

Interactions of Photosynthetic Pigments in Monolayers at a Water-Air Interface

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Summary. The compression of mixtures of photosynthetic pigments and phosphatidylcholine in monolayers at a water-air interface shows that (i) the phosphatidylcholine disperses the isolated pigment molecules; (ii) chlorophylls *a* and *b*, chlorophyll *a* and β -carotene, and chlorophyll *b* and β -carotene form associations which cannot be undone by the phospholipids; and (iii) when the two chlorophylls and β -carotene are mixed all together with or without phosphatidylcholine, the three types of associations are ideally mixed and they can be dispersed by phosphatidylcholine.

Trosper and Sauer (1968) have shown that galactosyldiglycerides and sulfolipids are ideal solvents for chlorophyll *a* at a water-air interface. Phytol seems to act similarly (Tweet, Bellamy & Gaines, 1964; Tweet, Gaines & Bellamy, 1964). Since such a research is always directed at understanding the structural and the functional properties of photosynthetic membranes, and since the latter contain mixtures of chlorophyll *a*, chlorophyll *b* and carotenoids, it appears necessary to test the possible interactions of photosynthetic pigments *in vitro*. Because the pigments are probably oriented *in vivo* (Breton & Roux, 1971), the *in vitro* set-ups should provide some orientation of the pigment molecules.

At widely used set-up, in this field, is the two-dimensional monolayer of pigments at water-air interfaces. Colmano (1962), aiming to mimic the absorption spectra of *in vivo* chloroplasts, has studied the absorption spectrum of monolayers containing mixtures of photosynthetic pigments. Aghion, Broyde and Brody (1969) have determined that the photochemistry of chlorophyll *a* monolayers proceeds with quantum yields ten times as high as chlorophyll *a* solutions. Many other examples of a more or less successful usage of photosynthetic pigment-monolayers can be found, for instance, in Gaines (1966) and in Ke (1966).

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The present paper describes pressure-area curves of two-dimensional systems at water-air interfaces. The systems used are the 2-by-2, 3-by-3 and 4-by-4 combinations of chlorophyll *a*, chlorophyll *b*, β -carotène and phosphatidylcholine (lecithin).

Materials and Methods

The phosphatidylcholine (type III, bovine liver), the β -carotene and the chlorophylls were purchased from Sigma. The purity of the chlorophylls was ascertained by visible absorption spectra, by thin-layer chromatography; in addition, their extrapolated area per molecule at zero pressure matched those published (*see* Tweet *et al.*, 1964). The phytol (also purchased from Sigma) was distilled three times under a 1.5 mm Hg pressure at 145 to 150 C. The second fraction (refractive index: 1.4610) was used. All those products were spread from ether solutions (ether from B.D.H. Analar). The water was distilled 3 times, the last two times in a quartz still. When mixtures of solutes were spread, the order of their mixing in solution was shown to have no importance in the experimental results.

The Langmuir trough was described previously (Leblanc *et al.*, 1974). The float system directly yields the surface pressure π , measured by the torsion of the wire to which it is tied (0.28 dyne/cm per degree of torsion). Knowing the number of solute molecules spread from the ether on the surface and the total area they cover, we can calculate the area σ , A², allotted to each molecule. Usually, 10^{15} chlorophyll molecules were spread from ether at the interface, over an area of 300 cm². The subphase was an aqueous phosphate buffer (4×10^{-3} M, pH=8.0). All the experiments were done at $20 \text{ }^\circ\text{C} \pm 1 \text{ }^\circ\text{C}$ in dim green light.

All the results given here are the arithmetic means of three to five repeats. The mixtures of solutes are described in terms of mole numbers of each component.

Results

All the solute mixtures used in this work form so-called liquid monolayers (Gaines, 1966). The apparent area per molecule of monolayer component, usually obtained by extrapolating to $\pi=0$ the steepest part of the isotherm, has therefore no meaning. It would not be very significant, either, to give the σ at an arbitrarily chosen π . For those two reasons it has been decided to show some typical isotherm compression curves. The other curves can be reconstructed from Table 1.

The compression of the films was pushed to the collapse of the monolayers, so that re-expansion was not feasible. Since compression and expansion cycles are frequently used to check for leaks of the monolayer under float or barrier, it was considered that the possibility of such leaks was eliminated by (i) reproducing the isotherm compression

Table 1. All the π - σ curves of this work bear a family resemblance. This is why only 5 sets of graphs are given in figures^a

Mixture	Molar composition	σ , Å ² /molecule for π =(dyne/cm)				π of collapse, dyne/cm
		2	6	12	20	
Phosphatidylcholine	—	119	104	88	75	37
Chlorophyll <i>a</i> / β -carotene	1:1	176	147	116	80	21
Chlorophyll <i>b</i> / β -carotene	1:1	176	146	111	76	21
Chlorophyll <i>a</i> /chlorophyll <i>b</i>	1:1	234	200	168	133	21
Chlorophyll <i>a</i> /chlorophyll <i>b</i> / phosphatidylcholine	1:1:1	203	177	147	122	21
	1:1:8	127	110	92	86	33
	1:1:33	—	101	95	82	35
Chlorophyll <i>a</i> /chlorophyll <i>b</i> /phytol	1:1:1	202	173	140	—	20
	1:1:8	101	87	76	64	21.5
	1:1:33	67	57	50	46	> 25
Chlorophyll <i>b</i> / β -carotene/ phosphatidylcholine	1:1:1	168	143	113	83	29
Chlorophyll <i>a</i> /chlorophyll <i>b</i> / phosphatidylcholine	1:1:1	216	180	147	98	20
Chlorophyll <i>a</i> /chlorophyll <i>b</i> / β -carotene/phosphatidylcholine	2:2:1:16	133	116	100	87	31

The experimental error on the σ values is of the order of 5%.

^a The table contains the data necessary to construct the other curves.

curves several times and (ii) sometimes stopping the compression at a given π during up to 5 min and verifying that π remained stable for a fixed value of σ .

Finally, when phytol is used in the place of phosphatidylcholine, it leads to the same conclusions as those reached hereafter.

I. Binary Mixtures

a) *Phosphatidylcholine and Pigments*. The π - σ curves of all three components are determined. Within experimental error, they match those published by Gaines (1966), by De Costa *et al.* (1972) and by Leblanc and Orger (1972). The curve for phosphatidylcholine can be drawn from Table 1.

Then, π - σ curves are run for the following mixtures: Chlorophyll *a*/Phosphatidylcholine 1:4; Chlorophyll *b*/Phosphatidylcholine 1:4; β -ca-

rotene/phosphatidylcholine 1:4 and 1:16. In all cases, at every value of π , the value of σ experimentally determined coincides with that calculated by

$$\sigma = \sum_{\text{mixture}} \sigma_i \times N_i$$

where N_i is the mole fraction of component i and σ_i its area per molecule, when it is the only component spread on the interface. This match is considered to mean that the phosphatidylcholine forms ideal bidimensional solutions with the pigments, just as galactolipids and sulfolipids do with chlorophyll a (Trosper & Sauer, 1968).

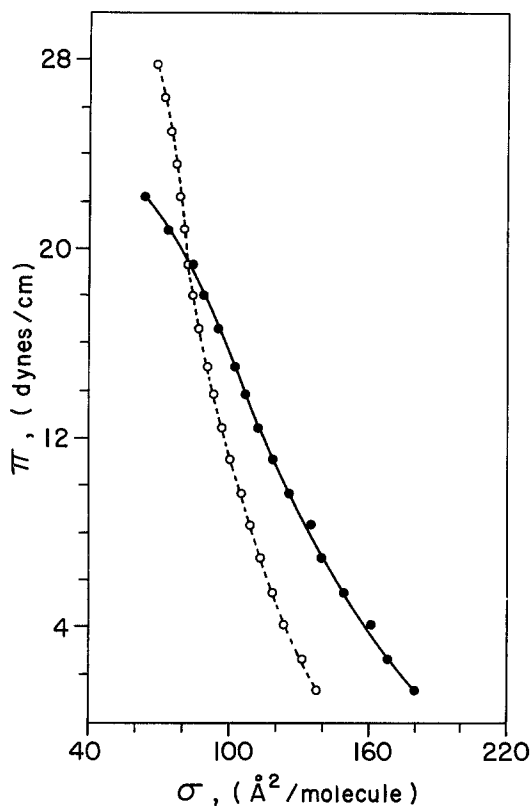


Fig. 1. Compression curve of a chlorophyll a and β -carotene monolayer on a water-air interface. The monolayer is spread from a 1:1 mixture dissolved in diethylether. The determination of σ takes into account the total number of molecules deposited in the water. Filled circles: experimental points. Open circles: theoretical curve calculated by $\sigma = \sum \sigma_i \times N_i$

b) *Chlorophylls and β -Carotene*. The experimental compression curves of chlorophyll *a*/ β -carotene 1:1 (mole numbers) and of chlorophyll *b*/ β -carotene 1:1 do not obey the ideal solution equation above. They are not as steep as could be predicted (see Figure 1, relative to chlorophyll *a*). Thus there is some interaction occurring between chlorophylls and β -carotene spread at the water-air interface.

c) *Chlorophylls a and b*. The same applies to a 1:1 mixture of the two chlorophylls (Fig. 2). They undoubtedly undergo some sort of an interaction in this two-dimensional state.

II. Ternary Mixtures

a) *Chlorophylls and Phosphatidylcholine*. This ternary mixture is assayed with 3 different molar compositions: chlorophyll *a*/chlorophyll *b*/phosphatidylcholine 1:1:1, 1:1:8, and 1:1:33. In all 3 cases the experimental curves fit the curves calculated considering that phosphatidylcholine disperses a molecular species consisting of chlorophyll *a*/chlorophyll

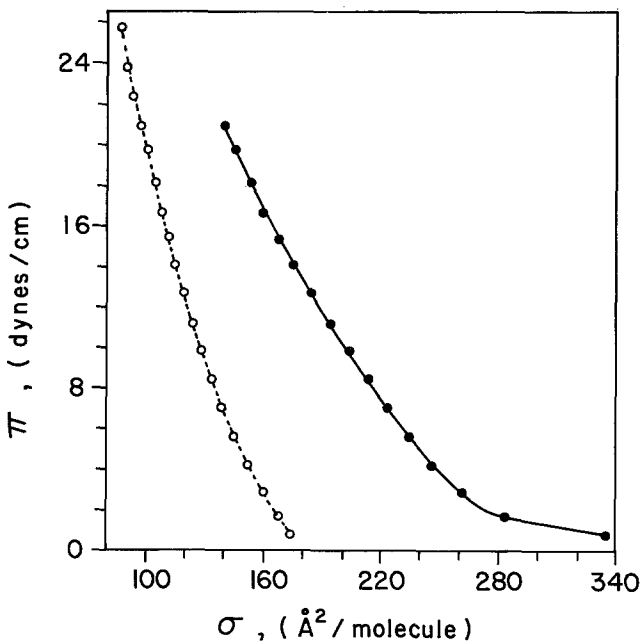


Fig. 2. Compression curve of a chlorophyll *a* and chlorophyll *b* monolayer at a water-air interface. The monolayer is spread from a 1:1 mixture in diethylether. The determination of σ takes into account the total number of molecules deposited on the surface

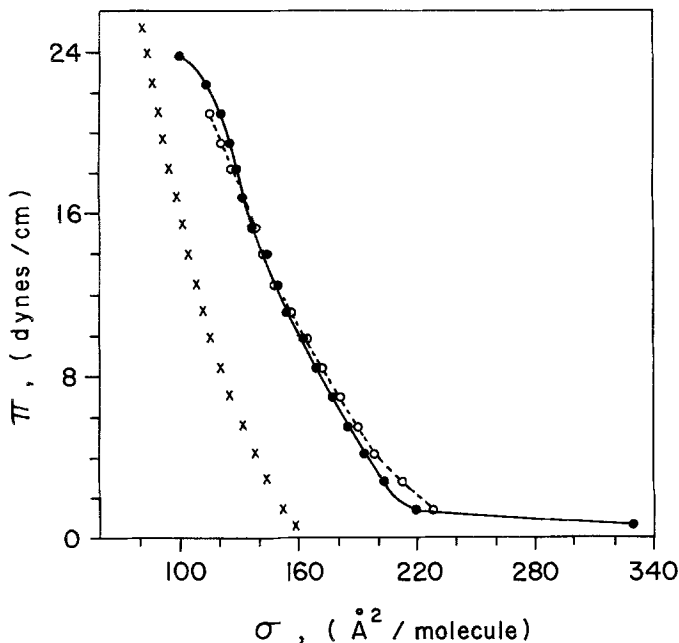


Fig. 3. Compression curve of chlorophyll *a*, chlorophyll *b*, and phosphatidylcholine monolayers at a water-air interface. The crosses are the theoretical points calculated by assuming a dispersion of the three components of the monolayer; the open circles are the theoretical points calculated assuming that phosphatidylcholine disperses the chlorophyll *a*, chlorophyll *b* "complex". The full circles are the experimental points: chlorophyll *a*/chlorophyll *b*/phosphatidylcholine 1:1:1

b 1:1. The curves do not at all fit the ones calculated, as if each of the 3 components were dispersed by the other two (Fig. 3).

b) *Chlorophyll*|*β-Carotene*|*Phosphatidylcholine* 1:1:1. The phosphatidylcholine disperses the "complex" of chlorophyll and *β*-carotene, whether the chlorophyll is *a* or *b*. Figure 4 shows the fit for chlorophyll *b*.

c) *Chlorophyll a*|*Chlorophyll b*|*β-carotene* 1:1:1. Two types of theoretical curves can be calculated here: one where the three components are ideally mixed and a second one where the three components are combined by pairs to form ideally mixed "complexes". The fit of the experimental curve is far better with the second type of graph than with the first.

III. Quaternary Mixture

Chlorophyll a|*Chlorophyll b*|*β-Carotene*|*Phosphatidylcholine* 2:2:1:16. As in section IIc, it appears that the three pigments are associated by

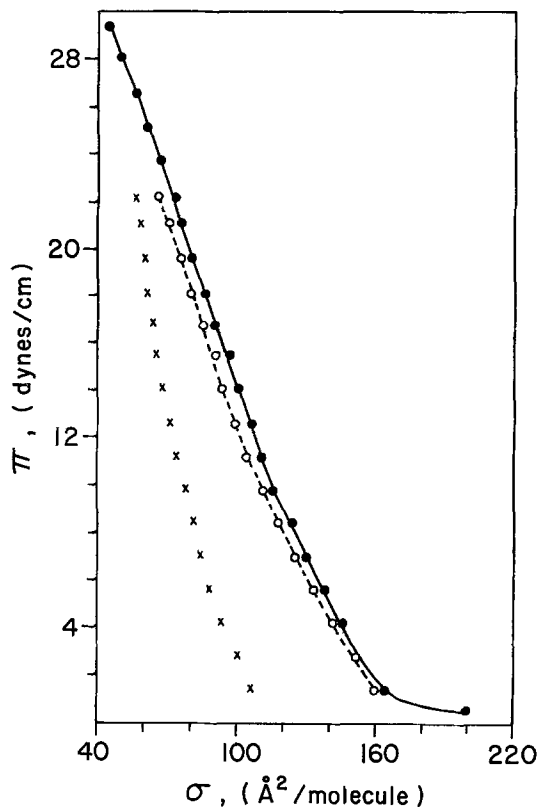


Fig. 4. Compression curve of a chlorophyll *b*/ β -carotene/phosphatidylcholine (1:1:1) monolayer at a water-air interface. Symbols are as in Fig. 3

pairs which are ideally dispersed by phosphatidylcholine (Fig. 5). Actually, it is not really possible to differentiate between the two following possibilities: (i) a chlorophyll *a*/chlorophyll *b* "complex" is ideally mixed with β -carotene and with phosphatidylcholine, or (ii) three pairs of pigments are ideally mixed with one another and with the lecithin. In view of the previous result, the latter possibility seems reasonable.

Discussion

It is shown that chlorophylls *a* and *b*, and each of them with β -carotene, interact in monomolecular layers at water-air interfaces. The results of the interactions are ideally dispersed by phosphatidylcholine and/or by phytol. In all cases, the interactions are displayed by values

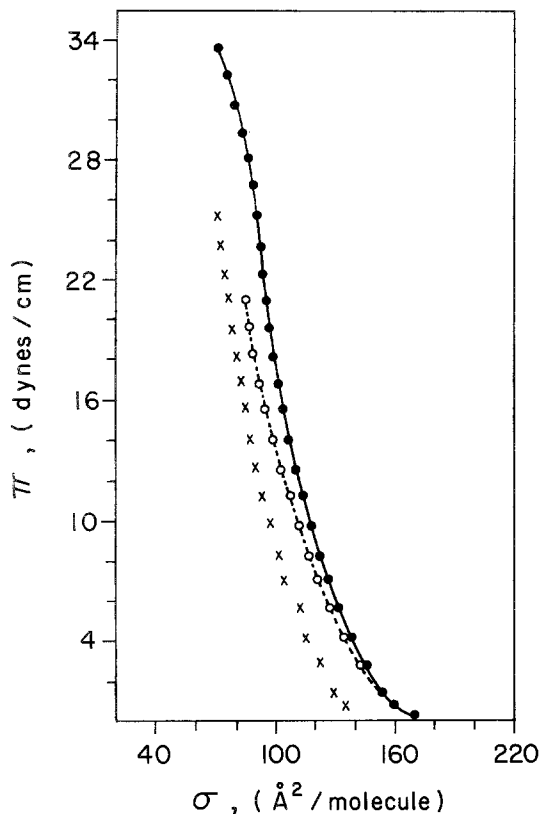


Fig. 5. Compression curve of a chlorophyll *a*/chlorophyll *b*/β-carotene/phosphatidylcholine (2:2:1:16) monolayer at a water-air interface. The crosses are theoretical points calculated, assuming that all 4 components are ideally mixed on the surface of the water. The open circles are theoretical points calculated by assuming that phosphatidylcholine dissolves ideally the three binary complexes formed by the pigments. The full circles are experimental points

of σ bigger than those which could theoretically have been predicted. In other words, it appears that the interacting molecules take more place at the interface. What kind of interaction could it be?

At $\pi = 15$ dyne/cm, the plane of the porphyrin ring of the chlorophylls is tilted $60^\circ \pm 2^\circ$ above the water. The ketone and carbonyl C=O (rings III, IV and V) are in the water. According to Reich *et al.* (1976), this is true of chlorophyll *a*, but for chlorophyll *b*, the aldehyde group of ring II is in contact with the water and the C=O groups of rings III, IV and V are in the air. In any of those cases, it seems difficult to imagine any other interaction than between the central Mg atom of chlorophyll

a and either the $\begin{array}{c} \text{H} \\ \diagdown \\ \text{C} \\ \diagup \\ \text{O} \end{array}$ or the C=O groups of chlorophyll *b*, pulling

both molecules down together. If this is true, there remains to explain why chlorophyll *b* molecules do not react together: one way to do so is to suggest that the aldehyde groups of two adjacent chlorophyll *b* molecules form preferential H-bonds together. It is also possible that the aldehyde group of ring II gives the π -electron clouds a shape that decreases the accessibility of the Mg atom.

The interaction between the cycle π -system of chlorophylls and the linear one of β -carotene is much more surprising and seems hardly figurable unless new experiments are done, for example with molecules having undergone deletions and/or substitutions.

The above results show that in layers of phosphatidylcholine, chlorophylls and β -carotene, associations of chlorophylls *a* and *b* and of chlorophyll with β -carotene are formed even at low surface pressures. This will necessarily have to be taken into consideration in any interpretation of the structure or function of photosynthetic membranes where chlorophylls, carotenoids, and amphipathic lipids co-exist.

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