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PHASE SEPARATION IN CHLOROPHYLL A CONTAINING DIPALMITOYLLECITHIN VESICLES A FLUORESCENCE AND PHOTOACOUSTIC STUDY

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SUMMARY. Chlorophyll a was incorporated into dipalmitoyllecithin vesicles in different concentrations. Depending on the physical state of the lipid, the chlorophyll can aggregate into domains. This phase separation was demonstrated by fluorescence as well as by photoacoustic measurements.

INTRODUCTION. Most of the chlorophyll in the chloroplast thylacoid membrane is organized as "antenna" chlorophyll (2). Obviously, the investigation of its structural order is a prerequisite for the understanding of the functioning of the photosynthetic unit. Among others, interactions between chlorophyll and chlorophyll, chlorophyll and lipid, or chlorophyll and water may be responsible for the self-organization of these assemblies. Lipid bilayer vesicles and liposomes containing chlorophyll are thought to be useful model systems to study properties of chlorophyll and chlorophyll aggregates at a water-lipid-interface (4,7,9).

Information about the organization of chlorophylls in artificial lipid membranes can be derived from fluorescence measurements because the process of reemission of absorbed photons turned out to be most sensitive to structural changes of chlorophyll and/or its environment. Another optical method that is not concerned, however, with the radiative but with the nonradiative deexcitation of a sample is the photoacoustic spectroscopy (12). In the present report we combine fluorescence and

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photoacoustic studies upon phase separation phenomena of chlorophyll a in dipalmitoyllecithin (DPPC)† vesicles. It can be demonstrated that both spectroscopical methods are complementary since the two techniques are sensitive to different deexcitation processes of light-excited chlorophyll.

MATERIALS AND METHODS. DPPC was purchased from R. Berchtold, Biochemisches Labor, Bern, Switzerland, and was used without further purification.

Chlorophyll a was obtained by isolating the plant pigments from fresh spinach. Purification was performed as described earlier (8). For absorption and fluorescence measurements, vesicles were prepared by solving lipid and chlorophyll a in the appropriate molar ratio in chloroform. After evaporating with nitrogen, the samples were kept in vacuo for 10 minutes to remove traces of the solvent. After addition of 2 mM aqueous solution of CsCl (final concentration 1 mg lipid per 1 ml solvent) the mixtures were slightly sonicated in the dark under nitrogen atmosphere at a temperature well above the main transition of the lipid.

Absorption measurements were performed with a Cary 81 absorption spectrometer. The reference cuvette contained a similar vesicle preparation but without chlorophyll a in order to correct the absorption spectra for light scattering effects.

Fluorescence spectra were taken with a Schoeffel M 460 photometer equipped with a cooled red sensitive photomultiplier. The intensity versus temperature curves were obtained by continous recording the fluorescence at the spectral maximum (680 nm) as a function of the temperature. Dispersions for the photoacoustic measurements were prepared in the concentration range of 10-15% (w/w, lipid to water). For measurements of the temperature dependence of the photoacoustic signal, the liquid sample was placed in a photoacoustic cell, whose temperature could be varied. It was illuminated by the white light of a halogen lamp (75W), chopped with a frequency of 37 Hz. The acoustic signal was detected by a condensor microphone (Bruel and Kjaer, Type 4166) followed by a lock-in amplifier (PAR, Type HR8). To measure the tangent of the difference of the phase angle between the acoustic signal and the modulated light intensity, a second lock-in amplifier of the same type and a ratiometer (Ithaco, Type 3512) were used.

RESULTS

Absorption Measurements:

Absorption spectra were taken at different chlorophyll/lipid ratios (from 1:300 to 1:3) at 23°C, that is below the phase transition where DPPC is crystalline and at 53°C where it is liquid crystalline. Within experimental error, there is no difference in the absorption spectra for both temperatures up to concentrations of 1 chlorophyll in 10 lipid molecules. This is shown in Figure 1A in the wavelength range from 500 to

†DPPC: 1,2-dipalmitoyl-sn-glycero-3-phosphorylcholin.

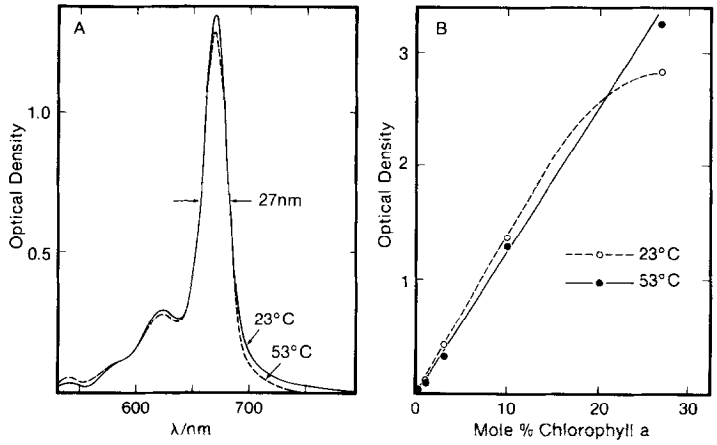

Figure 1: A) Absorption spectrum of 10 mole% chlorophyll a containing DPPC vesicles (lipid concentration 1 mg/ml CsCl solution) at 23°C and 53°C in the spectral range 530 nm<λ<780 nm. Width at half maximum of the α peak is around 27 nm.
B) The optical density of a 1 mg/ml DPPC (sonicated) dispersion with increasing chlorophyll a concentration at two different temperatures.

800 nm, the region where the chlorophyll a α peak appears. Not only the peak position (at 670 nm) but also its width (~27 nm) remains unaffected. This is in contrast to results of Lee, who found for the higher chlorophyll concentrations a shoulder at ca 685 nm which he attributed to aggregated chlorophyll (7). For chlorophyll in dimyristoyllecithin, Luisetti et al. (10), too, could not find an additional red shifted band for the higher chlorophyll concentrations.

Figure 1B shows the increase of the 670 nm peak height with increasing chlorophyll a concentration at the two temperatures. Up to molar ratio of 1:10 (chlorophyll:lipid) the optical density increases linearly with the chlorophyll concentration. Moreover, there is practically no temperature dependence of the absorption.

Fluorescence Measurements:

Although there is no influence of the temperature on the wavelength of the fluorescence emission of chlorophyll a containing vesicles, the physical state of the lipid can have a dramatic effect on the fluorescence intensity. This is shown in Figure 2 for different chlorophyll/DPPC ratios. While for 0.33% no abrupt change of the fluorescence intensity

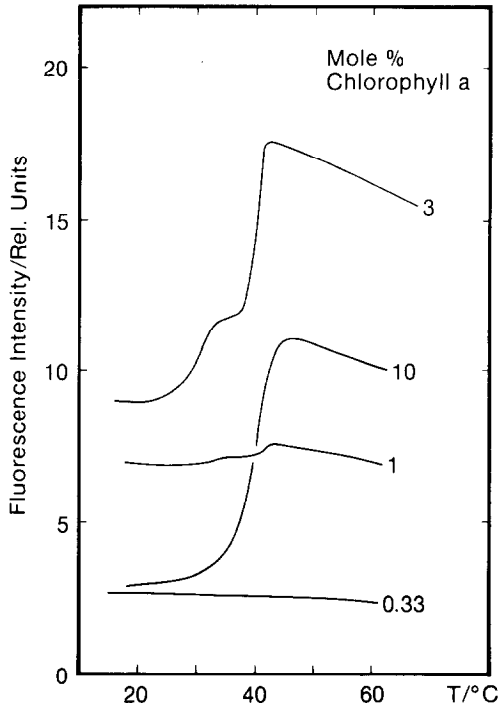

Figure 2: Fluorescence intensity at 680 nm as a function of the temperature of a 1 mg/ml DPPC dispersion at different chlorophyll a contents.

occurs by decreasing the temperature from 60°C to 20°C, a strong decrease of the intensity can be found for 3% and for 10% at temperatures which correspond to the main phase transition of DPPC (1). For 3 mole% chlorophyll a also the pretransition of DPPC at about 30°C can be detected, whereas for 10 mole% the main transition is considerably broadened. This agrees with results of Nicholls et al. (11), on chlorophyll b in DPPC liposomes.

Photoacoustic Measurements:

The temperature dependence of the amplitude and the phase angle of the photoacoustic signal of aqueous dispersions of pure DPPC and of chlorophyll a containing DPPC is shown in Figure 3. The scans for the pure lipid and the 0.33 mole% chlorophyll a containing lipid (A and B, respectively) do not differ significantly: The amplitude decreases slightly above the phase transition with decreasing temperature and remains constant below the transition, whereas the phase angle of the photoacoustic signal (with

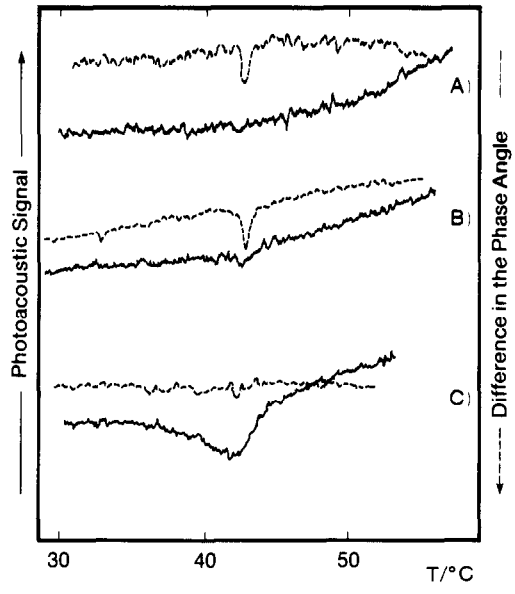

Figure 3: Photoacoustic intensity (full line) and phase angle (dashed line) of DPPC dispersions (15% wt/wt) without (A), with 0.33 mole% (B), and with 10 mole% (C) chlorophyll a.

respect to the modulation of the light intensity) remains constant over the whole temperature range except the narrow phase transition region where it shows a positive shift. A similar phase angle behavior has been found for first order phase transitions in VO_2 and $BaTiO_3$ (5).

The characteristic temperature behavior of the amplitude and the phase angle is essentially the same for increasing and for decreasing temperatures. It can be shown that for a sample that is very thick compared to the thermal diffusion length, the shift in the phase angle is always positive for each sort of first-order phase transition (6). The width of the transition region in the temperature scale in Figure 3 is a result of the temperature gradient in the photoacoustic cell and the sample.

A quite different result is obtained for a DPPC dispersion containing 10 mole% chlorophyll a (Figure 3C): The phase angle shows no temperature dependence at all and the decrease of the amplitude with decreasing temperature in the fluid state of the lipid is more pronounced. During the

(broadened) phase transition, however, the amplitude of the photoacoustic signal increases.

DISCUSSION

It is well known that chlorophylls, incorporated into artificial lipid membranes, may form domains of aggregated molecules. This can be demonstrated most sensitively by fluorescence measurements since oligomeric chlorophyll does not fluorescence (7,10). These phase separation phenomena depend not only on the chemical nature of the chlorophylls and of the lipids, but also on the relative concentration of the chromophore and on the physical state of the lipid: The critical solubility concentration is higher in the fluid phase of the lipid than it is in the ordered state. Accordingly, it is possible at certain concentrations to induce the formation of (more) chlorophyll aggregates by lowering the temperature below the phase transition of the phospholipid. As a consequence, the fluorescence intensity of the sample decreases because of the concentration quenching of the oligomeric organized chlorophyll (3). This was demonstrated for 1, 3 and 10 mole% chlorophyll a in DPPC. The sample containing only 0.33 mole% chlorophyll a shows no change of the fluorescence intensity at the phase transition temperature because the solubility limit is higher even in the crystalline state.

As for all investigated concentrations (except 27 mole% chlorophyll/lipid) the absorption of light does not change upon temperature variation, the decrease of the fluorescence intensity at the phase transition temperature is not a consequence of a reduction in photon uptake, but of a reduction in photon reemission. Accordingly, there should be an enhanced production of heat upon illumination. This, indeed, could be demonstrated by photoacoustic measurements: For a 10 mole% chlorophyll a containing DPPC dispersion, the decrease of the fluorescence intensity is accompanied by an increase of the photoacoustic intensity (see Fig. 3C). For 0.33 mole% chlorophyll a with no temperature dependence of the

fluorescence, the photoacoustic signal is not changed compared with an undoped lipid dispersion (Fig. 3B and A). The fact that the phase angle of the chlorophyll rich sample shows no variation with temperature, is probably a consequence of the broadened phase transition which levels out the shift of the phase angle.

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