Surface Reactions of Chlorophyll a Monolayers at a Water–Air Interface. Photochemistry and Complex Formation*

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ABSTRACT: Monolayers of chlorophyll a were spread at a water–air interface in a Wilhelmy plate film balance, and surface isotherms were measured under various conditions. On aqueous phosphate buffer at pH 7.8 the area per molecule (extrapolated to zero pressure) was 122 Å^2. With tetraethylammonium chloride or methyl viologen dissolved in the subphase, the area per molecule increased with the substrate concentration in accordance with a Langmuir-type adsorption isotherm. This increase in area was attributed to complex formation. Equilibrium constants for complex formation between chlorophyll and substrates were calculated to be 7.2 $\times$ 10^2 and 2.6 $\times$ 10^3 M^{-1} for methyl viologen and tetraethylammonium chloride, respectively. Mixed monolayers of chlorophyll a and cytochrome *c* or egg albumin were also prepared. These exhibited nonideal behavior, in that the area of the mixed monolayers is greater than the sum of the areas of the individual components. Either a complex is formed between chlorophyll and protein, or the protein undergoes a conformational change or a reorientation on the surface in the presence of the pigment, so as to enlarge its surface area.

Upon illumination in air, chlorophyll monolayers undergo photooxidation, which results in an increase in the area per molecule to 203 Å^2 when all the pigment is oxidized. The quantum yield for this reaction when the monolayer is compressed to 17 dyn/cm was calculated to be 0.06. This is 12 times greater than the yield for photooxidation of a dilute solution of chlorophyll a in benzene. For expanded monolayers (initial pressure of 2 dyn/cm), the reaction was at least seven times less efficient than for the compressed systems, which suggests that energy transfer is important in the photochemistry of these monolayers. For mixed monolayers of chlorophyll a and β-carotene in air, the photooxidation of chlorophyll was inhibited, either because chlorophyll sensitized the photooxidation of carotene or because carotene quenches the triplet state of chlorophyll.

In the chloroplast it is believed that chlorophyll exists in high concentration, as a partly ordered monolayer, situated between layers of lipid and protein (Rabinowitch, 1956). Thus, studies of the photochemistry of chlorophyll in dilute solution are of limited use as models for the situation *in vivo*. In dilute solution, for example, there are no restraints on the orientations of the molecule, whereas *in vivo* the pigment is anchored. Also, reactions with water-soluble substrates can be examined only by solubilizing the chlorophyll in aqueous media with detergents. Furthermore, the effect of interactions between the pigment molecules, and of energy transfer, on the photochemistry cannot be assessed in dilute solution. Monolayers of chlorophyll spread on water more closely simulate the *in vivo* state, and the photochemical properties of such monolayers are therefore of interest.

A number of workers (for review, see Ke, 1966) have investigated the physical properties of monolayers of chlorophyll and its derivatives. Some of these studies (Colmano, 1961; Bellamy *et al.*, 1963) included observations on the effect of light on the monolayers, but did not systematically examine the photochemistry nor correlate chemical and photochemical changes with changes in surface properties. The first results of a study of photochemical reactions of chlorophyll a monolayers, spread in a film balance at a water–air interface, are reported here. Under appropriate conditions, these monolayers were found to undergo photooxidation, to form complexes with materials dissolved in the aqueous subphase, and to undergo photoreactions with these materials.

Experimental Section

Monolayer studies were carried out in an automatic Wilhelmy plate film balance, housed in an environmental chamber, with provision for flushing with nitrogen and for evacuation. The sensitivity of the balance permitted measurement of film pressures with a precision of $\pm$0.2 dyn/cm. The trough, whose dimensions were 68 $\times$ 17 $\times$ 1 cm, was constructed of Teflon. While this material is not rigid, frequent measurements of its dimenstions were made and taken into account in the calculations, and reproducible isotherms were obtained. The barrier was made of brass coated with paraffin. The Wilhelmy blade was a sand-blasted platinum leaf. The monolayers were compressed slowly in order to keep the blade vertical.

Chlorophyll a was spread on 10^{-2} M phosphate buffer (pH 7.8) unless otherwise indicated. Benzene was used as the spreading solvent. The exact concentration of the spreading solution, usually about 10^{-5} M, was determined spectrophotometrically, with a recording spectrophotometer (Cary Model

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14R). The surface of the subphase was cleaned and then about 300 μl of solution was delivered slowly over it from a Hamilton microliter syringe. These operations were done in air unless otherwise specified. A few minutes were allowed for the benzene to evaporate, and then measurements were commenced. These measurements were made in subdued daylight, which is what we refer to as "dark." For experiments carried out under nitrogen, a positive pressure of the prepurified gas (99.996% pure) purchased from Matheson & Co., East Rutherford, N. J., was maintained in the environmental chamber.

For photochemical experiments, the light source was a slide projector with a 500-W tungsten lamp, mounted in front of a shutter in the box housing the balance. A KG-1 Chance filter (Chance-Pilkington Works, Flintshire, Great Britain) was used to eliminate infrared wavelengths longer than 800 nm, and a 3-73 Corning filter (Corning Glass Co., Corning, N. Y.) eliminated ultraviolet wavelengths shorter than 400 nm. The lamp was about 25 cm from the surface, and the average energy at the surface, measured with an Eppley Laboratories (Newport, R. I.) calibrated thermopile was 3.8×10^5 ergs/cm^2 sec. An area of 150 cm^2 was irradiated. Unless otherwise specified, monolayers were irradiated with white light. To obtain monochromatic red light (660 or 677 nm), interference filters (Farrand Optical Co., Yonkers, N. Y.) were employed. The average incident intensity at the surface was 4.1×10^{-10} einstein/cm^2 sec for 677 nm. For photoreactions in solution, the intensity of light (660 nm) at the sample was 4.0×10^{-9} einstein/cm^2 sec.

The procedure used in following reactions at the surface was to compress the film until reaching some predetermined surface pressure, π_0. The area was held constant and changes in π were measured as a function of time. After every reaction the surface was examined for leakage. If none was evident, the monolayer was retrieved on a microscope slide. Usually 95% of the monolayer was recovered.

Chlorophyll a was prepared as described previously (Broyde and Brody, 1966), and had extinction coefficients within 2% of published values (Seely, 1965). Nevertheless, with this preparation, the area per molecule on the surface was almost double that of previously published values (Bellamy *et al.*, 1963); the extinction coefficients are therefore not a sufficient criterion for purity. The following additional procedures yielded chlorophyll with an area per molecule as low as that given by previous workers, and with an extinction coefficient of 7.95×10^4 l./(mole cm) at 665 nm in dilute benzene solution, which is in agreement with the value reported by Bellamy *et al.* (1963). About 100 mg of crystalline chlorophyll a were dissolved in 10 ml of acetone, to which was added 90 ml of hexane. The hexane had been previously washed 20 times with distilled water, to remove acid impurities. The hexane–acetone solution was then washed (about 20 times) until all acetone had been removed and the solution became colloidal. The suspension was allowed to stand overnight at $-40°$, then centrifuged at 48,000g for 20 min, and the supernatant was discarded. This procedure, which is a variation of the method described by Strain and Svec (1966), removed colorless lipid impurities, present to the extent of a few per cent, and improved the surface area about 20%.

The pellet was then dissolved in 25 ml of acetone, and this solution was diluted with an equal volume of distilled water. The resulting suspension was again centrifuged as above, and the supernatant discarded. Since the resulting crystals were very fine and difficult to handle, they were redissolved in 25 ml of ethyl ether and recrystallized over 10^{-2} M phosphate buffer (pH 7.8); the crystals were stored in this suspension in the dark at 0°. The acetone–water recrystallization removed surface active contaminants presumably extracted from the sugar, which are present to such a small extent that only surface measurements give evidence of their presence. It was necessary to change the buffer about once a month, at which time the crystals were washed with distilled water.

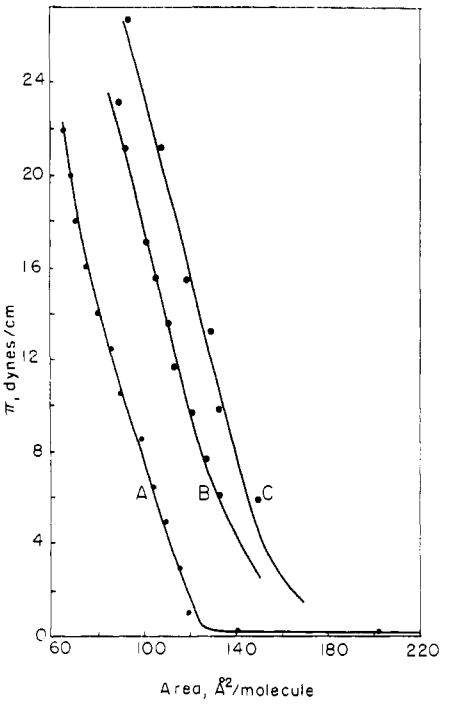

FIGURE 1: Surface pressure (π, dynes per centimeter) as a function of area per molecule (Å^2), subphase 10^{-2} M phosphate buffer (pH 7.8), N_2 atmosphere, 298°K. Curve A, chlorophyll a. Curve B, chlorophyll a with 10^{-3} M methyl viologen in subphase. Curve C, chlorophyll a with 10^{-1} M tetraethylammonium chloride in subphase.

Distilled water of satisfactory purity was obtained by deionizing with a Barnstead standard ion-exchange column, and distilling in a Corning (Corning, N. Y.) Model AG-2-Still. Organic solvents were Spectroquality Reagents (Matheson Coleman and Bell, East Rutherford, N. J.). It was found that the 300 μl of benzene used to spread the pigment contained no measurable surface active materials. Tetraethylammonium chloride was the "Red Label" product from Eastman Kodak (Rochester, N. Y.). Methyl viologen was obtained from British Drug House (Poole, England), β-carotene from Nutritional Biochemicals Corp. (Cleveland, Ohio), reagent grade ascorbic acid and cytochrome c, type III, 90–100% pure, from Sigma Chemicals (St. Louis, Mo.), and egg albumin from Fisher Scientific Co. (Milford, N. J.).

Results

Surface Isotherms in the Dark. ON BUFFER. In Figure 1 (curve A) is shown the surface pressure–area curve obtained for chlorophyll a on 10^{-2} M phosphate buffer at pH 7.8, under nitrogen atmosphere. The same isotherm is obtained on 5 ×

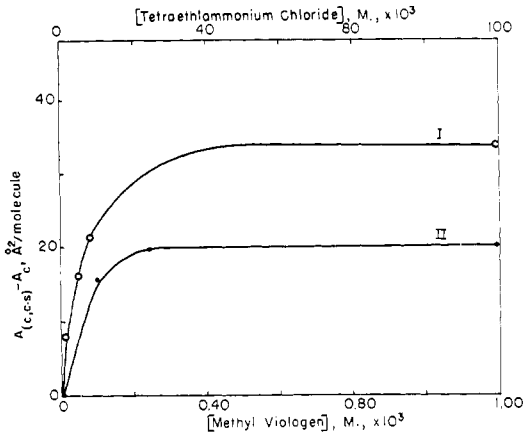

FIGURE 2: $A_{(c,c\cdot s)} - A_c$, the difference between the area per molecule of chlorophyll a in the presence of substrate and in its absence, as a function of substrate concentration. Curve I and upper axis, tetraethylammonium chloride. Curve II and lower axis, methyl viologen.

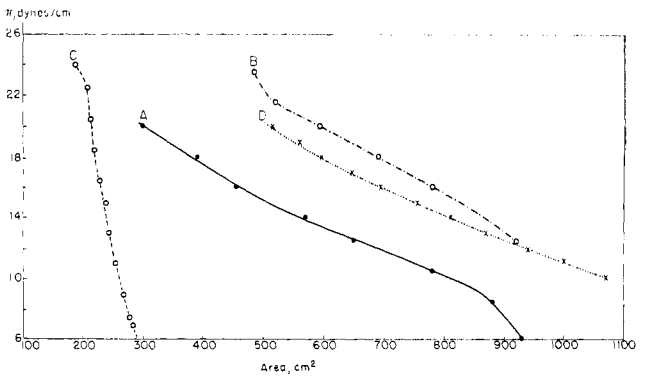

FIGURE 3: Surface isotherms on a subphase of 10^{-2} M phosphate buffer, pH 7.8, 298°K. Curve A, cytochrome *c*. Curve B, chlorophyll a–cytochrome *c* mixed monolayer. Curve C, chlorophyll a. Curve D, sum of curves A and C. For curves A, B, and D, the subphase contains 10^{-6} M cytochrome *c*. The number of chlorophyll molecules comprising the monolayer is the same for curves B, C, and D.

10^{-2} M buffer or in air. The area per molecule extrapolated to zero pressure (hereafter referred to as A_c) is 122 Å².

WITH ADDED MATERIALS DISSOLVED IN THE SUBPHASE. Surface isotherms of chlorophyll a have also been measured in the presence of tetraethylammonium chloride or methyl viologen dissolved in the buffered subphase. These are substances which have been found to complex with other porphyrins in aqueous solution (Mauzerall, 1965; Cann, 1967). In the concentration range employed (less than 10^{-1} M) these materials had no measurable effect on the surface tension of water, nor did they form a compressible film at the water–air interface. Figure 2 gives $A_{(c,c\cdot s)} - A_c$, where $A_{(c,c\cdot s)}$ is the area per molecule in the presence of substrate and A_c is the area per molecule of chlorophyll alone, as a function of substrate concentration. Curve I is for tetraethylammonium chloride and curve II is for methyl viologen. The area per molecule of chlorophyll increased with increasing concentration of these substances, reaching 143 Å² (Figure 1, curve B) and 154 Å² (Figure 1, curve C) at the highest concentration, for methyl viologen and tetraethylammonium chloride, respectively. These values are taken as the area of the chlorophyll substrate complex $A_{c\cdot s}$, at $\pi = 0$. From these data it is possible to calculate equilibrium constants for complex formation, as will be shown in the Discussion. A similar series of isotherms was measured with NaCl dissolved in the subphase, to ascertain the effect of increased ionic strength. With an ionic strength as high as 0.1, the area per molecule of chlorophyll was increased to 126 Å². Thus, a considerably lower concentration of methyl viologen than of quarternary ammonium salt is needed to produce a given change in $A_{c\cdot s}$ and the effect of NaCl is very much less than that of either viologen or quarternary ammonium salt. The value for $A_{c\cdot s}$ does not change even when the monolayer is permitted to remain for 1 hr in the dark, and in air at initial pressures, π_0, of either 2 or 17 dyn per cm, either in the presence of methyl viologen or tetraethylammonium chloride.

Experiments were also made with 10^{-6} mole/l. of cytochrome *c* in the subphase. This protein is, of course, surface active. Reproducible isotherms for the protein alone were obtained, under nitrogen and in the dark (Figure 3, curve A). Isotherms measured after the subphase had stood for 15 min were the same as those measured immediately after preparation of the solutions. Chlorophyll a was then deposited on the subphase, by sweeping a portion of the surface with the barrier and quickly applying chlorophyll, sweeping further and applying more chlorophyll, until the entire surface had been covered with the desired amount of chlorophyll. The resulting isotherm is shown in Figure 3 (curve B). A similar experiment was done with egg albumin in the subphase, with similar results. In both cases, the area at a given pressure was greater when chlorophyll was present. Irradiation appeared to have no effect on these isotherms.

Photoreactions. Surface isotherms were determined after the monolayers of chlorophyll a remained on buffer for 1 hr in the dark, in air or nitrogen. At π_0 equal to 2 dyn/cm, there was no change in A_c. At π_0 equal to 17 dyn/cm, there was a slight increase in A_c (about 1%).

After exposure to white light for 1 hr, under nitrogen, A_c is again increased only slightly, both at π_0 equal to 2 and 17 dyn per cm. In air, however, illumination causes A_c to increase markedly, at high π_0. At π_0 of 17 dyn/cm, A_c increased by 31% after 1 hr in light, to 160 Å². At π_0 of 2 dyn/cm, on the other hand, after 1 hr in light, A_c increased only 2%, to 124 Å². The kinetics of this transformation were monitored at constant area as a function of π, at π_0 of 17 dyn/cm (Figure 4, curve A). In 15 min π decreased to 13.4 dyn/cm, and then remained stable. Since the isotherm determined after irradiation showed an increase in A_c, the kinetics of the transformation would be expected to give an increase in π rather than a decrease. An explanation for this hysteresis effect is not evident.

In order to determine the quantum yield for this reaction, kinetics were also determined with monochromatic light (677 nm). In Figure 5 is shown the first-order plot $\log(\pi - \pi_f)/(\pi_0 - \pi_f)$, as a function of time, where π_f is the pressure at the end of the experiment. From these data a quantum yield of 0.06 can be calculated, as will be shown in the Discussion.

Absorption spectra of the monolayer pigment were determined after the reaction, by removing the monolayer and redissolving in benzene. The spectral changes consisted of subtle modifications in the ratios of the blue maximum to its shorter wavelength blue satellite, and of the blue/red absorbancies, in

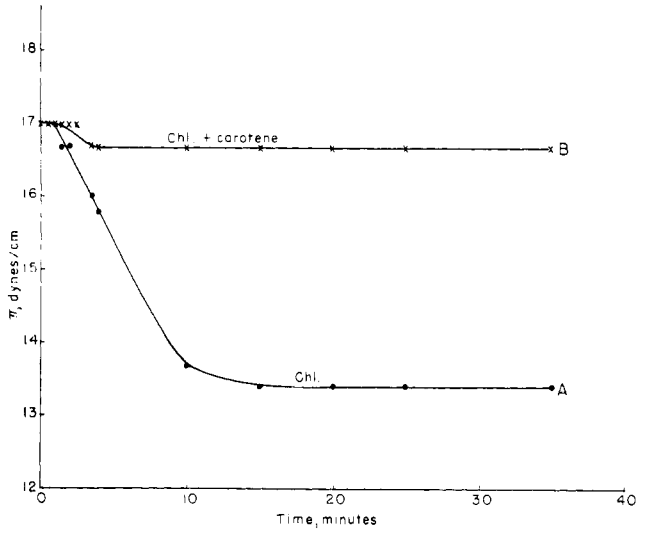

FIGURE 4: Variation of film pressure with time, in air, upon irradiation with white light, 3.8×10^6 ergs/cm² sec. Initial pressure, 17 dyn/cm, subphase 10^{-2} M phosphate buffer (pH 7.8). Curve A, chlorophyll a. Curve B, mixed monolayer of chlorophyll a and β-carotene. Ratio of concentration of chlorophyll to carotene in spreading solution is 2:1.

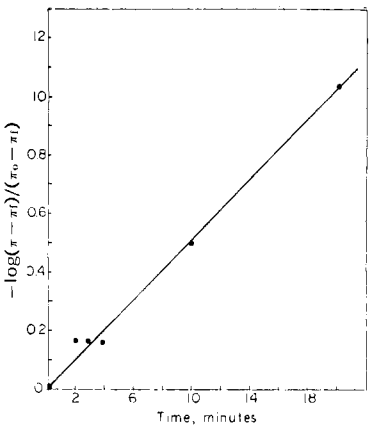

FIGURE 5: First-order plot for variation of pressure with time for monolayers of chlorophyll a, upon irradiation with red light (677 nm, 4.1×10^{-10} einstein/cm² sec) in air. Initial pressure 17 dyn/cm. π is the instantaneous pressure, π_0 the initial pressure, and π_f the pressure at the end of the reaction.

agreement with earlier findings (Bellamy *et al.*, 1963). Any bleaching of the pigment resulting from the 30-min irradiation was less than 5%.

For comparison, a 4.4×10^{-6} M solution of chlorophyll a in benzene was irradiated with red light (660 nm) for 2 min, in air, in a 1-cm path-length cuvet. The optical density at 665 nm, the red absorption maximum of chlorophyll a, decreased by 18%, and a quantum yield of 4.6×10^{-3} was calculated.

However, if the monolayers were allowed to remain in darkness for 15 min before irradiation, at π_0 of 17 dyn/cm, they were no longer susceptible to action by light. That is, the surface properties of the film remained constant under illumination in air, even after expansion and recompression.

When β-carotene is present in the monolayer, the photoreaction of chlorophyll in air is inhibited. A chlorophyll–β-carotene mixed monolayer, spread from benzene solution in a molecular ratio of 2:1, respectively, in air, at π_0 of 17 dyn/cm, showed almost no change in π, when illuminated for 30 min (Figure 4, curve B).

Ascorbic acid dissolved in the subphase also has the effect of inhibiting the photoreaction of the monolayer in air. This reductant did not show measurable surface activity at concentrations of 10^{-2} M or less. On irradiating in air, with 10^{-2} M ascorbic acid dissolved in the subphase (buffer concentration 5×10^{-2} M), π decreased from 17 to a final value of only 15.6 dyn/cm (compare with Figure 4, curve A).

With methyl viologen or tetraethylammonium chloride in the subphase, surface isotherms measured after the monolayers remained in the dark for 1 hr, in air or nitrogen, showed virtually no change in A_{c+s}. The same was true when irradiating under nitrogen. In air, on the other hand, illumination caused A_{c+s} to decrease when tetraethylammonium chloride was present in the subphase. With 5×10^{-3} M tetraethylammonium chloride in the subphase, irradiation for 1 hr at a π_0 of 2 dyn/cm produced a decrease in area per molecule from 137 to 125 Å². Under similar conditions but a π_0 of 17 dyn/cm, A_{c+s} decreased to 120 Å² in this time. The monolayers retrieved from the surface did not appear bleached. In the presence of 2.5×10^{-4} M methyl viologen, on the other hand, irradiation for 1 hr produced little change in area per molecule, but the retrieved monolayer was about 75% faded.

Discussion

Complex Formation. Tetraethylammonium chloride and methyl viologen present in the subphase produce an increase in area per molecule of chlorophyll, which is dependent upon the concentration of these substrates. This increase in A_c is much greater than that produced by a comparable concentration of sodium chloride, so that it is not merely the result of increased ionic strength. In light of the findings that porphyrins complex with quarternary ammonium salts and with methyl viologen in aqueous solution (Mauzerall, 1965; Cann, 1967) it seems reasonable to interpret the increased area per molecule of chlorophyll in the presence of these materials as the result of complex formation, since at the concentration employed here the substrates themselves showed no measurable surface activity. It is possible to calculate equilibrium constants for complex formation at the surface from our data.

Assuming that chlorophyll and chlorophyll–substrate complex form an ideal mixed monolayer, then

$$A_{(c,c\cdot s)} = A_c N_c + A_{(c\cdot s)} N_{(c\cdot s)} \quad (1)$$

where the subscripts c, (c·s), and (c,c·s) refer to chlorophyll, complex, and mixture, respectively, the A's are areas per molecule at $\pi = 0$, and the N's are mole fractions. Since $N_c + N_{(c\cdot s)} = 1$

$$N_{(c\cdot s)} = [A_{(c,c\cdot s)} - A_c]/[A_{(c\cdot s)} - A_c] \quad (2)$$

For the equilibrium Chl + S $\rightleftarrows$ Chl·S, where Chl is chlorophyll and S is substrate and the equilibrium constant is b, one can write the equilibrium relation as

$$N_{(c\cdot s)} = \frac{b(S)}{1 + b(S)} \quad (3)$$

which is of the same form as Langmuir's isotherm for the adsorption of gases on solids. Substituting this back into eq 2, we obtain

$$A_{(c,c\cdot s)} - A_c = [(A_{(c\cdot s)} - A_c)]b(S)/[1 + b(S)] \quad (4)$$

which becomes

$$A_{(c,c\cdot s)} - A_c = [(A_{(c\cdot s)} - A_c)]b(S) \quad (5)$$

at low substrate concentration.

From the slope of Figure 2 (curve I) at low (S), namely, 8 Å^2/molecule per 9×10^{-4} M, b can be evaluated for tetraethylammonium chloride. Taking for $A_{(c\cdot s)}$ the value at high substrate concentration, namely 154 Å^2, a value of 2.6×10^2 M^{-1} is obtained for b. For methyl viologen, a value of 7×10^3 M^{-1} is similarly calculated. Mauzerall (1965) estimated a binding constant of 6.3×10^2 M^{-1} for hexadecyltrimethylammonium bromide and 10^3 M^{-1} for γ,γ-dipyridyl (the parent compound for viologens) with uroporphyrin. In those systems, complexes occur with ratios of substrate to uroporphyrin higher than 1:1. This is less likely to occur with monolayers of chlorophyll where one side of the plane of the porphyrin is exposed to the aqueous subphase, while the other side is exposed primarily to phytol.

The isotherms obtained for chlorophyll with a surface active protein present in the subphase are less easily interpreted. To examine the possibility that chlorophyll and cytochrome or serum albumin form an ideal mixed monolayer, we have plotted the sum (Figure 3, curve D) for separate isotherms of chlorophyll (Figure 3, curve C) and cytochrome (Figure 3, curve A), and compared them with the isotherm for the mixtures obtained experimentally (Figure 3, curve B). The number of chlorophyll molecules was the same in the separate isotherms and in the mixture. The area determined experimentally for the mixture is greater than the sum of the areas calculated from the individual isotherms, so an ideal mixture is not formed. Similar results were obtained with egg albumin. If the chlorophyll were just replacing protein at the surface, or if the chlorophyll were lying above the surface protein, then the experimentally determined surface area of the mixture would be smaller than the calculated value. An interaction between chlorophyll and protein could lead to a larger experimental area for the mixture. A conformational change or a reorientation of the protein on the surface in the presence of chlorophyll could also give larger areas. Binding of chlorophyll to egg albumin has been previously noted in solution (Aghion, 1964).

Photoreactions. It is known that chlorophyll monolayers may be degraded with time, particularly in the light (Bellamy *et al.*, 1963), but the nature of the change has not been specified or correlated with surface properties. Under the conditions described here chlorophyll undergoes degradation in light and air on a buffered subphase that is neutral or slightly alkaline and contains no added materials; the transformation is manifested by changes in the area per molecule or in surface pressure. The small changes obtained in the "dark" are probably a slow light reaction, since we did not work in total darkness. This reaction is accompanied by subtle changes in the absorption spectra of the retrieved pigment. Since the reaction is inhibited in a nitrogen atmosphere, and in air by the presence of ascorbic acid in the subphase, it is concluded that the pigment is undergoing a photooxidation.

The oxygen dissolved in the subphase and present even in our "nitrogen" experiments is not effective in promoting this photooxidation, which takes place only when there is oxygen in the atmosphere above the monolayer. This suggests that the oxidation site is at the porphyrin–air rather than the porphyrin–water interface, perhaps at the vinyl substituent. Chlorophyll is believed to be situated on the aqueous surface with its cyclopentanone oxygen in the water, and the phytol and the plane of the porphyrin sticking out, with an angle of about 45–50° between the surface of the water and the plane of the porphyrin (Chasovnikova *et al.*, 1966). The oxidized state of the pigment is probably more hydrophilic, so that the angle between the plane of the porphyrin and the water would be smaller, resulting in a larger area per molecule. The area per molecule of the oxidized chlorophyll A_{ox} at $\pi = 0$ can be calculated, assuming that chlorophyll and its oxidation product form an ideal mixed monolayer. Equation 2 can be applied, with the new subscripts c, (ox), and (c,ox) referring to chlorophyll, oxidized chlorophyll, and their mixture, respectively. To obtain A_{ox}, assume that all the irradiated chlorophyll molecules are photooxidized when $A_{(c,ox)}$ in the mixed monolayer reached 160 Å^2. As noted before A_c is 122 Å^2. Since only 150 cm^2 of the 320-cm^2 monolayer was irradiated, the value of N_{ox} equals 0.47. Then, from eq 2, $A_{ox} = 203$ Å^2.

In order to calculate the quantum yield for photooxidation, a similar analysis can be used, substituting π for A in eq 1 and 2. This assumes that the film pressures are additive, which is true between 2 and 17 dyn per cm, where π varies almost linearly with A (see Figure 1). To calculate π_{ox}, the surface pressure which would obtain if all the chlorophyll were oxidized, eq 2 is used, with N_{ox} again equal to 0.47. The final value of the surface pressure in white light $\pi_{(c,ox)}$ equals 13.4 dyn/cm and the initial pressure $\pi_c = 17$ dyn/cm. π_{ox} is then calculated to equal 9.4 dyn/cm. In monochromatic red light (677 nm), after 2-min illumination, $\pi_{(c,ox)}$ was 16.3 dyn/cm. Using eq 2 again to solve for N_{ox}, the fraction of molecules oxidized after 2 min illumination with red light, a value of 0.092 is obtained for that quantity. This corresponds to 5.7×10^{15} molecules for this run, in which there were 6.3×10^{16} molecules on the surface. The energy of red light incident on the surface was 4.1×10^{-10} einstein/cm^2 sec. The fraction of light absorbed is 0.022, for a monolayer whose optical density is 0.010 at 677 nm (Chasovnikova *et al.*, 1966), so that in 120 sec, 9.8×10^{16} quanta are absorbed on an area of 150 cm^2, giving 0.06 for the yield of photooxidation. This may be compared with the 12-fold lower yield for photooxidation in benzene solution, namely, 0.0046. The oxidation product on the surface is apparently not the same as that in solution since the chlorophyll was bleached in solution, but not on the surface. However a comparison of the efficiency of photooxidation in the two systems is useful in assessing the reactivity of monolayers.

There have been conflicting reports in the literature (Trurnit and Colmano, 1959; Bellamy *et al.*, 1963; Rosoff and Aron, 1965) on the effect of film pressure on the reactivity of chlorophyll monolayers in the dark. In any event, the present data point to higher photoreactivity at high pressure. At 2 dyn/cm $A_{(c,ox)}$ reached a value of 124 Å^2 after irradiation for 1 hr, corresponding to a calculated value of 0.024 for N_{ox} (eq 2), whereas at 17 dyn/cm, N_{ox} was 0.47 or 20-fold greater. Taking into account that the number of molecules illuminated at 2

dyn/cm was only 0.6 times that illuminated at 17 dyn/cm (the expanded monolayer took up more area while the irradiated area remained constant), and that the optical density of the monolayer increases from 0.007 to 0.01 (Chasovnikova *et al.*, 1966) between these two pressures (fraction absorbed increases from 1.40 to 2.25%), a real rate enhancement of sevenfold is realized. Actually this represents only a minimum rate enhancement, since at 17 dyn/cm the reaction had gone to completion in 15 min, while it was not even measurable in that time at 2 dyn/cm. The actual rate increase is very likely much greater, but could not be estimated here. Thus the photoreactivity of chlorophyll monolayers appears to be promoted as the interaction between pigment molecules is increased. As the distance between pigment molecules decreases, there is greater probability of energy transfer, which according to Förster's (1951) mechanism varies inversely with the sixth power of the intermolecular distance. Without energy transfer, an absorbed photon can only produce a reaction at the absorbing molecule. Thus, if the absorbing molecule does not happen to be in the proper configuration for a reaction, no reaction will occur. With the possibility for energy transfer, the absorbed photon can still induce the reaction of another molecule, which is in the right configuration. The increased rate or quantum yield when the pigment is compressed can be understood, on this basis.

There are at least two possible explanations for the stability of the chlorophyll monolayers in air and light when β-carotene is present. This stability may be taken as evidence that chlorophyll is sensitizing the photooxidation of carotene and is thereby protected by it. Inhibition of chlorophyll photooxidation by carotene and other autoxidizable substrates is well known in solution (for review, see Seely, 1966). Such a reaction should produce a decrease in absorbance of carotene, due to its oxidation. However, in the mixed monolayer, which was retrieved from the surface, no such decrease was found. Since the chlorophyll-sensitized photooxidation of carotene is quite inefficient, at least in solution (Claes, 1961), a small change in carotene concentration relative to chlorophyll could have escaped detection. Another possibility is that the photooxidation of chlorophyll proceeds *via* the triplet state, which is quenched by β-carotene (Fujimori and Livingston, 1957), thus retarding the photooxidation. The presence of ascorbic acid in the subphase also inhibits photooxidation of chlorophyll, either by rapidly reducing the oxidation product back to the original pigment or by quenching the triplet state of chlorophyll. Nekrasov *et al.* (1967) have shown that chlorophyll monolayers can sensitize the photoreduction of methyl red by ascorbic acid.

When tetraethylammonium chloride is present in the subphase, chlorophyll is complexed with it and the area per molecule of the complex is 154 Å^2. Illuminating in air produces a decrease in area to 120 Å^2. Using eq 2 again, to correct for the presence of unirradiated complex in the film, an area per molecule of 86 Å^2 is obtained for the irradiated pigment. Porphyrins such as chlorophyllide (Gaines *et al.*, 1964) or hemin (Alexander, 1937) which lack the phytol ester "tail" of chlorophyll have small areas in the neighborhood of 80 Å^2/molecule. Perhaps in light the quarternary ammonium salt additionally binds the phytol, thereby effectively removing it from the surface. However, the requirement for oxygen is not at all clear.

When chlorophyll is complexed with methyl viologen, the course of the photoreaction is different in that the chlorophyll fades in air. Here, a direct electron transfer reaction between chlorophyll and viologen (MV) could take place, $\text{Chl}\cdot\text{MV} \rightleftharpoons \text{Chl}^+ + \text{MV}^-$, which is reversible. Under nitrogen, no product is detected. In air, however, the reduced viologen is reoxidized very quickly, which would permit an oxidation product of chlorophyll to accumulate. Since it is bleached, this oxidized form of chlorophyll on the surface is different from the one observed in the absence of MV. Studies in solution, of chlorophyll-sensitized photoreduction of methyl viologen, have also given evidence for a primary photoreaction in which chlorophyll is oxidized by the viologen (Brin *et al.*, 1967).

Conclusion

The ability of chlorophyll monolayers to undergo photoreactions and to interact with water-soluble materials present in the subphase shows that such systems are promising as photosynthetic models. The higher quantum yield for photooxidation of the monolayer compared to the yield in benzene solution, and the enhanced efficiency of this reaction when the monolayer is compressed, compared with when expanded, are relevant to an understanding of the highly efficient photochemistry of chlorophyll *in vivo*, where the pigments are partly oriented, and condensed in layers.

Surface properties have proved to be very sensitive to chemical changes in the chlorophyll molecule, and the kinetics of these changes are easily monitored, while only slight changes in the visible absorption spectra may accompany some of these reactions. In the case of photooxidation, it has been possible to make judgments on the site of reaction, which are not easily made from spectral observations.

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