pcyan) at an air-water interface varies with pH. At a pH of about 8.6 a maximum value is obtained for  $A$  and  $\Delta V$ . At a surface pressure of 3 dyn/cm  $A$  and  $\Delta V$  are 375 Å<sup>2</sup> and 325 mV, respectively. The dipole moment is 2050 milliDebyes.

Pcyan and chlorophyll  $a$  (chl) form a mixed film (free energy of mixing and interaction is negative). At a concentration of  $[\text{pcyan}]/[\text{chl}] \approx 1$  a maximum interaction is observed both for  $\Delta V$  and  $A$  (at pH 7.8). Irradiation of the mixed film, in a nitrogen atmosphere, results in an increase in  $A$  and  $\Delta V$ .

Plastocyanin (pcyan) is a protein containing two copper atoms per molecule which functions as an electron carrier in photosynthesis (Kato<sup>1</sup>, Kato<sup>2</sup>). It has been proposed that pcyan could be the primary electron donor for System I (Bishop<sup>3</sup>; Gorman and Levine<sup>4</sup>).

Before monomolecular model systems incorporating pcyan could be designed it was necessary to first determine the surface properties of oxidized pcyan. Mixed films of pcyan and chlorophyll (chl) were prepared; the surface properties and effect of light on these films were studied.

to measure surface pressure,  $\pi$ . The precision of measuring  $\pi$  is  $\pm 0.05$  dyn/cm.

To determine surface potentials  $\Delta V$ , a Keithley differential electrometer (Model 604) was used to measure the difference in potential between a radioactive Nickel electrode and a silver-silver chloride electrode (Gaines<sup>6</sup>, Brody<sup>7</sup>). The precision for measuring  $\Delta V$  is  $\pm 10$  mV. The mixed films were radiated at  $\pi = 6$  dyn/cm with a 100 W low pressure Hg lamp without filters; intensity of the surface was  $2 \cdot 10^3$  erg/cm<sup>2</sup> sec. After irradiation the films were expanded and the surface isotherms measured.

### Methods and Materials

The pcyan used for this study was the generous gift of Prof. N. Bishop of the University of Oregon, Corvallis; the sample was reported to be electrophoretically pure. The samples of pcyan used to form the film contained oxidized and reduced forms.

Over the pH range of 6.0 to 7.8 phosphate buffer was used; over the pH range 8.2 to 9. Tris buffer was used. The temperature of the subphase was maintained at 15 °C. Measurements of mixed films containing chl were carried out in a nitrogen environment. Chl  $a$  was prepared as described previously (Aghion *et al.*<sup>5</sup>).

The equipment used to study the surface properties of pcyan are similar to those described previously (Aghion *et al.*<sup>5</sup>). A Beckman electrobalance (LM 500) in conjunction with an x-t recorder was used

### Results and Discussion

Stable films and reproducible surface pressure-area ( $\pi$ - $A$ ) isotherms are obtained when an ionic strength of at least 0.5 is maintained in the aqueous phase. In this study a constant ionic strength of 0.6 was used.

In some cases high ionic strengths in the subphase have been shown to cause denaturation of protein monolayers (avies and Rideal<sup>8</sup>). To determine if pcyan monolayers were susceptible to this form of denaturation two experiments were run with phosphate buffer (pH 7.8) at ionic strengths of 0.6 and 0.0066. Denaturation apparently is not occurring at high ionic strength since in both cases the values of area/molecule,  $A$ , and surface potential,  $\Delta V$ , at  $\pi = 1$  dyn/cm were the same within experimental error. However, after compressing the film to 3 or 6 dyn/cm the areas at low ionic strength were 10 to 15% lower than at the higher ionic strength. It appears that with a low ionic strength in the

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subphase compression to high values of  $\pi$  force some of the pcyan into the subphase. A typical  $\pi$ - $A$  isotherm is shown in Fig. 1. A graph of  $\Delta V$  as a function of  $1/A$  is shown in the insert in Fig. 1. From the relationship  $\Delta V = \mu_{\perp} 12 \pi / A$ , the perpendicular component of the dipole moment,  $\mu_{\perp}$ , at pH 8.2 is 2050 milliDebyes.

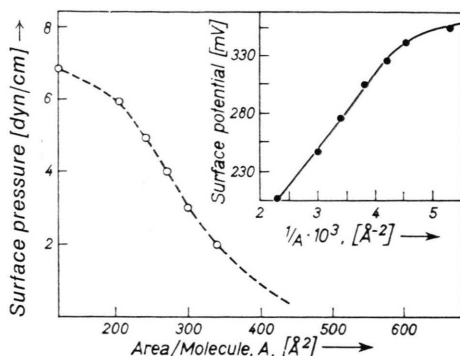


Fig. 1. Surface isotherm ( $\pi$ - $A$ ) of a monomolecular film of plastocyanin. The insert shows  $\Delta V$  as a function of molecules/ $\text{\AA}^2$ . Subphase contains 0.5 M phosphate buffer pH 7.4, temperature 15 °C.

A study was also carried out at low values of  $\pi$  where the intermolecular forces are small compared to the intramolecular forces. A typical surface isotherm and surface potential at pH 7.4 are shown in Fig. 2.

Monolayers of pcyan collapse at  $\pi$  of about 6 dyn/cm. Whether or not the collapse pressure varies with pH was not studied. When isotherms are

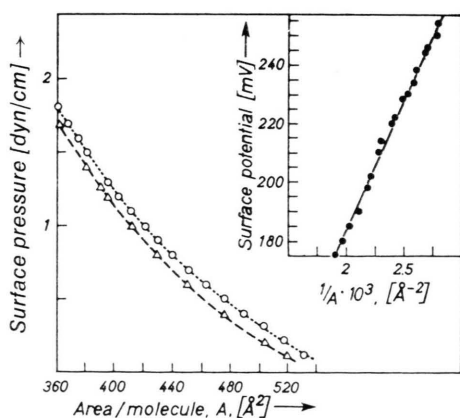


Fig. 2. Surface isotherm of plastocyanin at low surface pressure. The surface isotherms measured during the first and second compression are shown as dotted and dashed lines, respectively. Subsequent isotherms are the same as dashed lines. The insert  $\Delta V$  as a function of molecules/ $\text{\AA}^2$ . Subphase contains 0.5 M phosphate buffer pH 7.4.

measured repeatedly up to and beyond the collapse point, the size of the molecule is observed to decrease with each isotherm. However, if a surface pressure of 6 dyn/cm is not exceeded, then after the third isotherm the size of the molecule remains constant. The decrease in molecular area between the second and third isotherm is only 2 to 4%.

When a surface pressure of 2 dyn/cm is not exceeded in measuring successive isotherms there is only 2 to 4% decrease in area between the first and second isotherms. Between the second and third isotherms the decrease in area is less than 1%.

There is a time dependency for the molecules to reorient on the surface as  $\pi$  is increased. The time required for the molecular orientation to come to equilibrium at low values of  $\pi$  ( $< 2$  dyn/cm) is negligible. However, at high values of  $\pi$  ( $> 4$  dyn/cm) it takes 2 to 10 min for  $\pi$  to reach equilibrium (see Fig. 3).

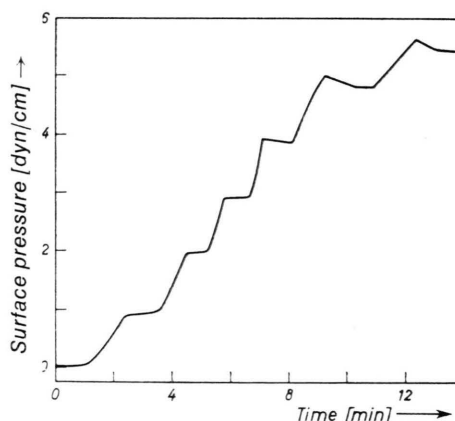


Fig. 3. The time dependence for the surface tension,  $\pi$ , of a film of plastocyanin to reach equilibrium at different stages of compression. Data was obtained by periodically interrupting the compression of the film and measuring  $\pi$  at constant area. At low values of  $\pi$  equilibrium is reached immediately, however, at high values of  $\pi$  (above 4 dyn/cm) it takes several minutes.

Surface isotherms and surface potentials of pcyan at an air-water interface were measured as a function of pH of the subphases. At a pH of about 8.6 a maximum is obtained for the area/molecule (at  $\pi = 3$  dyn/cm),  $A_3$ , equal to  $375 \text{ \AA}^2$ , see Fig. 4. At the same pH and value of  $\pi$  a maximum is also obtained for  $\Delta V_3$  equal to  $325 \pm 10$  mV. Between pH 7.2 and 6.8 a minimum value is obtained for  $A_3$  equal to  $220 \text{ \AA}^2$ ; at pH 7.2 a minimum  $\Delta V_3$  is obtained equal to 210 mV.

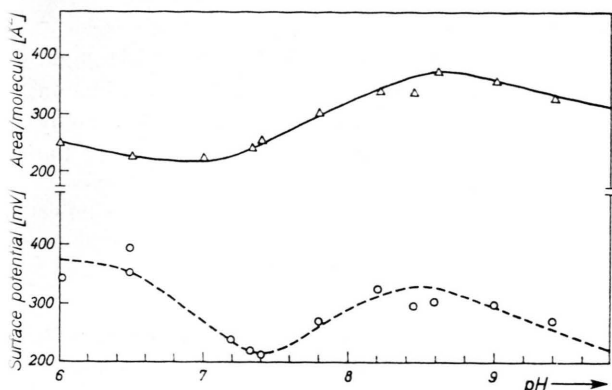


Fig. 4. Molecular area and surface potential at a surface tension of 3 dyn/cm as a function of pH.

One of the major parameters determining the shape of the protein molecule on the surface is intramolecular forces. These intramolecular forces result primarily from the peripheral charges on the molecule and the counter ions in the subphase. These forces can be varied by altering the concentration of free charges in the protein. Varying the pH from 7.0 to 8.6 results in a large change of the protein structure and/or orientation, *i.e.*, the area projected on the surface increases over 50%. While pH can change the charge distribution and size of the protein at an air-water interface, it has been shown that pH does not modify the oxidation-reduction potential of pcyan (Kato *et al.* <sup>2</sup>).

The molecular weight of pcyan (21,000) is more than 20 times heavier than that of chl. Nevertheless the projected area of pcyan is only about 2 times greater than chl. It would appear that the molecule

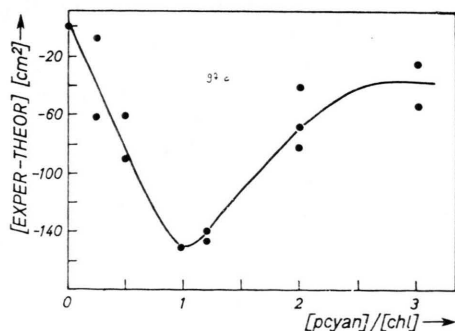


Fig. 5. [EXPER-THEOR] for chl and pcyan films at pH 7.8,  $\pi = 6$  dyn/cm. Difference between the experimentally measured isotherms of a mixed film [EXPER] and the theoretically calculated isotherm [THEOR] as a function of the ratio [pcyan]/[chl]. The difference [EXPER-THEOR] is measured at a surface pressure 6 dyn/cm. A maximum decrease in area is observed at [pcyan]/[chl]  $\approx 1$ .

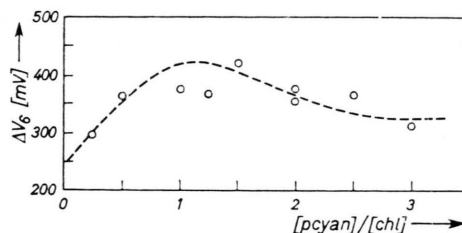


Fig. 6. Surface potential,  $\Delta V$ , at surface pressure of 6 dyn/cm, as a function of the ratio [pcyan]/[chl] in a mixed film.

of pcyan might be cylindrically shaped with the long axis (about 40 to 50 Å) oriented perpendicularly to the surface. The molecule may or may not be partially submerged in the aqueous phase.

Mixed films of pcyan and chl were studied (in a nitrogen environment). All measurements were made with the subphase at pH 7.8 where films of chl are rather stable. While films of pcyan collapse at  $\pi \approx 6$  dyn/cm, in mixed films with chl no evidence of a collapse is noted until  $\pi \approx 20$  dyn/cm. The theoretical area for a mixed monolayer (THEOR) is calculated from the expression  $\text{THEOR} = a(\text{chl}) + a(\text{pcyan})$ , where  $a(\text{chl})$  and  $a(\text{pcyan})$  are the areas (in cm²) of the chl and or pcyan added the surface in the absence of any interaction (Brody, 1972).

The difference between the experimentally measured area (EXPER) and THEOR at 6 dyn/cm (*i.e.*, [EXPER-THEOR]) as a function of the molecular ratio of [pcyan]/[chl] is shown in Fig. 5. The minimum value for [EXPER-THEOR] occurs at [pcyan]/[chl]  $\approx 1$ . Since [EXPER-THEOR] is

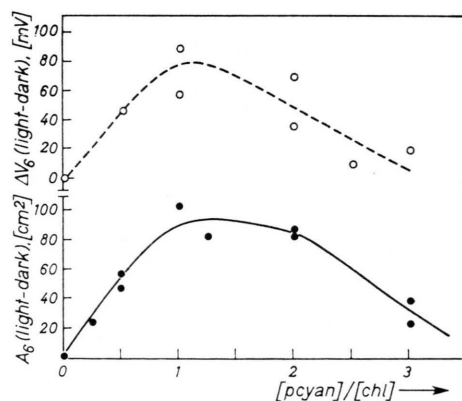


Fig. 7. Effect of light on area and surface potential. Irradiation of a mixed film of oxidized pcyan and chl results in an increase in area/molecule,  $A$ , and surface potential,  $\Delta V$ . Increase of  $A$  and  $\Delta V$  is shown as a function of the ratio of [pcyan]/[chl]. Measurements made at a surface tension of 6 dyn/cm.

negative it shows that the free energy is negative and that the mixing and interaction between chl and pcyan is spontaneous (Davies and Rideal, 1963).

A graph of surface potential, at 6 dyn/cm as a function of  $[\text{pcyan}]/[\text{chl}]$  is shown in Fig. 6. At  $[\text{pcyan}]/[\text{chl}] = 0$  the  $\Delta V_6$  of chl. is used in the figure; the  $\Delta V_6$  of pcyan is used at a ratio of  $[\text{pcyan}]/[\text{chl}] \geq 3$ . While there is considerable scatter to the  $\Delta V$  data it is nevertheless clear that there is a maximum in the vicinity of  $[\text{pcyan}]/[\text{chl}] \approx 1$  (corresponding to the minimum observed for [THEOR - EXPER] for the area).

The decrease in area observed when pcyan interacts with chl may reflect a conformational change or an orientational change or both. Probably both chl and pcyan are already oriented so as to project a minimum area on the surface so that a conformational change might be responsible for the interaction. The interaction might involve a binding or incorporation of chl by pcyan. Irradiation of the mixed film results in an increase in area and surface potential. The difference in  $A$  and  $\Delta V$  isotherms before and after irradiation (light-dark) are shown in Fig. 7. Maximum increases are observed at a  $[\text{pcyan}]/[\text{chl}]$  ratio of about one. All measurements are made at  $\pi = 6$  dyn/cm.

The role proposed for pcyan in photosynthesis is that of an electron donor to System I. The photo-reaction observed above in the monolayer could be

consistent with the *in vivo* reaction since reduced pcyan is probably present in the film. The light reaction results in the reduction of chl and oxidation of pcyan. Since the pcyan used in this study probably also contains oxidized [pcyan] the photoreaction on the surface might be  $\text{pcyan} + \text{chl} \longrightarrow \text{chl}^+ + \text{pcyan}^-$ . This light reaction is similar to the low efficiency triplet reaction suggested by Franck (Clayton<sup>9</sup>) between reaction center I chl and its oxidized electron donor (which he presumed to be a cytochrome), *i. e.*,  $\text{chl}^T \cdot \text{cyt}_{\text{ox}} \rightarrow \text{chl}_{\text{ox}} \cdot \text{cyt}$ . Additional work is planned to resolve the nature of the photoreaction.

It has been shown that when chl undergoes a photooxidation (in air) there is an increase in area from 122 to 203 Å<sup>2</sup> (Aghion *et al.*<sup>5</sup>). If the light induced changes in  $A$  (and  $\Delta V$ ) arose solely from an oxidation of chl then the maximum change would be expected at maximum concentration of chl. Since the maximum light effect is observed at the  $[\text{pcyan}]/[\text{chl}]$  ratio where maximum interaction occurred this would argue for a light reaction between chl and pcyan. Furthermore, since the mixed films were irradiated in nitrogen it is unlikely that chl is being photooxidized in air (Aghion *et al.*<sup>5</sup>).

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