Quantitative Analysis of Molecular Orientation in Chlorophyll *a* Langmuir Monolayer: A Polarized Visible Reflection Spectroscopic Study

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ABSTRACT Polarized visible reflection spectra of a chlorophyll *a* (Chl.a) Langmuir monolayer have been measured in situ at various surface pressures. By applying Hansen's optics to the three-phase plane-bounded system (air/Chl.a monolayer/water), the negative reflection absorbances observed were reproduced satisfactorily by the theoretical calculation. Molecular orientation of Chl.a in the monolayer was evaluated quantitatively as a function of surface pressure, from the reflection absorbance of *p*- and *s*-polarized spectra of the red (Q_y) band. It has been proven that Chl.a molecules in the monolayer form aggregates (islands) even in the low surface pressure region and that during the monolayer compression the molecules are gradually reorganized from inhomogeneous islands to ordered structures, with the chromophores oriented on the average vertically to the water surface.

INTRODUCTION

The study on reliable quantitative analysis of molecular orientation in monolayers at the air/water interface (so-called Langmuir films) nowadays has become crucial, because the structural studies of Langmuir films have been especially progressed for the last several years by applying various kinds of in situ techniques such as FT-IR external reflection (Dluhy, 1986; Dluhy et al., 1988; Mitchell and Dluhy, 1988; Hunt et al., 1989; Mielczarski, 1993; Sakai and Umemura, 1993; Flach et al., 1993) and UV absorption spectroscopy (Kawai et al., 1989; Grüniger et al., 1983; Orrit et al., 1986), Raman scattering (Matsuda et al., 1991; Kawai et al., 1989), sum-frequency vibrational spectroscopy (Guyot-Sionnest et al., 1987), synchrotron x-ray diffraction (Kajer et al., 1989) and neutron reflection (Vaknin et al., 1991), fluorescence (Knobler, 1990; Möhwald, 1990; Möhwald et al., 1990; McConnell, 1991), and Brewster angle microscopy (Hönig and Möbius, 1991).

So far, Orrit et al. have theoretically calculated polarized reflection and transmission amplitudes of dye monolayers on water from a point-dipole model (Orrit et al., 1986). They have also estimated chromophore orientations in monolayers, using the ratio of *p*-polarized reflection (or transmission) strength to *s*-polarized one, $\Delta R_p/\Delta R_s$ or $\Delta T_s/\Delta T_p$. In their model, the ratio $\Delta R_p/\Delta R_s$ or $\Delta T_s/\Delta T_p$ is much dependent on the magnitude of the reflection or transmission ΔR_s or ΔT_s, and the ratio from the reflection measurement, $\Delta R_p/\Delta R_s$, determines two solutions for orientation (Orrit et al., 1986).

The purpose of this study is to analyze quantitatively the molecular orientation of Langmuir monolayer of chlorophyll *a* in a photosynthetic plant by in situ polarized reflection spectroscopy.

The analysis in this study, based on Hansen's optical theory, is simple and convenient in the points that the orientation can be estimated independently from the measured reflection absorbance in *p*- or *s*-polarized spectra and that each reflection absorbance gives a unique solution of the orientation.

The structure and aggregation states of chlorophyll *a* films have been investigated for many years (Jacobs et al., 1954; Kreutz, 1968; de B.Costa et al., 1972; Le Brech et al., 1974; Leblanc et al., 1974; Chapados and Leblanc, 1977; Aghion and Leblanc, 1978; Chapados et al., 1980; Dodelet et al., 1980; Jones et al., 1980; Aghion et al., 1981; Lawrence et al., 1981; Frackowiak et al., 1983; Chapados and Leblanc, 1983; Lawrence et al., 1984; Désormeaux and Leblanc, 1985; Diarra et al., 1986; Bardwell and Dignam, 1987; Lamarche et al., 1991; Désormeaux et al., 1993) because of the potential utility for solar energy conversion into electrochemical energy. However, the structure and molecular orientation in Langmuir monolayers of chlorophyll *a* has not been fully understood yet.

In this paper, we first describe the experimental result on in situ polarized visible reflection spectra of chlorophyll *a* monolayers measured at various surface pressures. Then we present the method for quantitative evaluation of the molecular orientation of chlorophyll *a*. Finally, we discuss characteristic features of the structure and aggregation states in the Langmuir monolayer of chlorophyll *a* from the evaluation.

MATERIALS AND METHODS

Chlorophyll *a* from spinach, substantially free of chlorophyll *b*, was purchased from Sigma Chemical Co. (St. Louis, MO) and used without further purification. Benzene used as a spreading solvent was a specially prepared reagent from Nakalai Tesque, Inc. (Kyoto, Japan). Water was purified by a Mitamura Riken model PLS-DFR automatic lab-still consist-

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ing of a reverse osmosis module, an ion-exchange column, and a double distiller.

Surface-pressure (π)-area (A) isotherm measurements were carried out by the use of a Kyowa Kaimen Kagaku Model HBM-AP Langmuir trough with a Wilhelmy balance. Monolayers of chlorophyll *a* were prepared by spreading a 0.210 mg/ml benzene solution on water buffered with 1.0×10^{-3} M phosphate (pH 8.0) at 20°C.

Polarized visible reflection spectra of the Langmuir monolayer of chlorophyll *a* at a constant surface pressure were recorded on an Otsuka Electronics Model MCPD-1000 spectrophotometer equipped with optical fiber probes and a multichannel photodiode array detector. The experimental setup was schematically shown in Fig. 1. Both *p*- and *s*-polarized beams (electric vector parallel and normal to the plane of incidence, respectively), radiated from an I_2 lamp (150 W), were incident on the water surface at an angle of 37.5° from the surface normal (z axis) through a quartz fiber bundle positioned 2 mm above the water surface. The incident light was then reflected just on the surface, collected by the second fiber equipped with a scrambler, and finally transmitted to the multichannel detector. Five spectra were accumulated for both *p*- and *s*-polarizations with the observation time of 20 s/exposure. The spectra were corrected by subtracting the background of the subphase water surface with the fiber probe positioned at the same height. During spectral measurements, surface pressures were monitored simultaneously and kept constant within the experimental error of ± 0.1 mN m^{-1}. Because chlorophyll *a* is light-sensitive, all of the experiments were performed under dim incandescent light.

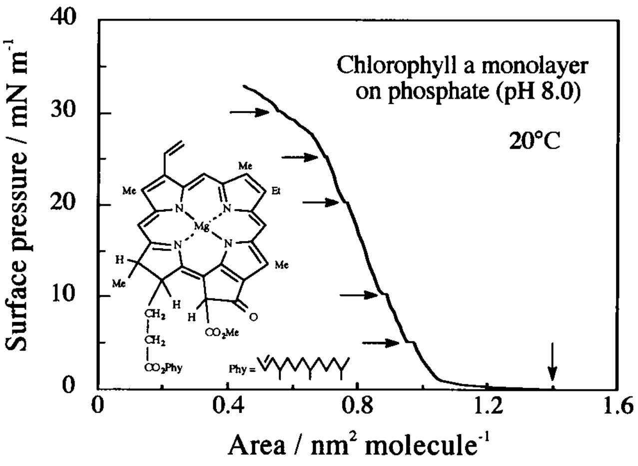

FIGURE 2 The π-A isotherm of the chlorophyll *a* monolayer on water (pH 8.0) simultaneously recorded with the spectral measurement. Arrows designate the surface pressures (or areas) at which the spectra were measured. Molecular structure of chlorophyll *a* is also shown inside the figure.

RESULTS

Visible reflection spectra of chlorophyll *a* monolayers at the air/water interface

To study surface-pressure dependence of molecular structure and orientation in Langmuir monolayers of chlorophyll *a*, polarized visible reflection spectra of chlorophyll *a* monolayers were measured in situ at the surface pressures of 0, 5, 10, 20, 25, and 30 mN m^{-1}, designated by the arrows in the π-A isotherm (Fig. 2). Typical spectra are shown in Fig. 3 at the surface pressure of 0 mN m^{-1} (A = 1.4 nm^2 $molecule^{-1}$). There are two peaks at 426 ± 1 and 674 ± 1 nm irrespective of the polarization. These peaks are called Soret and red bands and are assigned to B_x and Q_y transitions, respectively, both of the π-π^* transitions with the transition moment parallel to the porphyrin ring of chlorophyll *a* (Le Brech et al., 1974; Frackowiak et al., 1983; Tomita, 1968). In Fig. 3, both bands show negative values of reflection absorbance, $\log(R_0/R)$, where R and R_0 are the reflectivities of the water with and without a monolayer, respectively. Moreover, larger absolute values of reflection absorbance were obtained in the *p*-polarized spectrum than the *s*-polarized one.

In the spectra at high surface pressures, the absorption maxima of both B_x and Q_y bands are gradually decreased with the surface pressure increase and finally shifted to 423 ± 1 and 672 nm, respectively, at the surface pressure of 30 mN m^{-1}. Further, these bands also exhibit negative reflection absorbances, the absolute values of which are larger in the *p*-polarization. However, the absolute values of reflection absorbance are decreased with monolayer compression, as shown in Fig. 4 in the case of the Q_y band, for both *p*- and *s*-polarizations.

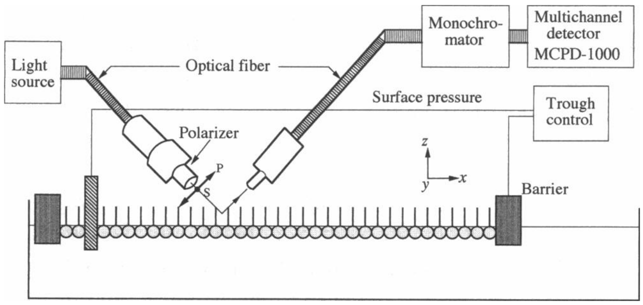

FIGURE 1 Schematic of the experimental setup for measuring polarized visible reflection spectra of a Langmuir monolayer with constant surface pressure. P and S denote the polarization with its electric vector parallel and normal to the plane of incidence, respectively.

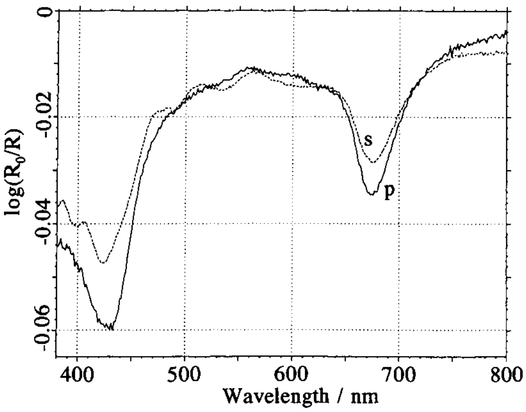

FIGURE 3 In situ polarized visible reflection spectra of a chlorophyll *a* monolayer on water (pH 8.0) measured at 0 mN m^{-1} (the area of 1.4 nm^2 $molecule^{-1}$). Solid and dashed lines correspond to the *p*- and *s*-polarizations, respectively.

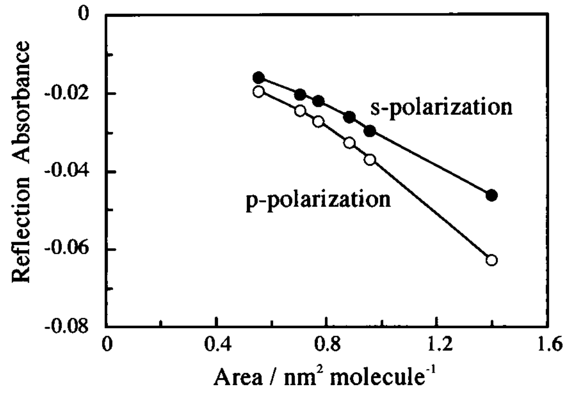

FIGURE 4 Change in the reflection absorbance of the Q_y band at 673 nm with monolayer compression. To eliminate the increase in the absolute values of absorbance, which is due to the surface density increase of chlorophyll *a* on the monolayer compression, the observed absorbance values were corrected and normalized to those per molecule.

Quantitative evaluation of the molecular orientation of chlorophyll *a* at the air/water interface

To determine quantitatively the orientation of the chromophore of chlorophyll *a* molecules in the Langmuir monolayer, we consider a three-phase plane-bounded system; phase 1 is the semiinfinite incident phase of air, phase 2 is the chlorophyll *a* monolayer, and phase 3 is the semiinfinite phase of water. By applying Hansen's optical theory for thin multilayer films (Hansen, 1970) to our system, the reflection absorbances for *s*-polarized beam (A_s) and *x* and *z* components of the *p*-polarized beam (A_{px} and A_{pz}) can be calculated, respectively, as follows (Mielczarski and Yoon, 1989, 1991; Hasegawa et al., 1993):

$$A_s = -\frac{4}{\ln 10}\left(\frac{\cos\theta}{n_3^2 - 1}\right)n_2\alpha_{2y}h_2, \tag{1}$$

$$A_{px} = -\frac{4}{\ln 10}\left(\frac{\cos\theta}{\xi_3^2/n_3^4 - \cos^2\theta}\right)\left[-\frac{\xi_3^2}{n_3^4}\right]n_2\alpha_{2x}h_2, \tag{2}$$

$$A_{pz} = -\frac{4}{\ln 10}\left(\frac{\cos\theta}{\xi_3^2/n_3^4 - \cos^2\theta}\right)\frac{\sin^2\theta}{(n_2^2 + k_{2z}^2)^2}\,n_2\alpha_{2z}h_2 \tag{3}$$

Here θ is the angle of incidence, n_2 and n_3 are the refractive indices for the chlorophyll *a* monolayer and water, respectively, α_{2i} and k_{2i} (i = x, y, and z) are the i components of the absorption and extinction coefficients of the chlorophyll *a* monolayer, and h_2 is the thickness of the monolayer. Further, α_{2i} and ξ_3 are given by

$$\alpha_{2i} = 4\pi k_{2i}\nu \tag{4}$$

and

$$\xi_3 = (n_3^2 - n_1^2\sin^2\theta)^{1/2}. \tag{5}$$

where ν and n_1 are the wavenumber of the incident beam in vacuum and the refractive index of the air, respectively.

We evaluate the orientation of the chromophore in the chlorophyll *a* monolayer for the Q_y band at 673 nm (ν = 14,859 cm^{-1}), because the shape of the B_x band is rather complicated because of the overlapping of more than two components. The optical constants used are $\theta = 37.5°$, n_1 = 1.0 (air), n_3 = 1.33 (water), and h_2 = 2.13 nm (Chapados and Leblanc, 1983). The parameters n_2 and k_{2i} of the monolayer were determined from the experimental data as described next.

First, reflectances for *p*- and *s*-polarizations (R_p and R_s) are given in Eqs. 6 and 7, respectively, by using Hansen's equations for the isotropic N-phase plane-bounded system (Hansen, 1968).

$$R_p = \left|\frac{(m_{11} + m_{12}q_N)q_1 - (m_{21} + m_{22}q_N)}{(m_{11} + m_{12}q_N)q_1 + (m_{21} + m_{22}q_N)}\right|, \tag{6}$$

$$R_s = \left|\frac{(m_{11} + m_{21}p_N)p_1 - (m_{21} + m_{22}p_N)}{(m_{11} + m_{12}p_N)p_1 + (m_{21} + m_{22}p_N)}\right| \tag{7}$$

Here, $q_j = \cos\theta_j/n_j$ and $p_j = n_j\cos\theta_j$, where θ_j and n_j are the angle of incidence or refraction and the refractive index of the *j*th layer, respectively. Moreover, m_{vw} is the *vw*th element of the following 2×2 matrices

$$\mathbf{M}_p = \mathbf{M}_2^p\mathbf{M}_3^p\mathbf{M}_{N-1}^p \tag{8}$$

$$\mathbf{M}_s = \mathbf{M}_2^s\mathbf{M}_3^s\mathbf{M}_{N-1}^s, \tag{9}$$

with

$$M_j^p = \begin{bmatrix} \cos\beta_j & -(i/q_j)\sin\beta_j \\ -iq_j\sin\beta_j & \cos\beta_j \end{bmatrix}, \tag{10}$$

$$M_j^s = \begin{bmatrix} \cos\beta_j & -(i/p_j)\sin\beta_j \\ -ip_j\sin\beta_j & \cos\beta_j \end{bmatrix}. \tag{11}$$

Here, $\beta_j = 2\pi\nu h_j n_j\cos\theta_j$, where ν is the wavenumber of the incident light and h_j is the thickness of the *j*th layer.

Because N is three in the present case and θ_j, n_1, n_3, ν, and h_2 are the known values as stated above, we can obtain refractive indices n_{2p} and n_{2s} from the reflectances R_p and R_s using Eqs. 6–11. Based on the fact that the refractive index n_j is the real part of the complex refractive index $\tilde{n}_j$ ($=n_j + i \cdot k_j$), we estimate optimized refractive indices of the chlorophyll *a* monolayer for *p*- and *s*-polarizations (n_{2p} and n_{2s}) with trial and error methods by fitting calculated values of R_p and R_s in the case of k_{2i} = 0 at 673 nm to the experimentally obtained values (baseline) of those in the same conditions.

Considering the possibility of the variance of n_{2p} and n_{2s}, which is caused by the change in molecular aggregation upon monolayer compression, we obtain optimized n_{2p} and n_{2s} at all of the surface pressures examined. The precision of the measurement of reflectances due to the temporal fluctuations was better than ±0.4%, giving the experimental error of the estimation of refractive indices within ±0.10 irrespective of the surface pressure. The results are shown in Fig. 5 against the surface area of the monolayer. It is evident

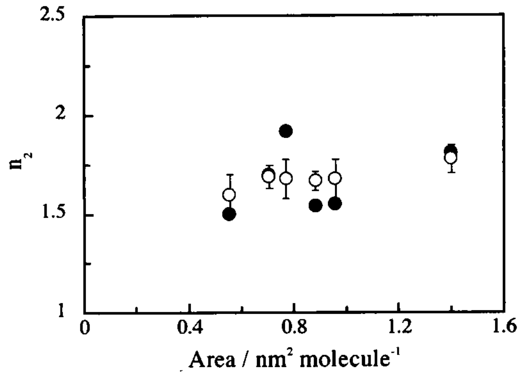

FIGURE 5 Change in the refractive indices n_{2p} (○) and n_{2s} (●) of the chlorophyll *a* monolayer with monolayer compression.

that 1) n_{2p} and n_{2s} are substantially the same within the experimental error, i.e., the isotropic assumption of the refractive indices $n_{2p} = n_{2s} = n_2$ is valid in this case, and 2) the values of n_2 are not changed appreciably at each surface area examined, resulting in the average n_2 value of 1.68.

Concerning the evaluation of the extinction coefficients k_{2x}, k_{2y}, and k_{2z} of the monolayer, we measured the transmission spectrum of a cast film of chlorophyll *a* with Q_y peak maximum at 674 nm. The isotropic extinction coefficient k_2 of 1.27 was obtained at 674 nm, from which the anisotropic k_{2x}, k_{2y}, and k_{2z} were evaluated by the equation $3k_2 = k_{2x} + k_{2y} + k_{2z}$ for the perfect parallel ($k_{2x} = k_{2y}$ and $k_{2z} = 0$) and normal ($k_{2x} = k_{2y} = 0$) orientation of the transition moment (Hasegawa et al., 1993).

We assumed uniaxial orientation of the transition moment around the surface normal (*z* axis), because it was confirmed experimentally that no dichroism was found in the polarized transmission spectra of chlorophyll *a* Langmuir monolayers. On this assumption, the observed reflection absorbances A_p (obsd) and A_s (obsd) can be related to the orientation angle against the surface normal ϕ and the theoretical values A_{px}, A_{pz}, and A_s as

$$A_p(\text{obsd}) = A_{px}\sin^2\phi + A_{pz}\cos^2\phi, \tag{12}$$

$$A_s(\text{obsd}) = A_s\sin^2\phi. \tag{13}$$

We can evaluate, therefore, the reflection absorbances of the Q_y band as a function of the orientation angle of the chromophore in the chlorophyll *a* monolayer, as shown in Fig. 6. For both *p*- and *s*-polarizations, the reflection absorbances are negative irrespective of the molecular orientation, which accords well with the experimental results described above. Further, the absorbances monotonously decrease their absolute values with the decrease in the orientation angle ϕ, i.e., the normal orientation of the chromophore.

Using the observed values of reflection absorbance, we can determine the orientation angle of the chromophore in the Langmuir monolayer of chlorophyll *a* from Fig. 6. The results are depicted in Fig. 7 against the surface area of the chlorophyll *a* monolayer. It is clear that the orientation angles calculated from the reflection absorbances for both *p*- and *s*-polarizations are almost equal irrespective of the surface area and that the angles are decreased with monolayer compression. These facts mean that the chromophores (the porphyrin rings) of the chlorophyll *a* monolayer gradually stand up from the water surface with compression.

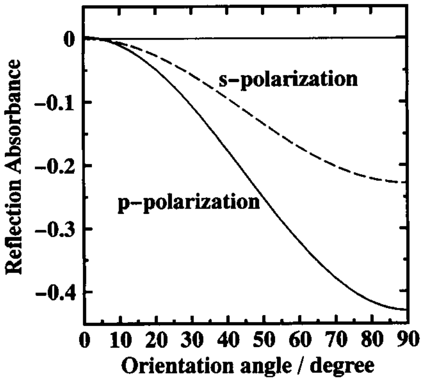

FIGURE 6 Relation between the reflection absorbances of the Q_y band and the molecular orientation of chlorophyll *a* in the Langmuir monolayer.

DISCUSSION

The present result on the polarized visible reflection spectroscopy reveals noticeable features characteristic of chlorophyll *a* Langmuir monolayers. First, reflection absorbances are negative both *p*- and *s*-polarizations, which can also be verified by Hansen's optical theory for three-phase plane-bounded system. The negative values of reflection absorbance are already predicted theoretically in visible external reflection spectroscopy (Grüniger et al., 1983; Orrit et al., 1986) as well as infrared external reflection spectroscopy for transparent or low absorption substrates with extinction coefficient $k_3 < 1$ (Dluhy, 1986; Flach et al., 1993; Mielczarski and Yoon, 1989; Hasegawa et al., 1993).

Next it should be pointed out that anisotropy of the refractive indices of the monolayers can be disregarded in this study. It is reasonable to consider optical anisotropy for the molecules with transition moments oriented parallel to

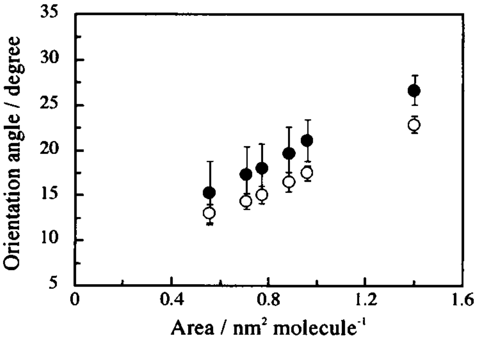

FIGURE 7 Change in the orientation angle of the chromophore in the chlorophyll *a* monolayer with monolayer compression. Symbols (○) and (●) refer to the angles evaluated from the reflection absorbances of the Q_y band in *p*- and *s*-polarizations, respectively.

the flat-shaped and ordered chromophores like chlorophyll *a*. The most probable reason for the lack of noticeable anisotropy in the present study is that the effect of optical anisotropy is apparently small as a result of the following two facts: 1) the refractive indices n_{2p} and n_{2s} are given by two axes of the index ellipsoid, and 2) the chromophore in the chlorophyll *a* monolayer is not completely ordered but tilted at angles 15°–25° against the surface normal. However, it is still possible that the optical anisotropy is missed, unfortunately, because of the limitation of this kind of measurement targeting very thin monolayers on the fluid liquid water surface.

The present result of the quantitative evaluation demonstrates that the orientation angle of the chromophore in the chlorophyll *a* monolayer changes gradually from ~25° to ~15° with the surface pressure increase from 0 to 30 mN m^{-1}. According to the π-A curve of the monolayer (Fig. 2), the surface area per molecule of chlorophyll *a* varies from 1.40 to 0.96, 0.88, 0.77, 0.71, and 0.55 nm^2 with the surface pressure increase. Considering that the cross sectional area of the porphyrin plane is 1.987 nm^2 (Chapados and Leblanc, 1983), these values of surface area correspond to the orientation angles of the chromophore against the surface normal, 45°, 29°, 26°, 23°, 21°, and 16°, respectively. Although the angles are almost equal within the experimental errors at high surface pressure or low surface area, the angles determined from the spectral measurement are much smaller than those estimated from the π-A curve, especially at low surface pressures. The small values of orientation angles indicate that the chlorophyll *a* molecules in the monolayer form ordered aggregates (islands) even in the low surface pressure region. The existence of the islands is also supported by the time-dependent stepwise variation of reflection absorbance observed for the Q_y band at the surface area of 2.4 nm^2 $molecule^{-1}$ shown in Fig. 8. It is difficult to determine directly the island sizes from the time dependence data in Fig. 8. However, considering that the diameter of the radiated area on the water surface is ~2 mm, it is roughly estimated that the islands with diameter as large as millimeter to sub-millimeter order are at least distributed, because islands with diameter smaller than micrometer order are macroscopically homogeneous compared with the radiated area and cannot cause time-dependent spectral change as in Fig. 8.

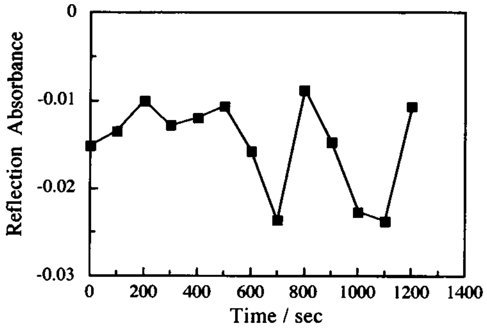

FIGURE 8 Time-dependent change in the reflection absorbance in the *s*-polarized spectra for the Q_y band of the chlorophyll *a* monolayer at the molecular area of 2.4 nm^2 $molecule^{-1}$.

The orientation angle ϕ is the average value, and no information is obtained with respect to the distribution of the angle associated with the calculated average. Nevertheless, the facts that at high surface pressures the orientation angles, evaluated by the reflection absorbance, deviate slightly from those estimated from the π-A curve and that in the area smaller than 1.5 nm^2 $molecule^{-1}$ there is no stepwise time dependence of reflection absorbance shown in Fig. 8 indicate that the chlorophyll *a* molecules are gradually reorganized from inhomogeneous islands to ordered structures on the average during the monolayer compression.

Finally, it should be noted that the aggregation state of the Langmuir monolayer of chlorophyll *a* is different from that of crystal structures with the orientation angle of the chromophore ~65° with respect to the surface normal (J-aggregates). This is because the Q_y band maximum observed is not bathochromically shifted to 740 nm, which is characteristic of the crystals (Kratky and Dunitz, 1977). Kratky and Dunitz have reported that in vivo chlorophyll in a photosynthetic plant shows Q_y band maximum near 680 nm (Kratky and Dunitz, 1977). Therefore, the aggregation state of the Langmuir monolayer of chlorophyll *a* in the present study is considerably similar to that of in vivo chlorophyll with regard to the absorption maximum.

CONCLUSION

By applying Hansen's optics to the three-phase system (air/chlorophyll *a*/water), the polarized spectral features in Langmuir monolayers of chlorophyll *a* can be well reproduced by the theoretical calculation. Refractive index of the chlorophyll *a* monolayer is not substantially changed upon monolayer compression. Moreover, the anisotropy of the refractive index is not appreciably observed. By the quantitative evaluation of the chromophore orientation in the chlorophyll *a* monolayer, it is proven that chlorophyll *a* molecules form aggregates (islands) even in the low surface pressure region, and during the monolayer compression the molecules are reorganized from inhomogeneous islands to ordered structures, with the chromophores oriented on the average vertically to the water surface.

REFERENCES

Aghion, J., and R. M. Leblanc. 1978. interactions of photosynthetic pigments in monolayers at a water-air interface. *J. Membr. Biol.* 42:189–198.

Aghion, J., I. Dupont, and R. M. Leblanc. 1981. Monolayers of chlorophyll and membrane lipids at an air-water interface: pressure and potential stability in red light. *J. Colloid Interface Sci.* 82:569–571.

Bardwell, J. A., and M. J. Dignam. 1987. Infrared spectra of Langmuir-Blodgett chlorophyll *a* films resolved into normal and tangential components. *J. Colloid Interface Sci.* 116:1–7.

Chapados, C., D. Germain, and R. M. Leblanc. 1980. Aggregation of chlorophylls in monolayers. IV. The reorganization of chlorophyll *a* in multilayer array. *Biophys. Chem.* 12:189–198.

Chapados, C., and R. M. Leblanc. 1977. Aggregation of chlorophylls in monolayers. Infrared study of chlorophyll a in a mono- and multilayer arrays. *Chem. Phys. Lett.* 49:180–182.

Chapados, C., and R. M. Leblanc. 1983. Aggregation of chlorophylls in monolayers. V. The effect of water on chlorophyll *a* and chlorophyll *b* in mono- and multilayer arrays. *Biophys. Chem.* 17:211–244.

de B.Costa, S. M., J. R. Froines, J. M. Harris, R. M. Leblanc, B. H. Orger, and G. Porter. 1972. Model systems for photosynthesis. III. Primary photoprocesses of chloroplast pigments in monomolecular arrays on solid surfaces. *Proc. R. Soc. Lond. Ser. A.* 326:503–519.

Désormeaux, A., and R. M. Leblanc. 1985. Electronic and photoacoustic spectroscopies of chlorophyll *a* in monolayer and multilayer arrays. *Thin Solid Films.* 132:91–99.

Désormeaux, A., J. J. Max, and R. M. Leblanc. 1993. Photovoltaic and electrical properties of Al/Langmuir-Blodgett films/Ag sandwich cells incorporating either chlorophyll *a*, chlorophyll *b*, or zinc porphyrin derivative. *J. Phys. Chem.* 97:6670–6678.

Diarra, A., S. Hotchandani, J.-J. Max, and R. M. Leblanc. 1986. Photovoltaic properties of mixed monolayers of chlorophyll a and carotenoid canthaxanthin. *J. Chem. Soc. Faraday Trans.* 82:2217–2231.

Dluhy, R. A. 1986. Quantitative external reflection infrared spectroscopic analysis of insoluble monolayers spread at the air-water interface. *J. Phys. Chem.* 90:1373–1379.

Dluhy, R. A., M. L. Mitchell, T. Pettenski, and J. Beers. 1988. Design and interfacing of an automated Langmuir-type film balance to an FT-IR spectrometer. *Appl. Spectrosc.* 42:1289–1293.

Dodelet, J. P., J. Le Brech, C. Chapados, and R. M. Leblanc. 1980. Photovoltaic action spectra and efficiencies of chlorophyll *a* species absorbing near 700 nm. *Photochem. Photobiol.* 31:143–155.

Flach, C. R., J. W. Brauner, and R. Mendelsohn. 1993. Calcium ion interactions with insoluble phospholipid monolayer films at the A/W interface. External reflection-absorption studies. *Biophys. J.* 65: 1994–2001.

Frackowiak, D., S. Hotchandani, and R. M. Leblanc. 1983. Effect of electric field on polarized absorption spectra of chlorophyll *a* and *b* in nematic liquid crystals. *Photobiochem. Photobiophys.* 6:339–350.

Grüniger, H., D. Möbius, and H. Meyer. 1983. Enhanced light reflection by dye monolayers at the air-water interface. *J. Chem. Phys.* 79:3701–3710.

Guyot-Sionnest, P., J. H. Hunt, and Y. R. Shen. 1987. Sum-frequency vibrational spectroscopy of a Langmuir film: study of molecular orientation of a two-dimensional system. *Phys. Rev. Lett.* 59:1597–1600.

Hansen, W. N. 1968. Electric fields produced by the propagation of plane coherent electromagnetic radiation in a stratified medium. *J. Opt. Soc. Am.* 58:380–390.

Hansen, W. N. 1970. Reflection spectroscopy of adsorbed layers. *Symp. Faraday Soc.* 4:27–35.

Hasegawa, T., J. Umemura, and T. Takenaka. 1993. Infrared external reflection study of molecular orientation in thin Langmuir-Blodgett films. *J. Phys. Chem.* 97:9009–9012.

Hönig, D., and D. Möbius. 1991. Direct visualization of monolayers at the air-water interface by Brewster angle microscopy. *J. Phys. Chem.* 95: 4590–4592.

Hunt, R. D., M. L. Mitchell, and R. A. Dluhy. 1989. The interfacial structure of phospholipid monolayer films: an infrared reflectance study. *J. Mol. Struct.* 214:93–109.

Jacobs, E. E., A. S. Holt, and E. Rabinowitch. 1954. The absorption spectra of monomolecular layers of chlorophyll *a* and ethyl chlorophyllide *a*. *J. Chem. Phys.* 22:142–143.

Jones, R., R. H. Tredgold, and J. E. O'Mullane. 1980. Photoconductivity and photovoltaic effects in Langmuir-Blodgett films of chlorophyll-*a*. *Photochem. Photobiol.* 32:223–232.

Kajer, K., J. Als-Nielsen, C. A. Helm, P.Tippman-Krayer, and H. Möhwald. 1989. Synchrotron x-ray diffraction and reflection studies of arachidic acid monolayers at the air-water interface. *J. Phys. Chem.* 93:3200–3206.

Kawai, T., J. Umemura, and T. Takenaka. 1989. UV absorption spectra of azobenzene-containing long-chain fatty acids and their barium salts in spread monolayers and Langmuir-Blodgett films. *Langmuir.* 5:1378–1383.

Kawai, T., J. Umemura, and T. Takenaka. 1989. Non-resonance Raman studies on spread monolayers of stearic acid-d_{35} and cadmium stearate-d_{35} on water surfaces and thin LB films. *Chem. Phys. Lett.* 162:243–247.

Knobler, C. M. 1990. Recent developments in the study of monolayers at the air-water interface. *Adv. Chem. Phys.* 77:397–449.

Kratky, C., and J. D. Dunitz. 1977. Ordered aggregation states of chlorophyll a and some derivatives. *J. Mol. Biol.* 113:431–442.

Kreutz, W. 1968. On the state of chlorophyll in vivo. *Z. Naturforsch.* 236:520–527.

Lamarche, F., G. Picard, F. Téchy, J. Aghion, and R. M. Leblanc. 1991. Complex formation between chlorophyll a and cytochrome c: surface properties at the air-water interface. Absorbance, fluorescence and fluorescence-lifetime in Langmuir-Blodgett films. *Eur. J. Biochem.* 197: 529–534.

Lawrence, M. F., J. P. Dodelet, and M. Ringuet. 1981. Photovoltaic effect of chlorophyll *a*-quinone systems in multilayer arrays. *Photochem. Photobiol.* 34:393–401.

Lawrence, M. F., J. P. Dodelet, and L. H. Dao. 1984. Improvement of Al/Al_2O_3/multilayer array of chlorophyll a/Ag photovoltaic cell characteristics by treatment of the aluminum electrode. *J. Phys. Chem.* 88: 950–955.

Leblanc, R. M., G. Galinier, A. Tessier, and L. Lemieux. 1974. Laser spectrofluorometry of chlorophylls. *Can. J. Chem.* 52:3723–3727.

Le Brech, J., R. M. Leblanc, and A. F. Antippa. 1974. The absorption spectrum of chlorophyll a and the chlorophyll/dioxane and chlorophyll/benzoquinone interactions. *Chem. Phys. Lett.* 26:37–44.

Matsuda, H., T. Kawai, J. Umemura, and T. Takenaka. 1991. Resonance Raman study of spread monolayers of cetyl orange on water and thin LB films. *J. Mol. Struct.* 242:39–48.

McConnell, H. M. 1991. Structures and transitions in lipid monolayers at the air-water interface. *Annu. Rev. Phys. Chem.* 42:171–195.

Mielczarski, J. A. 1993. External reflection infrared spectroscopy at metallic, semiconductor, and nonmetallic substrates. 1. Monolayer films. *J. Phys. Chem.* 97:2649–2663.

Mielczarski, J. A., and R. H. Yoon. 1989. Fourier transform infrared external reflection study of molecular orientation in spontaneously adsorbed layers on low-absorption substrates. *J. Phys. Chem.* 93: 2034–2038.

Mielczarski, J. A., and R. H. Yoon. 1991. Spectroscopic studies of the structure of the adsorption layer of thionocarbamate. 2. On cuprous sulfide. *Langmuir.* 7:101–108.

Mitchell, M. L., and R. A. Dluhy. 1988. In situ FT-IR investigation of phospholipid monolayer phase transitions at the air-water interface. *J. Am. Chem. Soc.* 110:712–718.

Möhwald, H. 1990. Phospholipid and phospholipid-protein monolayers at the air/water interface. *Annu. Rev. Phys. Chem.* 41:441–476.

Möhwald, H., R. M. Kenn, D. Degenhardt, K. Kjaer, and J. Als-Nielsen. 1990. Partial order in phospholipid monolayers. *Phys. A* 168:127–139.

Orrit, M., D. Möbius, U. Lehmann, and H. Meyer. 1986. Reflection and transmission of light by dye monolayers. *J. Chem. Phys.* 85:4966–4979.

Sakai, H., and J. Umemura. 1993. Infrared external reflection spectra of Langmuir films of stearic acid and cadmium stearate. *Chem. Lett.* 2167–2170.

Tomita, G. 1968. Dimer and polymers in solutions of chlorophyll a. *Biophysik.* 4:296–301.

Vaknin, D., K. Kjaer, J. Als-Nielsen, and M. Lösche. 1991. Structural properties of phosphatidylcholine in a monolayer at the air/water interface. *Biophys. J.* 59:1325–1332.