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AGGREGATION OF CHLOROPHYLLS IN MONOLAYERS.
PART IV. THE REORGANISATION OF CHLOROPHYLL *a* IN MULTILAYER ARRAY

Camille CHAPADOS, Denis GERMAIN and Roger M. LEBLANC

Groupe de Recherche en Biophysique, Département de Chimie-Biologie, Université du Québec à Trois-Rivières, Trois-Rivières, Quebec, Canada, G9A 5H7

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The nature of the interaction between the chlorophyll *a* molecules in multilayer arrays obtained by the Langmuir-Blodgett technique is examined by electronic and infrared spectroscopies. Following the deposition of the multilayers, we observed a blue shift with time in the electronic spectra. This effect is monitored by infrared spectroscopy. The intensity of the coordinated ketone band is decreased while the intensity of the free ketone band is increased. These modifications are explained by the reorganization of the chlorophyll *a* molecules from an organized to a less organized one. The influence of H_2O, D_2O and SO_2 vapors on the chlorophyll *a* multilayers give some informations on the role of water molecules in the aggregation of chlorophyll *a* in this ordered system. From these observations, a model is proposed for the multilayer arrangement implying two molecules of water per molecule of chlorophyll *a*.

1. Introduction

The photosynthetic process is very dependent on the aggregation state of the chlorophyll *a* (chl *a*) molecules. For studying this aggregation state, several models have been used, for example, the mulls obtained from a dispersion of chl *a* in paraffin oil [1,2]; the solid film made by evaporation of the solvent from a solution of chl *a* [3]; the chl *a* solutions at room temperature and at low temperatures [4]; the chl *a* in a microemulsion [5] or in a liquid crystal [6]; the monolayer of chl *a* [7,8] and the multilayer of chl *a* [7,9a,9b]; the vesicles of chl *a* [10]. Depending on the preparation technique used, the organisation and therefore the aggregation state of the chl *a* molecules will be different.

In the build-up of a multilayer of chl *a*, a blue shift of about 2 nm in the band's maxima has been observed in the electronic spectrum [11]. This shift is accompanied by a decrease in the long-wavelength tail of the red band. In samples of monolayer or multilayer of chl *a* at a solid/air interface obtained by the Langmuir-Blodgett technique, the molecules of chlorophyll are initially in contact with water in the float trough. The question arises as to whether some water remains in the monomolecular arrays and, if so, what is the aggregation state of the chlorophyll in these systems.

In this paper, we examine the reorganisation of the chl *a* molecules in a multilayer arrangement using electronic and infrared spectroscopies. We also report on the influence of SO_2, H_2O and D_2O vapors on the multilayer array in order to pinpoint the aggregation state of the multilayer of chl *a*.

2. Experimental

The extraction and purification of chl *a* was done from spinach leaves by the standard method of Strain and Svec [12].

The materials, the preparation of slides, the deposition of monolayers and the Langmuir film balance apparatus have previously been described in detail [11].

2.1. Electronic spectra

Forty layers of chl *a* were deposited on a quartz slide at a surface pressure of 20 mN m^{-1}, and inserted in a vacuum-tight brass cell having quartz windows.

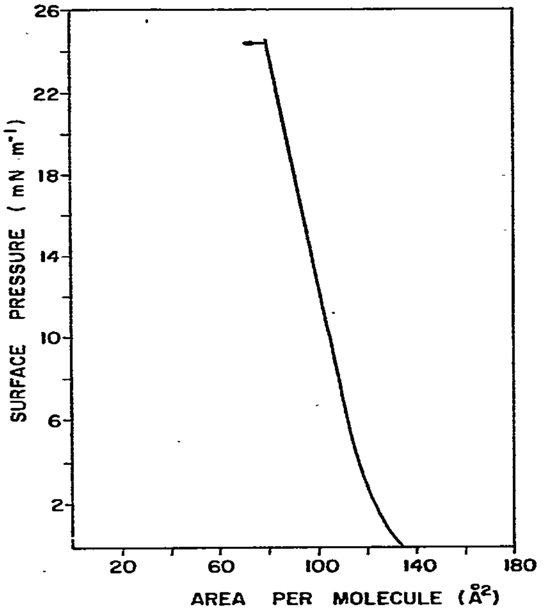

Fig. 1. Surface pressure-area curve for chlorophyll *a*.

The deposition of the chl *a* layers onto slide was monitored by the Langmuir film balance.

The absorption measurements were carried out on a Unicam SP 1800 spectrophotometer. The spectra were measured after the deposition of the sample, and after it was put in a vacuum at 10^{-3} Torr for two days.

2.2. *Infrared spectra*

Forty layers of chl *a* were deposited at a surface pressure of 20 mN m^{-1} on each side of a germanium multiple internal reflexion (MIR) plate using the Langmuir film balance to monitor the deposition.

Immediately after the deposition, the sample was placed in the sample compartment of the ir spectrophotometer where successive scans were made.

The infrared spectra were recorded on a Perkin—Elmer model 180 spectrophotometer using a FMIR accessory to increase the intensity of the signal of the absorbing bands.

For the study of the influence of SO_2 vapors on the multilayer of chl *a*, the ir spectrum of the sample was taken after the multilayer had reached equilibrium.

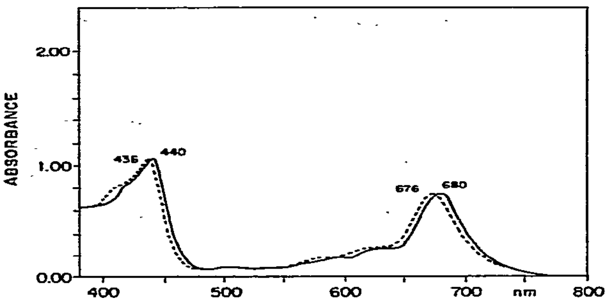

Fig. 2. Absorption spectra of chlorophyll *a* multilayer deposited on a quartz slide: initial spectrum (——); after a vacuum (10^{-3} Torr) for two days (- - - -).

The sample was placed in a dessicator for ten minutes where a vacuum of 10^{-3} Torr was obtained. Then dry SO_2 gas (~300 Torr) was introduced and reacted with the chl *a*. After a certain period of time, the ir spectrum was obtained. The sample was then washed with hot water to get rid of any inorganic water soluble substances and the ir spectrum was taken. To study these inorganic water soluble substances, a new sample obtained from the reaction of chl *a* with SO_2 vapors was washed with diethyl ether to get rid of any organic substances, and its ir spectrum was taken.

For studying the influence of a D_2O atmosphere on a freshly prepared multilayer of chl *a*, a special closed cell fitting the FMIR accessory and capable of accepting the MIR plates has been constructed. The cell can be used either as a FMIR accessory or as a transmission gas cell and was equipped with BaF_2 windows. More details on the cell will be given elsewhere [13].

3. Results and discussion

3.1. *Surface pressure-area curve*

The surface pressure curve was determined to characterize the purity of the chlorophyll *a* sample and to monitor its deposition onto slide. A typical curve which is the mean of five determinations is shown in fig. 1, this curve following very closely that of Gaines [14]. From the pressure-area isotherm, we know that the sample is pure enough for monolayer work. Another criteria of purity which was also used is the absorbance

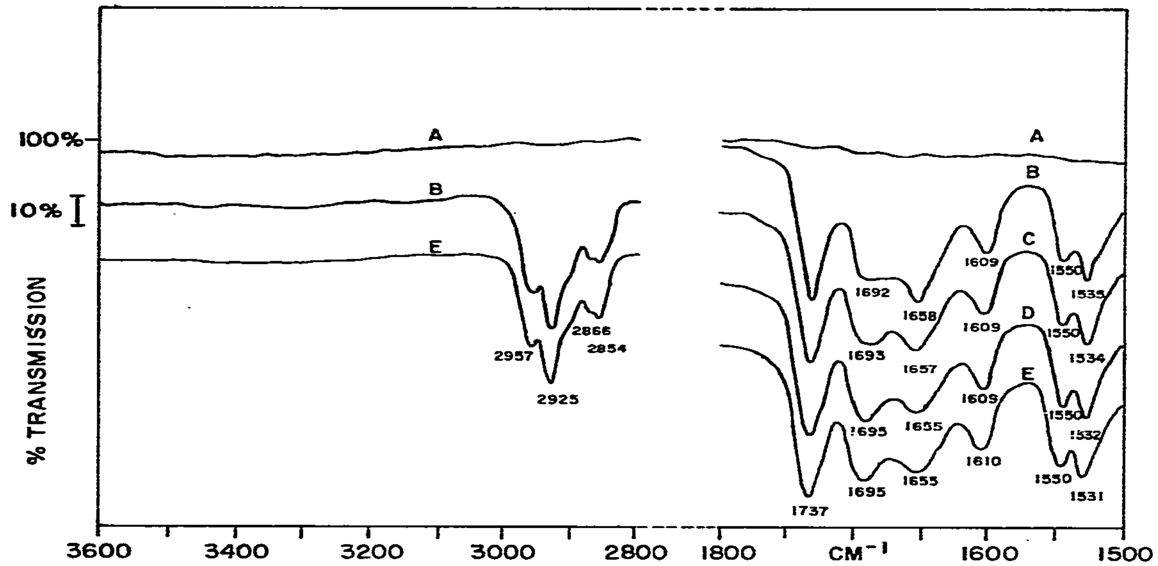

Fig. 3. Time effect on the infrared multiple internal reflexion (MIR) spectra of chlorophyll *a* multilayer deposited on a germanium plate. A: background spectrum; B: multilayer, 15 min after the end of deposition; C: 50 min after; D: 120 min after; E: 360 min after.

ratio at 428.4 nm to 660.8 nm for chl *a* in diethyl ether. The value of 1.27 obtained by us is comparable to the ratio of 1.31 obtained by Seely and Jensen [15]. The chromatogram from thin layer chromatography showed one minor spot besides that of chl *a*. The secondary spot is probably due to chl *a'*.

3.2. Electronic spectra

The electronic spectrum of forty layers of chl *a* on both side of a quartz slide, obtained after deposition, is presented in fig. 2. The spectrum is similar to the one obtained by de Costa et al. [11]. We observed, after aging the sample for two days, a blue shift of the band maxima of 4 nm. Of particular interest is the fact that there is no band at 740 nm a typical position for the hydrates of chl *a*. These modifications are attributed to a reorganisation of the chlorophyll molecules with time. The nature of this reorganisation will be elucidated by the ir spectra.

3.3. Infrared spectra

The spectra of 40 layers of chl *a* on a germanium MIR plate is presented in fig. 3. The positions and assignments of the bands are tabulated in table 1. The spectrum A is the background spectrum of the MIR plate. The spectrum B is taken 15 min after deposition: spectrum C, D and E are taken at 50, 120 and 360 min, respectively. Putting the sample in vacuum does not modify the bands any further.

The background spectrum, fig. 3A, is quite straight and free of any spurious bands. In the 3600–2800 cm^{-1} region, this background is much better than the one used previously [7]. The results in the 3400 cm^{-1} region are therefore much more reliable. In this region, for the multilayer of chl *a* (see fig. 3B), there is no absorption that can be attributed to the OH vibration of some water molecules: also there is no modification of the results with time (see fig. 3E). In the 2900 cm^{-1} region, the C–H vibrations of chl *a* show no significant modification three hours after deposition.

Table 1
Frequencies (cm^{-1}) of a multilayer of chlorophyll *a*

Spectrum	Description	Ester C=O	Free Keto C=O	Coordinate Keto C=O- - -Mg	Skeletal C=C C=N	Skeletal	
a)	Anhydrous chl *a* solid film	1736	1695	1655	1610	1550	1530
B	Multilayer of chl *a* on a germanium plate, 15 min after deposition	1737	1692	1658	1609	1550	1535
C	50 min after deposition	1737	1693	1657	1609	1550	1534
D	120 min after deposition	1737	1695	1655	1609	1550	1532
E	360 min after deposition	1737	1695	1655	1610	1550	1531
b)	Multilayer of chl *a* on an AgBr plate	1737	1692	1658	1609	1550	1535

a) Ballschmitter and Katz [3].
b) Leblanc and Chapados [9b].

In the carbonyl region, 1800–1500 cm^{-1}, the esters C=O band at 1737 cm^{-1}, and the skeletal C=C, C=N band at 1609 cm^{-1} show no significant modification with time except for a slight broadening. The skeletal doublet at 1550–1535 cm^{-1} suffers a little modification; the 1550 cm^{-1} branch is not modified while the 1535 cm^{-1} branch is broadened and displaced to 1531 cm^{-1}.

Most of the changes arise in the carbonyl ketone bands. Fifteen minutes after deposition, the free ketone C=O band appears as a shoulder situated at 1692 cm^{-1}, while the coordinated ketone C=O band appears as a neat band at 1658 cm^{-1}. With time, the free ketone band increases in intensity, becomes sharper, and is slightly displaced to 1695 cm^{-1}, while the coordinated ketone band decreases in intensity, becomes less sharp, and is slightly displaced to 1655 cm^{-1}. If we could extrapolate to time zero, the free ketone band would be even less intense and the coordinated ketone band would be more intense. These matters will be considered later. These modifications can be explained if we assume that at time zero most of the ketone C=O of the chl *a* molecules are coordinated to the Mg of adjacent chl *a* molecules in a polymeric organisation. With time, some of these coordinated bands are ruptured, a situation caused by the falling of the build-up stack of chl *a* molecules, a new stable organisation arising which is more stable than the previous one. The intensity pattern of this stable organisation (fig. 3E) is similar to the one which was used for the dioxane effect, fig. 4B of ref. [9b], but the positions of the bands are slightly displaced (table 1), a circumstance caused probably by the plates being different in the two cases. In the present work, we used germanium plates while AgBr plates were used in ref. [9b]. Different plates may cause some slight differences in the build-up pattern which will be accentuated when the reorganisation of the molecules takes place. Even when we used the same plate, the build-up pattern is always slightly different. So the reorganisation will also be slightly different. Since the carbonyl ketone bands have approximately the same intensities, the new stable organisation involves mostly dimers.

What is the aggregation state of the chl *a* multilayer, and what causes the collapse of the build-up of the multilayer? Ballschmitter and Katz [3] have proposed three models for a film of chl *a* interacting in various degree with water: the chl *a* hydrate (chl a_2–H_2O), the bi-chl *a* monohydrate (chl a_2–H_2O) and the amorphous chl *a*. When we compared the spectra of the multilayer with the spectra of these possibilities, we should obtained a clue of the aggregation pattern of the multilayer in different states of aggregation, and the reasons for the collapse. First, in the electronic spectra, the red band of the chl *a* hydrate is situated at 738 nm [3], and the one of a freshly prepared multilayer is at 680 nm and is displaced to 676 nm upon aging (fig. 2). In the ir spectra of the chl *a* hydrate, the aggregated C=O ketone band is situated at 1640 cm^{-1} [3]. For a fresh multilayer, this band is situated at 1658 cm^{-1} and for an aged one, it is sutiated at 1655 cm^{-1} (fig. 3). The chl *a* hydrate is therefore excluded as a model of the aggregation state of chl *a*, and

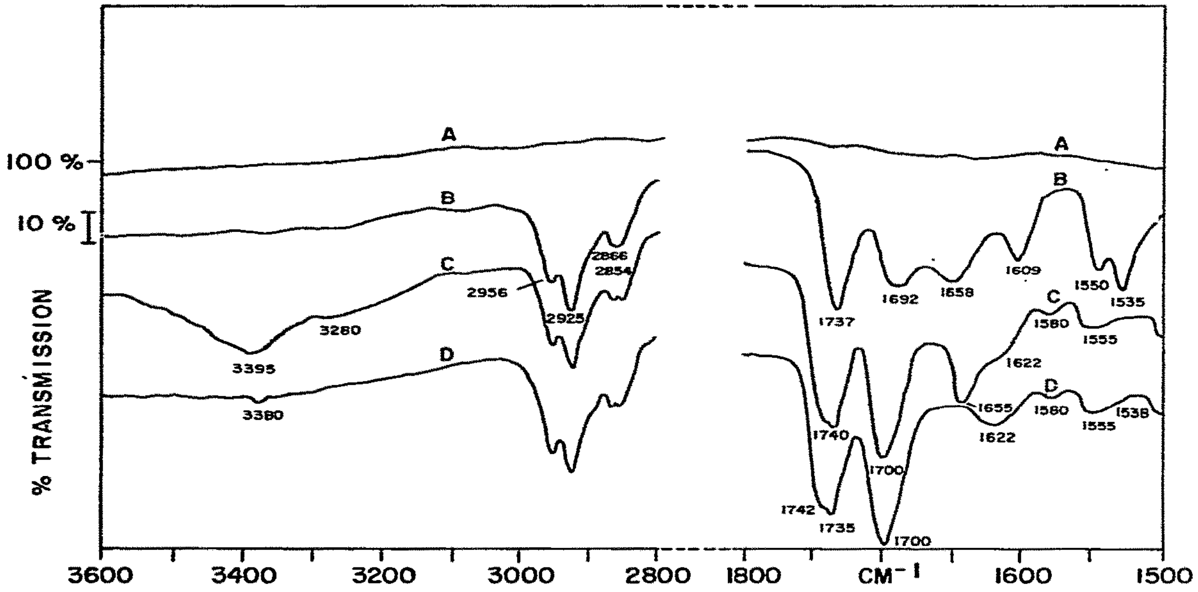

Fig. 4. Infrared spectra of the effect of SO_2 vapors on chlorophyll *a* multilayer deposited on germanium plate. A: background spectrum; B: multilayer, 4 hours after deposition; C: 36 hours in SO_2 vapors; D: sample washed with hot water.

this is true for both the freshly prepared multilayer as well as the aged one. Secondly, when we compared the ir spectra of the chl *a* multilayer (fig. 3), with the spectrum of the bi-chl *a* monohydrate (fig. 2B of ref. [3]), we noticed that the intensity pattern and the position of the ketone bands of a fresh chl *a* multilayer (fig. 3B) are different than the ones of the bi-chl *a* monohydrate. However the position and the intensity pattern of bi-chl *a* monohydrate and the stable chl *a* multilayer organisation (fig. 3E), are comparable, except in the OH region at ~3300 cm^{-1}, where there is no band in the multilayer of chl *a*, while there is a small band in the monohydrate. The aggregation pattern of the bi-chl *a* monohydrate is therefore not compatible with the multilayer of chl *a*. Thirdly, we compared the electronic and the ir spectra of the multilayer of chl *a* with those of an anhydrous solid film. We noted that with a freshly prepared chl *a* multilayer, the position of the band's maxima is situated at 440 and 680 nm, and is displaced to 436 and 676 nm with aging. These latter positions are the same as those of the solid film. The positions of the ir bands are also the same in both cases, but the intensity pattern of the ketone carbonyl bands is different. In the multilayer, the bands are larger and the coordinated C=O- - -Mg band at 1655 cm^{-1} is less intense than in the dry film. From these facts, we conclude that the aggregation pattern is different in both cases. Since none of the three models are compatible with either a freshly prepared multilayer of chl *a* or an aged one, we wanted to determine with certainty the presence or absence of water in the multilayer.

To investigate the presence of water in the stable multilayer, we have performed an experiment involving SO_2 vapors interacting with the stable multilayer. If water is present, it would interact in the following way:

$$SO_2 + H_2O \rightleftharpoons H_2SO_3 \qquad (1)$$

$$H_2SO_3 + \text{Chl } a \rightleftharpoons \text{Pheophytin } a + MgSO_3 . \qquad (2)$$

The following experiments were performed and monitored by infrared spectroscopy: after the chl *a* multilayer had stabilized, the ir spectrum was taken (fig. 4B). This spectrum is similar to the one given in fig. 3E. Then SO_2 vapors were put in contact with the multilayer for 36 hours (fig. 4C). The multilayer exhibited a strong band situated at 3395 cm^{-1} and a weaker

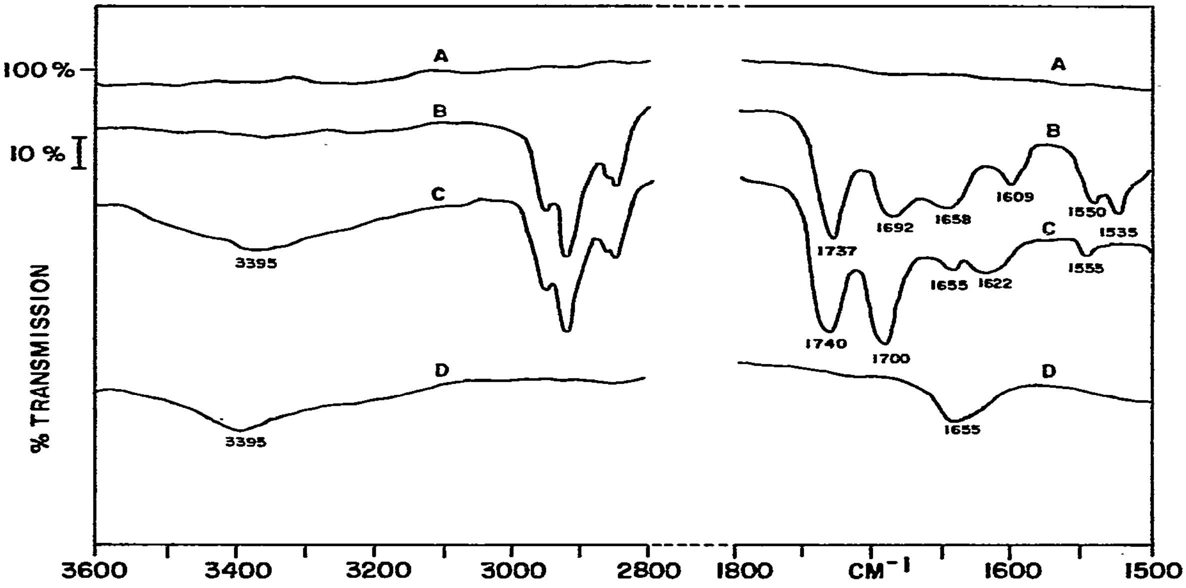

Fig. 5. Infrared spectra of the effect of SO_2 vapors on chlorophyll *a* multilayer deposited on a germanium plate. A: background spectrum; B: multilayer, 4 hours after deposition; C: 18 hours in SO_2 vapors; D: sample washed with diethyl ether.

one at 3280 cm^{-1}. These two bands can be attributed to water bands. In the C–H region, there is no modification. In the carbonyl region, the situation is more complex, and to clarify it the multilayer is washed with hot water to get rid of any water soluble substances that were formed. In the spectrum given in 4D, we notice that the large band at 3400 cm^{-1} is gone and a small weak band at 3380 cm^{-1} has been resolved. This can be attributed to N–H stretching vibrations. In the carbonyl region, the spectrum is much clearer and we notice that the C=O- - -Mg coordinated band is gone and the free ketone band is displaced to 1700 cm^{-1}. The C=O ester band is broadened, probably due to a splitting of the two C=O esters. When we compare this spectrum with the one given by Ballschmitter and Katz [3] for pheophytin *a*, we find that the two are compatible. An atomic absorption study of the water washings has revealed the presence of magnesium.

When we repeated this experiment (fig. 5) with SO_2 vapors in contact with a chl *a* multilayer, but washing the reaction products with diethyl ether instead of hot water to remove the organic substrate, we have obtained the spectrum of water (fig. 5D). Since these bands do not disappear in the dry compartment of the spectrometer, the water must be bound to an inorganic matrix. An electronic spectrum of the diethyl ether washing revealed the presence of pheophytin *a* (fig. 6). A TLC of the washings have revealed that the reaction product is mostly pheophytin *a*.

All these facts support the idea that the reaction of SO_2 with a multilayer of chl *a* produces pheophytin *a* with water bound to an inorganic matrix, confirming the reactions (1) and (2) proposed previously. This water must come from the multilayer even after the reorganisation following the deposition. Since the conversion by the SO_2 vapors of chl *a* to pheophytin *a* is almost complete, as seen by the TLC technique, it must be concluded that, after the molecules have reorganized in the multilayer, there is at least one molecule of water per molecule of chl *a*.

What then may cause the reorganisation of the multilayer immediately after it has been prepared? Would there be more than one water molecule bound to the chlorophyll molecule? To answer these questions, we have performed the following experiment: immediately after the deposition, the plate containing the multilayer of chl *a* is put in a closed vacuum-tight cell equipped with the appropriate windows and filled with

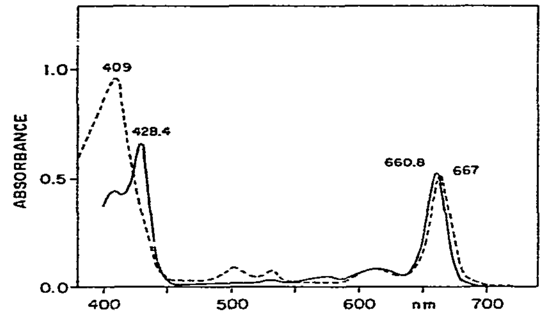

Fig. 6. Absorption spectra in diethyl ether of chlorophyll *a* and of the reaction products of SO_2 vapors on chlorophyll *a* multilayer: initial spectrum (——); spectrum of the reaction products (- - - -).

moist atmosphere in order to minimise the evaporation from the multilayer. For the ir spectra, the moist atmosphere is composed principally of D_2O. The addition of D_2O in the closed cell was done in order to minimise the rotational-vibration band of water in the carbonyl region and it has also permitted us to monitor the O–H and O–D exchange in the region of the O–H and O–D stretch vibration. The electronic spectra and the ir spectra are presented in figs. 7 and 8, respectively.

Immediately after deposition, the multilayer of chl *a* is put in its appropriate cell and the spectra taken. The results are presented in figs. 7A and 8A for the electronic and infrared regions, respectively. In the electronic region, the band maxima are situated at 437 and 678 nm, slightly lower than the maxima presented in fig. 2. This is due to the deposition ratio which was not as good in the former, as compared to the latter case. This indicates that the organisation of the multilayer is affected. For the same reason, the bands in the ir spectra (fig. 8A) are larger than the ones presented previously (fig. 3B). When we compare these two ir spectra, we notice that the intensity of the ester carbonyl band is almost the same in both cases, while the positions of the band maxima differ by 4 cm^{-1}. We can attribute these differences to some slight hydrogen bond of the carbonyl group to water molecules, when these molecules leave (fig. 8C), the band is displaced to higher values. The free ketone carbonyl band at 1696 cm^{-1} is almost absent while the coordinated C=O- - -Mg is most intense (fig. 8A).

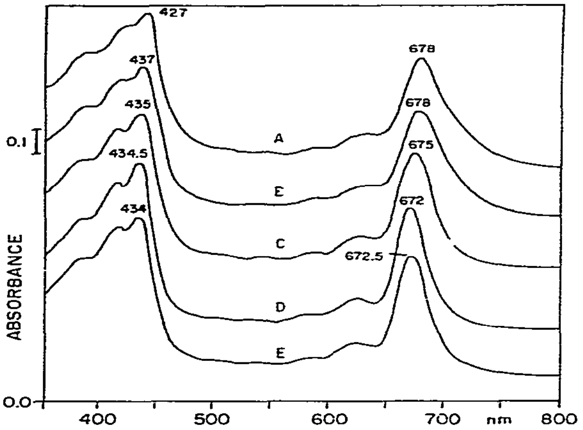

Fig. 7. Absorption spectra of the effect of humid air (approx. 28 Torr water vapor at 28°C) and of freezing on chlorophyll *a* multilayer. A: initial spectrum; B: at 28°C, after the thaw; C: after 6 hours under vacuum (10^{-4} Torr); D: after 23 hours under vacuum (10^{-6} Torr); E: after 4 hours under SO_2 vapors.

The C=C, C=N band at 1605 and the skeletal bands at 1546 and 1533 cm^{-1} (fig. 8A) are somewhat lower than the ones presented in the previous spectra (fig. 3B) indicating that the π electron network is slightly different in both cases.

Immediately after the spectra were taken, the cells were put in a deep freezer (−18°C) overnight to find out if the reorganisation of the multilayer was only a matter of time or if it depended also on the molecules present in the multilayer. The next day when the cells were again at room temperature the spectra were taken. The electronic spectrum (fig. 7B) is almost coincident with the previous one. Six hours after the thaw, an ir spectrum was taken (fig. 8B). In the 4000–2200 cm^{-1} region, we notice some O–H and O–D stretch vibrations indicating that water, as well as heavy water, are present in the multilayer. In the carbonyl region, on the other hand, the intensity pattern of the ketone C=O band shows a slight changed from the previous spectra (fig. 8A). The coordinated ketone band at 1662 cm^{-1} is more intense than the free ketone band at 1683 cm^{-1}. This has to be contrasted with the spectrum 3E where, after 6 hours, the free ketone band is more intense than the coordinated one.

Six hours after the thaw, the sample was put under a vacuum of 10^{-3} Torr, and immediately an ir spectrum

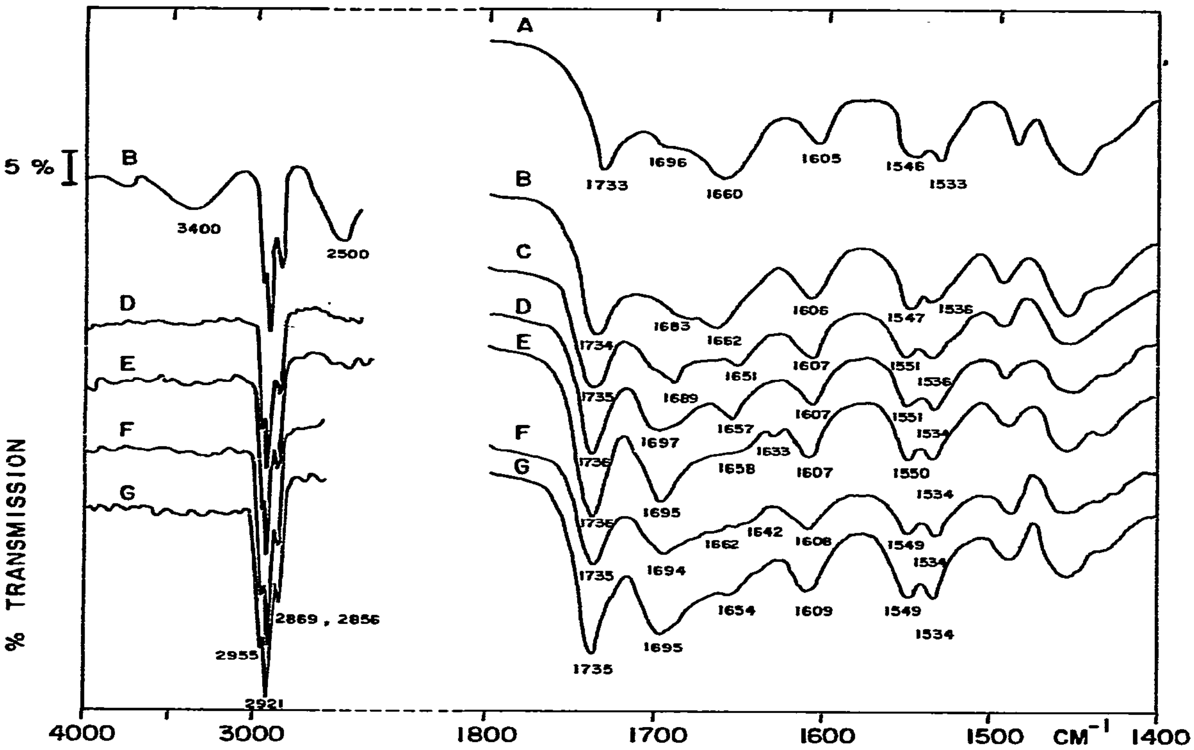

Fig. 8. Infrared spectra of the effect of humid air (approx. 40 Torr water at 34°C) and of freezing on a chlorophyll *a* multilayer. A: initial spectrum of chlorophyll *a* in D_2O saturated atmosphere of the closed chamber immediately after deposition; B: at 34°C, 6 hours after the thaw; C: immediately after putting the vacuum; D: after 5 hours under vacuum (10^{-3} Torr); E: after 16 hours under vacuum (10^{-6} Torr); F: after the introduction of SO_2 vapors; G: after 24 hours under SO_2 vapors.

was taken, fig. 8C. The O—H and O—D bands have disappeared and the ketone bands have started to reverse their intensity pattern. After five hours under vacuum (fig. 8D), the modifications are accentuated and after 16 hours, it is completed (fig. 8E). This spectrum can be compared with fig. 3E, where the reorganisation of the multilayer has reached an equilibrium. The position of the bands in the C—H and in the carbonyl region is almost the same. The only great difference is shown on the coordinated ketone band where in fig. 8E it is less intense than in fig. 3E, so there is less aggregated species in the latter than in the former. This can be understood by the fact that the original organisation was better in the multilayer of fig. 3 than the one in fig. 8, also when a vacuum is made to get rid of the water molecules in the multilayer, the stress is greater than just letting the molecules rearrange themselves in the multilayer.

The effect of vacuum on the electronic spectrum of the multilayer (fig. 7C and D) produces a blue shift of the band maxima from 437 and 678 to 434 and 672 nm, respectively. These positions are lower than those of fig. 2 and can be explained, as it was done in the case of the ir spectra, by assuming that the reorganisation of the sample corresponding to fig. 7D is more complete than that corresponding to fig. 2.

By adding SO_2 to the samples to find out if there is still some water left in the multilayer, we find that there is none left. This is because the bands in the electronic spectrum (fig. 7E) and the ir spectra (fig. 8F and G) showed no modification after several hours of contact with SO_2. From this result, we see that by using a vacuum of 10^{-6} Torr for several hours, we remove all the water from the multilayers.

We conclude from these experiments that the reorganisation of the multilayer of chl *a* with time is due to water molecules that leave the multilayer. These molecules, which are slightly hydrogen bonded to the other molecules in the multilayer, leave easily when the multilayer is left in open atmosphere.

3.4. A model for the multilayer of chl a arrays

From the previous discussion, we have determined that there are three kinds of water molecules present in the multilayer of chl *a* immediately after the deposition of the molecules onto slide. The first kind of water, which is seen as a mist when the plate is coming out the subphase, is bounded to the ester C=O groups. This water leaves readily when the plate is out of the trough, or when the plate is immediately exposed to D_2O atmosphere. These H_2O molecules exchange rapidly with D_2O. The second kind of water is present in the multilayer after the reorganisation is completed. This water has been detected by the SO_2 experiments. These water molecules, which are not bounded to any carbonyl groups, are nevertheless strongly bounded to chl *a* molecules and leave the multilayer only under drastic situations, like high vacuum or the action of SO_2 vapors. These water molecules must therefore be bounded to the magnesium atom through a coordinate bound with the oxygen. The third kind of water, which is present at time zero of the reorganisation, is responsible for maintaining the build-up stack of the multilayer of chl *a*. These water molecules are not bounded to any carbonyl groups of the chl *a* molecules, and contrary to the water molecules of the second kind, they leave easily the multilayer, and as they do, the build-up collapses. Also, these water molecules are not bounded to the Mg atom of the chl *a* molecules. The only groups left to make some bounds are the hydrogen atoms of the water molecules of the second kind. These would make some hydrogen bonds with the oxygen of the water molecules of the third kind, while the hydrogen would make some hydrogen bounds with the π electron network of the chl *a* molecule. These hydrogens are almost at right angle with the porphyrin plane of the chl *a*. This model has been proposed for the case of the adsorption of benzene on silica surface [16] where the benzene molecules interact specifically with the surface hydroxyl group through the formation of an OH- - -π bonds, with the molecular plane of the benzene molecule approximately "parallel" to the adsorbent surface. The energy of interaction in the benzene–silica surface is around 2 kcal/mol making these bonds weak hydrogen bonds. In the model proposed for the multilayer of chl *a*, the situation is very similar. There the OH groups come from the water molecules which are quite mobile and the π electron network comes from the porphyrin ring of the chlorophyll molecules.

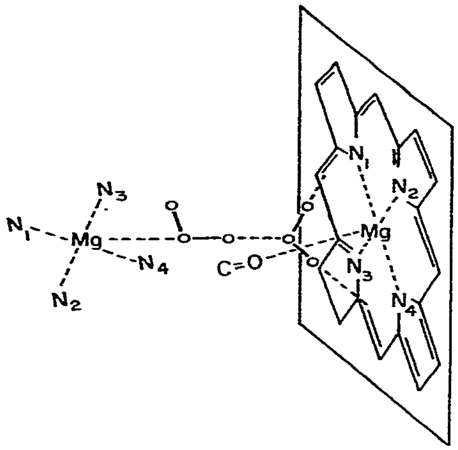

Fig. 9. Schematic representation of the water molecules bounded to the chlorophyll *a* molecules.

A schematic representation of the water molecules bonded to chl *a* multilayer is illustrated in fig. 9. With a coordination of 6 [17], the magnesium of the chl *a* molecule has 4 coordination number taken by the nitrogen groups, the fifth taken by the C=O ketone group of another chl *a* molecule through a coordination bond, and the sixth one taken by the oxygen of the tightly bond water molecule. One hydrogen of this water molecule is hydrogen bounded to the oxygen of the other water molecule where the hydrogen atoms form hydrogen bond with the π electron network of the other chl *a* molecule. When this mobile water molecule leaves the aggregated state of the chl *a*, the multilayer array collapses and the original polymeric state of the chl *a* molecules is replaced mostly by dimers of chl *a*.

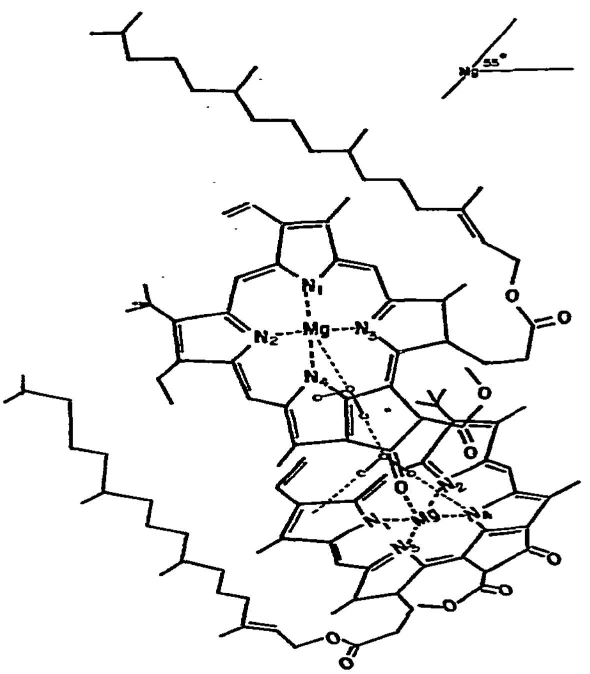

Fig. 10. Model of aggregation of a freshly prepared multilayer of chlorophyll *a*.

A model of the aggregation state of a freshly prepared multilayer and polymeric organisation of chl *a* is shown in fig. 10. For the sake of clarity, only two molecules of chlorophyll are shown. The distance between the Mg of one molecule of chl *a* and the Mg of the other is around 8 Å, a distance sufficient to place two molecules of water with the appropriate bonding. The angle between the two planes is 55°, which is about the same as the one for the porphyrin plane of the chl *a* at the air–water interface.

4. Conclusion

In the electronic spectrum of a chlorophyll *a* multilayer, we observe a blue shift of the band maxima with time. The shift, as explained by the ir spectra, is caused by the collapse of the build-up of chlorophyll *a* molecules when the coordination bond between the C=O ketone bond of one chl molecule with the Mg of the adjacent chl is ruptured. This rupture is attributed to the departure of one slightly bonded water molecule which holds together the build-up stack of the chl *a* multilayer. The model we propose for a freshly prepared multilayer of chl *a* is composed of a polymer of chlorophyll *a* molecules linked together through a C=O- - -Mg coordinate bond, and held together by two molecules of water, hydrogen bonded together, and linked to the chl *a* molecules by a Mg- - -O coordinate bond on one side, and by a OH- - -π hydrogen bond on the other side.

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References

[1] G. Sherman and H. Linschitz, Nature 215 (1967) 511.
[2] G. Sherman and S.-F. Wang, Photochem. Photobiol. 6 (1967) 239.
[3] K. Ballschmitter and J.J. Katz, Biochim. Biophys. Acta 256 (1972) 307.
[4] T.M. Cotton, P.A. Loach, J.J. Katz and K Ballschmitter, Photochem. Photobiol. 27 (1978) 735.
[5] C.E. Jones and R.A. Mackay, J. Phys. Chem. 82 (1978) 63.
[6] D. Frackowiak, D. Dauman, H. Manikowski and T. Martynski, Biophys. Chem. 6 (1977) 369.
[7] C. Chapados and R.M. Leblanc, Chem. Phys. Letters 49 (1977) 180.
[8] J. Aghion and R.M. Leblanc, J. Membrane Biol. 42 (1978) 189.
[9] a. W. Sperling and B. Ke, Photochem. Photobiol. 5 (1966) 865;
b. R.M. Leblanc and C. Chapados, Biophys. Chem. 6 (1977) 77.
[10] H. Dijkmans, R.M. Leblanc, F. Cogniaux and J. Aghion, Photochem. Photobiol. 29 (1979) 367.
[11] S.M. de B. Costa, J.R. Froines, J.M. Harris, R.M. Leblanc, B.H. Orger and G. Porter, Proc. Roy. Soc. Lond. A 326 (1972) 503.
[12] H.H. Strain and W.A. Svec, in: The chlorophylls, eds. L.P. Vernon and G.R. Seely (Academic Press, New-York, 1966) ch. 2.
[13] C. Chapados, A. Tessier and R.M. Leblanc, unpublished.
[14] G.L. Gaines, Jr., in: Insoluble monolayers at liquid–gas interfaces (Interscience Pub., New-York, 1966).
[15] G.R. Seely and R.G. Jensen, Spectrochim. Acta 21 (1965) 1835.
[16] H. Knozinger, in: The hydrogen bond, eds. P. Schuster, G. Zundel and C. Sandorfy (North Holland Publ. Co., Amsterdam, 1976) ch. 27.
[17] J. Le Brech, R.M. Leblanc and A.F. Antippa, Chem. Phys. Letters 26 (1974) 37.