Biochimica et Biophysica Acta, 314 (1973) 360–371

BBA 46593

MONOMOLECULAR FILMS OF BACTERIOCHLOROPHYLL AND DERIVATIVES AT AN AIR–WATER INTERFACE: SURFACE AND SPECTRAL PROPERTIES*

P. REINACH, B. B. AUBREY and S. S. BRODY

Department of Biology, New York University, Washington Square, New York, N. Y. 10003 (U.S.A.)

(Received March 19th, 1973)

SUMMARY

Monomolecular films of bacteriochlorophyll, bacteriopheophytin and 2-desvinyl-2-acetyl chlorophyll *a* were prepared and studied on aqueous subphases containing pH 7.8 buffer and $4 \cdot 10^{-4}$ M ascorbate. These monolayers are mechanically stable in the dark and light at 15 °C. At surface pressures below about 18 dynes/cm the slope of the surface isotherm of bacteriochlorophyll is steeper than at pressures greater than 18 dynes/cm. The surface dipole moments of bacteriochlorophyll are less than half that reported for chlorophyll *a*. Compression of bacteriochlorophyll or bacteriopheophytin monolayers result in changes of their absorption spectra.

Compression of bacteriochlorophyll monolayers to 18 dynes/cm results in a shift of the pigment's red peak from 787 to 749 nm as well as the appearance of a new absorption maximum at 896 nm. Continued compression to 24 dynes/cm results in a slight decrease in peak height of the 794-nm maximum and further increase in the absorbance of the 896-nm maximum. With bacteriopheophytin the red maximum at 760 nm starts to shift when the film is compressed to a surface pressure of only 2 dynes/cm; further compression yields a new absorption maximum at 846 nm. Compression of a film of 2-desvinyl-2-acetyl chlorophyll *a* results in only a 10-nm shift of the absorption maximum at 690 nm.

An orientation of bacteriochlorophyll at an air–water interface is proposed that is different from that for chlorophyll *a*. Like chlorophyll *a* bacteriochlorophyll monolayers are closely packed, but different in that bacteriochlorophyll allows greater interaction between pigment molecules. In compressed monolayers bacteriochlorophyll appears to aggregate differently than in other model systems.

INTRODUCTION

The infrared absorption maxima of bacteriochlorophyll *a*, bacteriochlorophyll, was shown to occur at considerably longer wavelengths in chromatophores than in organic solvents[1]. The specialized environment giving rise to the red shift of bacterio-

* This paper is based upon a dissertation submitted by Peter Reinach in partial fulfillment of the requirements for the Ph. D. degree of New York University, New York, N.Y., U.S.A.

chlorophyll in the condensed *in vivo* state has been attributed to interaction of bacteriochlorophyll with other bacteriochlorophyll molecules, proteins, and lipids[2,3]. In bacteriochlorophyll–protein complexes it has been shown that interaction of at least five bacteriochlorophyll molecules are responsible for both the dichroic properties and the observable exciton splitting[4]. Since bacteriochlorophyll contains both hydrophobic and hydrophillic groups, it has been suggested that most of the molecules are arranged at interfaces between proteins and lipids or between a lipid double layer[5].

A well defined model system that lends itself to quantitative interpretation an reproduces, in part, the *in vivo* structure is a bacteriochlorophyll monolayer at an air–water interface. In such a system the pigment packing appears to approximate the *in vivo* situation better than other model systems. Heretofore, the lability of bacteriochlorophyll monolayers has precluded their systematic study. Recently it has been demonstrated that by the addition of sodium ascorbate to the aqueous subphase, monomolecular films of bacteriochlorophyll can be stabilized[6].

The physical, spectral, and chemical properties of bacteriochlorophyll and several of its derivatives in monomolecular films at an air–water interface are presented in this paper. The spectral properties of bacteriochlorophyll monolayers are found to resemble those of photosynthetic bacteria. The spectral transformations observed with compressed bacteriochlorophyll monolayers are sufficient to be indicative of strong exciton interactions. These spectral transformations appear to be related to characteristic changes in the surface isotherm and surface potential of monomolecular films of bacteriochlorophyll.

METHODS AND MATERIALS

Monolayer and spectral studies were carried out using the apparatus described previously[7–9]. *Rhodospirillum rubrum* was grown under conditions described by Eimhjellen[10]. Bacteriochlorophyll, oxidized bacteriochlorophyll and bacteriochlorophyll pheophytin were prepared by methods described by Smith and Calvin[11]. All monolayer studies were carried out at 15 °C.

The subphase contained $2 \cdot 10^{-2}$ M phosphate buffer (pH 7.8) and $4 \cdot 10^{-4}$ M sodium ascorbate. Bacteriochlorophyll was spread onto the aqueous surface using benzene as a spreading solvent. The exact concentration of the spreading solution was determined spectrometrically (Cary 14R). The concentration of pigment in benzene was calculated from the absorbance using the molar extinction coefficients which were determined to be $75 \cdot 10^3$ $M^{-1} \cdot cm^{-1}$ for bacteriochlorophyll (at 780 nm), $60 \cdot 10^3$ $M^{-1} \cdot cm^{-1}$ for oxidized bacteriochlorophyll (at 682 nm). The surface of the subphase was cleaned and then about 300 μl of solution was delivered slowly over the surface from a Hamilton microliter syringe. The environmental chamber housing the surface balance was evacuated with a vacuum pump and then flushed several times with nitrogen.

Three parameters were used to characterize bacteriochlorophyll monolayers: (π–A) surface isotherms, surface potentials, ΔV, and absorption spectra. The area per molecule extrapolated to zero surface pressure ($\pi = 0$) is referred to as A_0. A has dimensions of Å^2/molecule. The surface pressure, π, at which bacteriochlorophyll monolayers collapse is referred to as π_c. ΔV is the difference in surface potential between a clean water surface, V_{H_2O}, and film on the water surface, V (*i.e.* $\Delta V = V - V_{H_2O}$).

The perpendicular component of the molecular surface dipole moment $\mu_\perp$ can be calculated from the data using the relationship

$$\Delta V = 12\,\pi\,\mu_\perp/A + V_w \qquad (1)$$

where $1/A$ is pigment concentration on the surface. From the slope of the line determined by Eqn 1, $\mu_\perp$ can be calculated in millidebyes, mD. V_w, the surface potential of water under the bacteriochlorophyll film, is equal to the y intercept of Eqn 1.

The spectral behavior of bacteriochlorophyll in monlayers was studied by measuring the absorbances of the pigments' absorption maxima as a function of $1/A$. The absorption maxima are given as $A\,\lambda$ (*e.g.* A 896) where λ is the wavelength (in nm) of the absorption maxima. In cases where the wavelength of absorption maxima are obtained from the deconvolution of the absorption spectra the bands are designated as $B\,\lambda$ (*e.g.* B 870). The absorption bands were deconvoluted using the program RESOLV[12].

Potassium phosphate salts were purchased from Fisher Chemical, sodium ascorbate from Fluka, A. G. (Bucks, S. G.), benzene spectral grade from Mallinckrodt, oleyl alcohol and lecithin from Applied Science Laboratories (State College Park, Pa.).

RESULTS

Bacteriochlorophyll in benzene solution

The characteristic spectra of bacteriochlorophyll, bacteriopheophytin, and oxidized bacteriochlorophyll in benzene are shown in Fig. 1. Spectral data for the above pigments are summarized in Table I.

Bacteriochlorophyll in benzene decomposes rapidly when exposed to air and light. In air and darkness the pigment undergoes a slow oxidation to 2-desvinyl-2-acetyl chlorophyll a[11]. Under a nitrogen atmosphere and in darkness bacteriochlorophyll can be stored in wet benzene without signs of decomposition; no change in the red/blue ratio is observed after storage for 24 h.

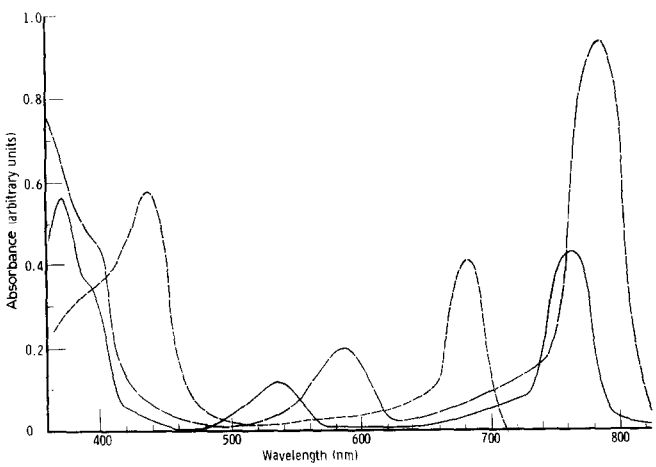

Fig. 1. Absorption spectra of benzene solutions of bacteriochlorophyll is shown by long dashes (— — —), bacteriopheophytin by a solid line (———), and 2-desvinyl-2-acetyl chlorophyll *a* by short dashes (- - - - -).

TABLE I

SPECTRAL PROPERTIES OF BACTERIOCHLOROPHYLLOUS PIGMENTS IN BENZENE SOLUTION

	Wavelengths of maxima (nm)	*Extinction coefficient of red band* ($M^{-1} \cdot cm^{-1}$)	*Red/blue ratio*
Bacteriochlorophyll	357, 396, 578, 780	$75 \cdot 10^3$	1.30 (780/357)
Bacteriopheophytin	364, 390, 530, 749	$60 \cdot 10^3$	0.65 (749/364)
Oxidized bacteriochlorophyll	436, 682	$68 \cdot 10^3$	0.71 (682/436)

Bacteriochlorophyll isotherms

A monolayer of bacteriochlorophyll on a subphase containing only $2 \cdot 10^{-2}$ M phosphate buffer (pH 7.8) is not stable. Isotherms of bacteriochlorophyll show a time-dependent increase in the value for A_0 even if the environmental chamber is kept in a nitrogen atmosphere and darkness. In Fig. 2 are shown two isotherms measured 20 and 90 min after the bacteriochlorophyll monolayer is formed. A_0 is seen to increase 15Å^2/molecule during this time. However, with $4 \cdot 10^{-4}$ M sodium ascorbate added to the subphase A_0 is invariant. In the presence of ascorbate bacteriochlorophyll monolayers are chemically stable for at least 6 h in either air or nitrogen. Furthermore, the films are stable in light.

One of the techniques used to examine the spectral stability of bacteriochlorophyll monolayers is to collect the film and measure their absorption spectra in a benzene solution. A criterion of bacteriochlorophyll purity is the value of the peak ratio 780/357 nm; pure bacteriochlorophyll in benzene has a peak ratio of 1.30. The rate of formation of oxidized bacteriochlorophyll (*i.e.* decrease in the peak ratio of 780/357

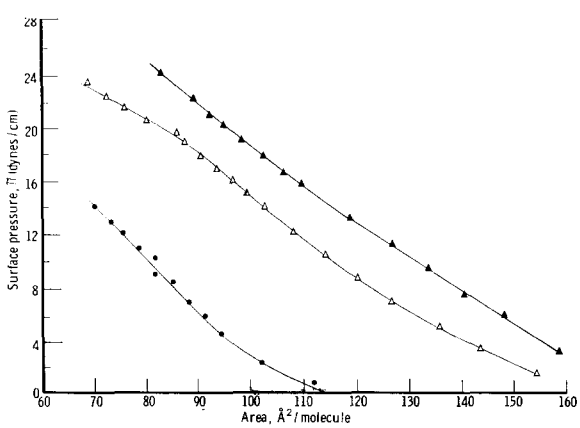

Fig. 2. Surface pressure (π, dynes/cm) as a function of area per molecule (Å^2) in a N_2 atmosphere and darkness. Initial bacteriochlorophyll isotherm is shown by △—△; isotherm after film has been on the surface for 1.5 h with no ascorbate in subphase is shown by ▲—▲. The subphase contains 0.02 M phosphate buffer (pH 7.8) at 15 °C. The surface isotherm of bacteriopheophytin measured with a subphase containing $2 \cdot 10^{-2}$ M sodium acetate buffer (pH 3.7) and no ascorbate is shown by ●—●.

nm) can be correlated with the time-dependent increases in A_0. Only when the subphase contains $4 \cdot 10^{-4}$ M sodium ascorbate is the value of the ratio 780/357 nm of the recovered bacteriochlorophyll in agreement with that for pure bacteriochlorophyll. In all the subsequent monolayer studies the subphase contains $2 \cdot 10^{-2}$ M phosphate buffer (pH 7.8) and $4 \cdot 10^{-4}$ M sodium ascorbate.

Isoterms of bacteriochlorophyll monolayers have an A_0 of 147 Å^2/molecule. When these monolayers are slowly compressed at the rate of 0.5–25 Å^2/molecule per min they collapse at a π_c between 24 and 25.5 dynes/cm. Between 18 dynes/cm and π_c the isotherm appears to have a shallower slope than between 0 and 18 dynes/cm (Fig. 2).

When the pH of the subphase is < 4 monolayers of bacteriochlorophyll are rapidly converted into bacteriopheophytin. Monolayers of bacteriopheophytin collapse at a π_c of 16 dynes/cm. Pheophytinization of bacteriochlorophyll on subphases more alkaline than pH 4 results in bacteriopheophytin monolayers that a only compressible to between 8 and 12 dynes/cm. In Fig. 2 is shown the isotherm of bacteriopheophytin on a subphase of pH 3.7; the A_0 is 105 Å^2/molecule.

Surface potential measurements of bacteriochlorophyll monolayers

The surface potential, ΔV, of bacteriochlorophyll monolayers increases gradually upon compression. In Fig. 3b ΔV increases for concentrations between $6.5 \cdot 10^{13}$ and $9 \cdot 10^{13}$ molecules/cm^2 and also between $10 \cdot 10^{13}$ molecules/cm^2 and π_c; ΔV appears to remain constant between $9 \cdot 10^{13}$ and $10 \cdot 10^{13}$ molecules/cm^2. Within the precision of the experiments (*i.e.* $\pm$ 10 mD) the values of $\mu_\perp$ between $6.5 \cdot 10^{13}$ and $9 \cdot 10^{13}$ molecules/cm^2 and between $10 \cdot 10^{13}$ molecules/cm^2 and π_c are 310 and 283 mD, respectively.

The surface potential measured for water in the absence of a bacteriochlorophyll monolayer, V_{H_2O}, (*i.e.* -30 mV) does not agree with V_w (*i.e.* 180 mV) which is obtained from Eqn 1 and the data in Fig. 3b. (The value of V_{H_2O} is a little high; values of -80 mV or less are more common.)

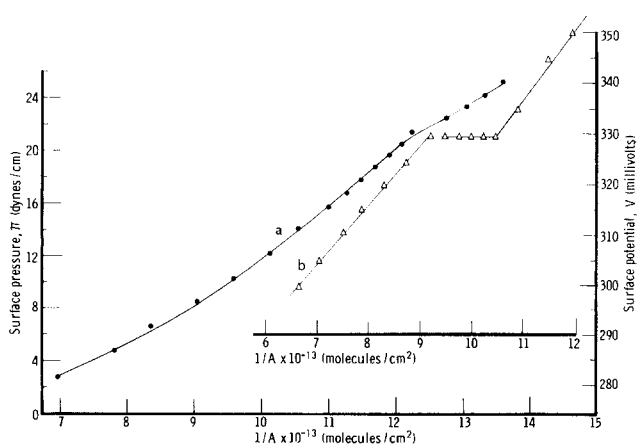

Fig. 3. Surface potential (Curve b) and surface pressure (Curve a) for a film of bacteriochlorophyll as a function of pigment concentration, $1/A$. The subphase contained 0.02 M phosphate buffer (pH 7.8) and $4 \cdot 10^{-4}$ M ascorbate at 15 °C.

Absorption spectra of bacteriochlorophyll monolayers

The absorption spectrum of bacteriochlorophyll monolayers at surface pressures of 1.5, 22.3 and 26.5 dynes/cm are shown in Fig. 4, respectively. At 1.5 dynes/cm absorption maxima are at 584 and 787 nm. Compressing the film to π = 18 dynes/cm results in broadening of the absorption band and a red shift of the 584 and 787 nm maxima to 592 and 794 nm, respectively. From the difference in the absorption spectrum between expanded and slightly compressed bacteriochlorophyll monolayers it is found that a pigment species is formed having an absorption maximum around 825 nm (see Fig. 9). At π = 20 dynes/cm a new band with an absorption maximum at about 882 nm is clearly resolved. With further compression this latter band appears to shift toward longer wavelengths reaching a limiting value of 896 nm at π = 22 dynes/cm. The absorbance of A_{896nm} continues to increase even after the film is compressed beyond its collapse point.

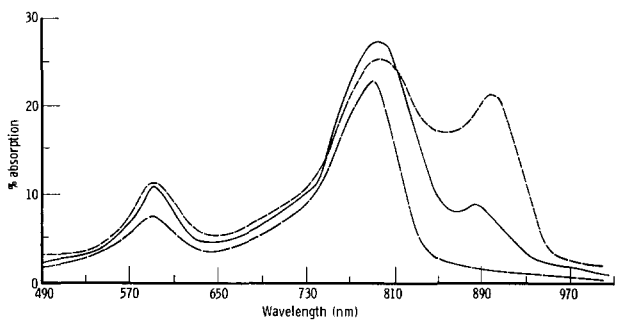

Fig. 4. Absorption spectra of a monolayer of bacteriochlorophyll at different surface pressures, π; the subphase is the same as in Fig. 3. The spectrum at π = 1.5 dynes/cm is shown by (– – –); the spectrum at π = 22.3 dynes/cm is shown by (———); the spectrum at π = 26.5 dynes/cm is shown by (– – – – –). Percent absorption is for light passing through the film 18 times.

In Fig. 5 are shown the absorbances at 592, 794, and 896 nm as a function of molecules/cm^2, $1/A$. Compression of bacteriochlorophyll monolayers results in complex changes of the absorbances of these absorption bands. Both A 592 and A 794 increase rather linearly to about 18 dynes/cm ($1/A = 10.5 \cdot 10^{13}$ molecules/cm^2, *i.e.* 100 Å^2/molecule). In the region between $10.5 \cdot 10^{13}$ and $13.7 \cdot 10^{13}$ molecules/cm^2 the values of A 592 and A 794 are found to decrease slightly. At still higher concentrations the absorbance of these bands increase again. A 896 first appears at 20 dynes/cm, its absorbance continues to increase with compression even beyond π_c.

Absorption spectra of mixed monolayers

Mixed monolayers of bacteriochlorophyll with lecithin and bacteriochlorophyll with oleyl alcohol were studied to determine whether these diluants prevent the pigment interactions which lead to the appearance of A 896. A 592 and A 794 for mixed films of bacteriochlorophyll and oleyl alcohol or lecithin as a function of bacteriochlorophyll concentration in the film, $1/A_m$, are shown in Fig. 5. The absorbances for a mixed monolayer of bacteriochlorophyll and oleyl alcohol (pigment mole fraction 0.24) or lecithin (pigment mole fraction 0.42) are slightly greater than the extrapolated values for pure bacteriochlorophyll monolayers. With either diluent compressed

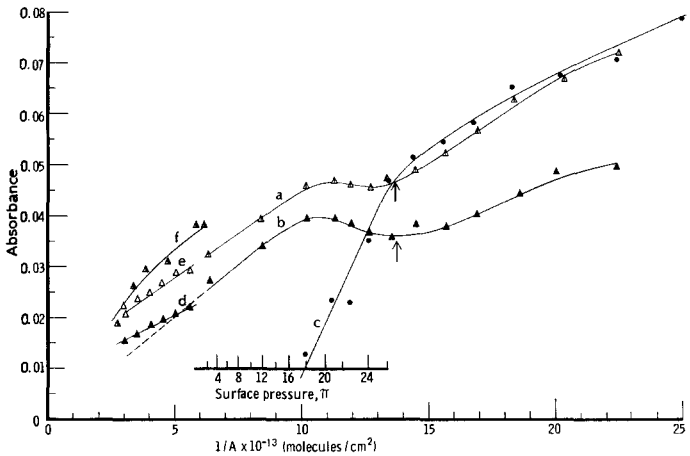

Fig. 5. Absorbance at 592 nm (a), 794 nm (b) and 896 nm (c) for a film of bacteriochlorophyll as a function of pigment concentration, $1/A$. The absorbance axis must be multiplied by 2 for the 896-nm band an by 4 for the 794-nm band. For a mixed film of bacteriochlorophyll and oleyl alcohol the absorbance at 592 nm is shown by Curve f; the mole fraction of bacteriochlorophyll is 0.24. For a mixed film of bacteriochlorophyll and lecithin the absorbance at 592 nm and 794 nm are shown by Curves e and d, respectively; mole fraction of bacteriochlorophyll is 0.42. The collapse pressure is indicated by an arrow, ↗. The surface pressure corresponding to $1/A$ for a bacteriochlorophyll isotherm is indicated by the horizontal axis inserted in the figure. The absorbance is for light passing through the film 18 times. The subphase is the same as in Fig. 3.

films do not show the characteristic long wavelength band at 896 nm found in compressed monolayers of bacteriochlorophyll.

Absorption spectra of bacteriopheophytin monolayers

A film of bacteriopheophytin is found to have absorption maxima at 530, 762 and 846 nm. In Fig. 6 is shown the spectra at π equal to 2, 6, and 10 dynes/cm. At π = 10 dynes/cm, films of bacteriopheophytin collapse.

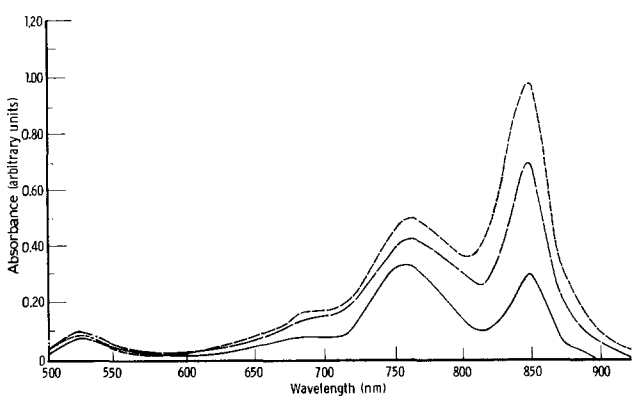

Fig. 6. Absorption spectra of a film of bacteriopheophytin, at surface pressure of 2 (———), 6 (– – –) and 10 (– – – – –) dynes /cm. The absorbance is for light passing through a film 18 times. The subphase is the same as in Fig. 3.

Deconvolution of absorption spectra of bacteriochlorophyll monolayers

Absorption spectra may be deconvoluted by fitting Gaussian and mixed Gaussian and Lorentian curves to the spectra[12]. In Fig. 7 is shown the result of one curve fitting carried out by French on an absorption spectrum of a compressed film of bacteriochlorophyll. This analysis showed that the spectra of bacteriochlorophyll

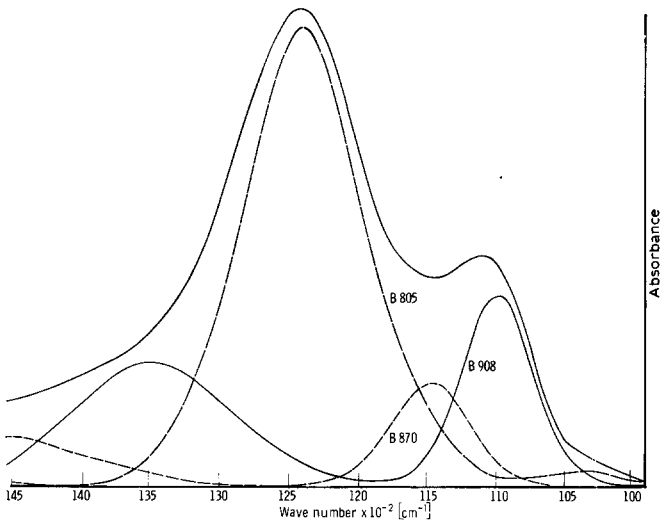

Fig. 7. Deconvolution of the absorption spectrum of the bacteriochlorophyll monolayer shown in Fig. 4 at π = 26.5 dynes/cm (courtesy of C.S. French). Absorbance is plotted as a function of wave number.

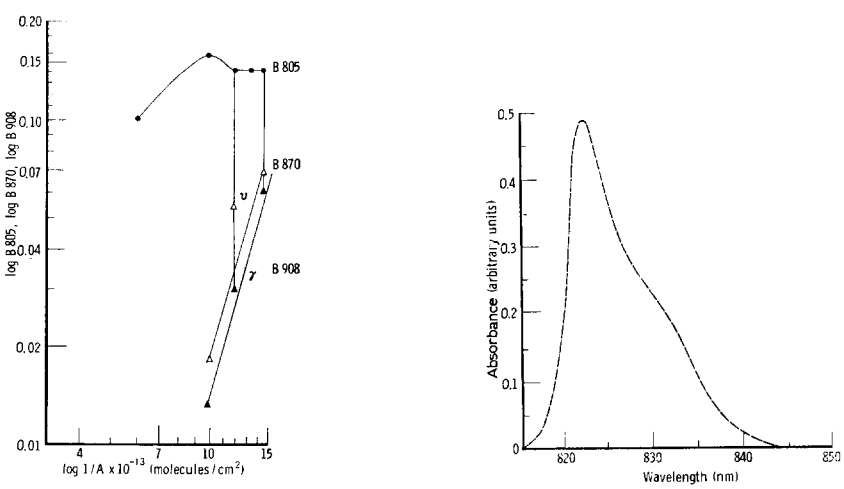

Fig. 8. Log–log plot of *B* 805, *B* 870 and *B* 908 as a function of surface concentration $1/A$. Absorbances are obtained from deconvolution of the absorption spectra shown in Fig. 4. The absorbance is for light passing through a film 18 times. The subphase is the same as in Fig. 3.

Fig. 9. Difference in absorption spectra of a bacteriochlorophyll monolayer at two different surface concentrations, *i.e.* $1/A$ is equal to $6 \cdot 10^{13}$ and $8 \cdot 10^{13}$ molecules/cm^2. The subphase is the same as in Fig. 3.

film (Fig. 4) can be deconvoluted into three absorption maxima in the 800–910-nm region. These maxima are designated *B* 805, *B* 870, and *B* 908 where the numerics indicate the peak position of the red absorption maximum. In Fig. 8 is shown a log–log plot of *B* 805, *B* 870, and *B* 908 as a function of $1/A$. The slopes of *B* 870 and *B* 908 are the same and the ration of *B* 908/*B* 870 is constant. This indicates that *B* 870 and *B* 908 are probably characteristic of a single absorbing species. At higher values of $1/A$ there is indication of still another absorbing species *B* 950

DISCUSSION

It is of interest to compare the properties of bacteriochlorophyll monolayers with those reported for chlorophyll monolayers (Bellamy *et al.*[13]). The A_0 for bacteriochlorophyll is 147 Å^2/molecule while for chlorophyll it is 122 Å^2/molecule[13]. Unlike the uniform slope of the chlorophyll isotherm the slope of the bacteriochlorophyll isotherm changes in value at around 18 dynes/cm. This change in slope of bacteriochlorophyll isotherms may reflect a reorientation of the pigment on the surface. A shallower slope indicates a decrease in intermolecular repulsive forces. A change in the orientation of the porphyrin planes with respect to the water surface or change in points of attachment may account for the increase in compressibility.

The surface dipole moments of bacteriochlorophyll and chlorophyll are very different. Bellamy *et al.*[13] obtained a single value of about 700 mD for chlorophyll. In the case of bacteriochlorophyll the $\mu_\perp$ appears to vary with $1/A$. The values of $\mu_\perp$ for bacteriochlorophyll are about 400 mD smaller than for chlorophyll. From Fig. 3b it was calculated that $\mu_\perp$ has values of 210 and 283 mD. In Fig. 3a where π is also plotted as a function of $1/A$ it can be seen that the transition region in the behavior of ΔV lies between 10 and 16 dynes/cm. That the above change in ΔV (or $\mu_\perp$) occurs at a lower value of $1/A$ than the change in slope of the surface isotherm (18 dynes/cm) might indicate that ΔV is a more sensitive parameter for detecting changes in molecular orientation. The smaller values of $\mu_\perp$ for bacteriochlorophyll compared to chlorophyll could, in part, be accounted for by bacteriochlorophyll lying flatter on the surface and having a larger A_0 than chlorophyll.

Bellamy *et al.*[13] have suggested that the chlorophyll porphyrin rings are anchored to the water surface by the ester linkages on Rings IV and V. It appears that Ring I of bacteriochlorophyll might be closer to the aqueous surface than is the case for chlorophyll's Ring I. This suggestion stems, in part, from the fact that the measured potential for water, V_{H_2O}, does not agree with V_w, the surface potential of water under the bacteriochlorophyll film. Similar suggestions of reorientation of subphase molecules have been made for other surface active materials[14,15]. An orientation for bacteriochlorophyll which could account for the difference between V_w and V_{H_2O} is one where the acetyl group of Ring I as well as the ester linkages of the molecule serve as anchorage points for bacteriochlorophyll to the aqueous surface. The strong electronegative charge of oxygen in the acetyl group of Ring I might induce a reorientation of the water molecules at the interface. The fact that V_w is about +180 mV might indicate that the more electropositive hydrogens are closer to the surface than would be the case in the absence of a bacteriochlorophyll film (where V_{H_2O} is −30 mV).

This suggestion of an orientation for bacteriochlorophyll different from that

of chlorophyll is consistent with a suggestion made previously on the basis of the oxidative properties of bacteriochlorophyll monolayers[16]. Since addition of an oxidant to the subphase resulted in a dehydrogenation of Ring II (to form 2-desvinyl-2-acetyl chlorophyll *a*, oxidized) bacteriochlorophyll it was suggested that Ring II of bacteriochlorophyll lies close to the aqueous surface. The proximity of Ring II to the aqueous surface may result from an additional anchorage point for bacteriochlorophyll at Ring I.

Jacobs *et al.*[17] described the spectral transformations of chlorophyll from a solution to a crystalline phase. With methyl bacteriochlorophyllide they observed a progressive shift of the red band from 770 to about 850 nm. The band with absorption maximum at 850 nm had a long wavelength shoulder extending to nearly 950 nm. They calculated the interaction energy $\Delta E/hc$ for the formation of this spectral shift to be 1800 cm^{-1}. They interpreted their observed spectral shifts as resulting from interaction between molecules in a monolayer.

Bacteriochlorophyll dissolved in benzene has a red peak at 780 nm while in an expanded monolayer a maximum at 787 nm is observed. This red shift is a result of solvent and pigment interaction with the aqueous subphase. Upon compression of bacteriochlorophyll monolayers to 18 dynes/cm an additional red shift from 787 to 794 nm (and band broadening) results from weak interaction between pigment molecules. Interaction energies, $\Delta E/hc$ are qual to $(1/\lambda_1 - 1/\lambda_2)$, where λ_1 is the red absorption maximum in a gaseous state, λ_2 is the red absorption maximum in a monolayer. For bacteriochlorophyll at low compression $\lambda_2 = 794$ nm; according to Rabinowitch[18] $\lambda_1 = 740$ nm. In this case $\Delta E/hc$ is weak only about 10 cm^{-1}. This value for $\Delta E/hc$ is close to that obtained for chlorophyll monolayers compressed to 15 dynes/cm[13]. The spectroscopic properties of compressed chlorophyll monolayers were interpreted as resulting from weak chromophore interaction[19], which are not sigificant enough to modify the pigment's singlet states.

At $\pi > 18$ dynes/cm, the absorbance of A 592 and A 794 decreases even though $1/A$ continues to increase. This complex behavior of A 592 and A 794 is not fully explained simply by assuming that the absorption dipoles change orientation so that they are more parallel to the direction of the incident light. The absorption transition dipoles for A 592 and A 794 are perpendicular to each other and both are in the porphyrin plane[20]. Depending upon the points of attachment of bacteriochlorophyll to the aqueous surface the projections of the absorption dipoles normal to the incident light could decrease as the porphyrin plane is compressed into a vertical orientation. Furthermore, it is to be expected that the increasing intermolecular interaction upon compression could modify the intensity of the absorption transitions.

At $\pi > 18$ dynes/cm the spectra are characteristic of a strong type of pigment interaction. The interaction energy in compressed bacteriochlorophyll monolayers is 2500 cm^{-1}; this is greater than that observed in condensed phases of methyl bacteriochlorophyllide. Such strong interaction energy is characteristic of an exciton mechanism where the interaction between absorption dipoles is large enough to affect the energy levels of bacteriochlorophyll. The strong interaction in bacteriochlorophyll monolayer probably results form pigment aggregation. The intermolecular forces resulting in the spectral changes of bacteriochlorophyll are probably weak, since the spectral transformation are completely reversible, even if the film is compressed beyond π_c and then re-expanded.

The spectral behavior may result from weak long range dipole interactions at low values of π while in more compressed states stronger short range dipole interaction prevails. These weak and strong interactions may be associated with different types of pigment aggregates or orientations. Formation of aggregates could readily result in orientation changes of bacteriochlorophyll at the surface, as well as alterations of energy levels and absorption transition probabilities. Further data is required to develop a detailed model correlating spectral transformations with the changes in orientation and aggregation accompanying compression of bacteriochlorophyll monolayers.

The appearance of an absorption maximum at 846 nm in monolayers bacteriopheophytin of upon compression to only 2 dynes/cm indicates a stronger type of pigment interaction than that found in bacteriochlorophyll monolayers. Removal of Mg from the porphyrin nucleus apparently permits strong interaction even when $1/A$ is comparatively small.

The spectral transformations observed upon compression of oxidized bacteriochlorophyll monolayers are similar to those found with chlorophyll monolayers. These changes include a slight increase of the half-band width of the red band and a linear increase in absorbance of the red band with pigment concentration. The spectral changes indicate a weak type of pigment interaction (*i.e.* $\Delta E/hc < 10\ cm^{-1}$). Unlike the change in slope of the surface isotherm and potential observed with bacteriochlorophyll, uniform behavior of both parameters are obtained with oxidized bacteriochlorophyll.

The fact that in monolayers bacteriochlorophyll and its pheophytin show stronger pigment interaction than chlorophyll and oxidized seems to result from differences in chemical structure and possibly also pigment orientation. The removal of two hydrogens from bacteriochlorophylls tetrahydroporphyrin nucleus to form the dihydroporphyrin derivative, oxidized bacteriochlorophyll, eliminates the possibility of strong interaction. In oxidized bacteriochlorophyll a double bond substistitutes for the two hydrogens on Ring II of bacteriochlorophyll. Perhaps this double bond is important for shielding the dipoles of contiguous chromophores from one another so that only weak interactions result.

The absorption spectrum of a compressed film of bacteriochlorophyll is similar to the spectra of *Chromatium* and *Rhodopseudomonas palustris* in that the absorption band of *A* 896 is less than *A* 794. In some species of photosynthetic bacteria the major absorption band is around 880 nm (*Rhodospirillum rubrum*) or 850 nm (*Rhodopseudomonas spheroides*). The similarity of the absorption spectra of bacteriochlorophyll monolayer with *in vivo* spectra makes the bacteriochlorophyll monolayers attractive as a model for *in vivo* phenomena. The preceeding supports the finding that pigment–pigment interactions in a membrane account to a large extent for the spectral properties observed *in vivo*[21].

REFERENCES

1 Clayton, R. K. (1963) in *Bacterial Photosynthesis* (Gest, A., San Pietro, A. and Vernon, L. P. eds), pp. 495–500, Antioch Press, Yellow Springs, Ohio

2 Olson, J. M. and Stanton, E. K. (1966) in *The Chlorophylls* (Vernon, L. P. and Seeley, G. R., eds), pp. 381–382, Academic Press, New York, N.Y.

3 Clayton, R. K. (1965) *Molecular Physics in Photosynthesis*, p. 150, Blaisdell Publishing Co., New York, N.Y.
4 Phillipson, K. and Sauer, K. (1972) *Biochemistry* 11, 1880–1885
5 Oelze, N. and Drews, G. (1972) *Biochim. Biophys. Acta* 265, 209–241
6 Reinach, P. (1972) Ph. D. Thesis, New York University, New York, N.Y.
7 Aghion, J., Broyde, S. and Brody, S. S. (1969) *Biochemistry* 8, 3120–3126
8 Brody, S. S. (1971) *Z. Naturforsch.* 26b, 134–139
9 Brody, S. S. (1971) *Z. Naturforsch.* 26b, 922–929
10 Eimhjellen, K. E., Aasmundrud, O. and Jensen, A. (1963) *Biochem. Biophys. Res. Commun.* 10, 232–236
11 Smith, J. R. L. and Calvin, M. (1966) *J. Am. Chem. Soc.* 88, 4500–4506
12 Tunnicliff, D. D. (1970) *Shell Development*, Oakland, Calif. and French, C. S., Stanford University, Palo Alto, Calif., private communication
13 Bellamy, W. D., Gaines, G. L. and Tweet, A. G. (1963) *J. Chem. Phys.* 39, 2528–2538
14 Gaines, G. L. (1966) *Insoluble Monolayers at Liquid–Gas Interfaces*, pp. 191–192, J. Wiley–Interscience, New York, N.Y.
15 Ghosh, S. and Bull, H. B. (1963) *Biochemistry* 2, 411–415
16 Reinach, P. and Brody, S. S. (1972) *Biochemistry* 11, 92–96
17 Jacobs, E. E., Holt, A. S., Kromhout, R. and Rabinowitch, E. (1957) *Arch. Biochem. Biophys.* 72, 495–511
18 Rabinowitch, E. (1956) *Photosynthesis and Related Processes*, p. 1823, Interscience, New York, N.Y.
19 Gaines, G. L., Tweet, A. G. and Bellamy, W. D. (1965) *J. Chem. Phys.* 42, 2193–2199
20 Goedheer, J. C. (1957) Ph. D. Thesis, Univ. Utrecht, Utrecht
21 Olson, J. M. and Stanton, E. K. (1966) in *The Chlorophylls* (Vernon, L. P. and Seeley, G. R., eds), p. 387, Academic Press, New York, N.Y.