EVIDENCE FOR THE LOCALIZATION OF CHLOROPHYLL IN LIPID VESICLES: A SPIN LABEL STUDY*

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SUMMARY

Fatty acid spin labels containing nitroxide groups at different positions in the fatty acid chain have been incorporated into lipid vesicles. Changes in esr parameters of the spin labels in the presence in the membrane of phytol, propionic acid phytol ester or chlorophyll a and the kinetics of chlorophyll a mediated photodestruction of the spin labels suggest a localization of the macrocyclic ring of the chlorophyll molecule in the polar head group region of the membrane.

INTRODUCTION

Lipid vesicles containing chlorophyll a (Chlorophyll-liposomes) can serve as model systems of the properties and localization of chlorophyll and to electron transfer mechanisms in the photosynthetic membrane (1,2). The location of chlorophyll within the membrane of chlorophyll-liposomes has not yet been conclusively established. It is generally accepted that the porphyrin ring of the chlorophyll is tilted towards the membrane surface at an angle between 45 and 54° (3-8). Four possibilities exist for the position of the chlorophyll macrocycle: a) the ring protrudes into the water phase outside

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the membrane and the chlorophyll itself is anchored by its phytol chain inside the hydrophobic region of the membrane; b) the ring is located within the polar head group region of the lipid; c) the ring is bent backwards into the fatty acid chain region; or, d) the ring is buried completely in the space between the hydrocarbon chains of the two lipid layers in the bilayer.

We wish to report here some new evidence for the location of chlorophyll in lipid vesicles from nitroxide fatty acid spin labels that are substituted at different positions in the fatty acid chain. Such spin labels probe the environment at different levels of the symmetric bilayer membrane and yield information on the micro-environment at that point (9,10).

MATERIALS AND METHODS

Dl-α-dipalmitoyl-phosphatidylcholine was purchased from Sigma. Spin labels 5-Doxyl (2-(3-Carboxypropyl)-2-tridecyl-4,4-dimethyl-3-oxazolidinyloxyl), 12-Doxyl (2-(10-Carboxydecyl)-2-hexyl-4,4-dimethyl-3-oxazolidinyloxyl) and 16-Doxyl (2-(14-Carboxytetradecyl)-2-ethyl-4,4-dimethyl-3-oxazolidinyloxyl) were obtained from Syva Corp. Phytol (Aldrich) was freshly distilled in vacuum before use. Chlorophyll a was prepared according to Strain and Svec (11) and propionic acid phytol ester according to Katz et al. (12). Liposomes (lipid, Dl-α-dipalmitoyl-phosphatidyl-choline) in 0.1 M sodium phosphate, 0.1 M sodium pyrophosphate and 1 mM sodium EDTA were prepared as described recently (2). All components were incorporated into the starting lipid solution prior to evaporation. Esr measurements were made with a Varian E 9 X-band spectrometer connected to a Nicolet Model 1072 time-averaging computer. For photodestruction experiments, illumination of the cavity was provided by a 300 W Eimac xenon arc lamp (Model X6263). Light was passed through a 5 cm water filter and a 3850 Corning filter (cut off ∿330 nm).

RESULTS AND DISCUSSION

Differences between the esr spectra of the spin labels 5- to 16-Doxyl in liposomes (Fig. 1A) are due to the partial immobilization of the spin labels at different levels of the membrane and the long known fact of increasing fluidity of the

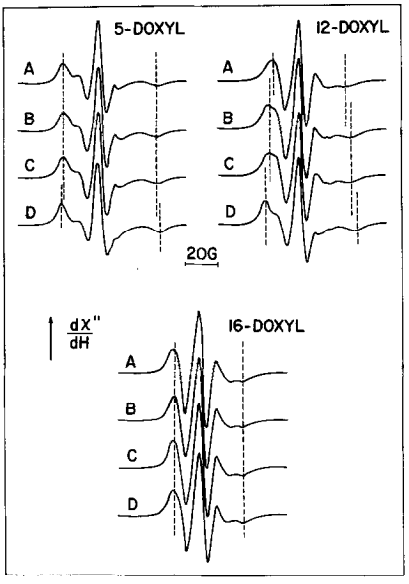

Figure 1. Esr spectra of Doxyl spin labels incorporated into liposomes with various additives at 20°. The concentration of lipid was 45 mM, that of the spin labels 0.15 mM and that of the additives 1.5 mM. A. no addition, B. phytol, C. propionic acid phytol ester, D. chlorophyll a. Esr spectra were recorded at a frequency of 9.09 GHz, modulation frequency 100 kHz, modulation amplitude 2.0 gauss, power setting 10 mW. 8 30 sec scans were collected.

membrane towards its center (9,10). Changes in spectral parameters of spin labels directly reflect changes in membrane properties and permit conclusions on the location of components incorporated. Introduction of the isoprenoid alcohol phytol, which anchors the chlorophyll molecule within the membrane into the liposomes, causes no significant changes in the polar head group region, where 5-Doxyl probes, nor at the hydrophobic interface, where 16-Doxyl probes (Fig. 1B). Changes are observed only at the middle level of the membrane, the site of probing of 12-Doxyl (Fig. 1B) ($\Delta T_{||}'$ 2.5; Table I). Incorporation of propionic acid phytol ester, which is the substituent of the 7-position of porphyrin in chlorophyll, causes changes

TABLE I. Experimental values of nuclear hyperfine tensors of Doxyl spin labels in liposomes. Experimental conditions are those of Fig. 1.

Additives	Spin Labels					
	5-Doxyl		12-Doxyl		16-Doxyl	
	$T_{\|\|}'$	$T_{\perp}'$	$T_{\|\|}'$	$T_{\perp}'$	$T_{\|\|}'$	$T_{\perp}'$
none	27.9	8.4	22.0	9.4	20.3	9.6
phytol	27.8	8.5	24.5	9.0	20.3	9.7
propionic acid phytol ester	28.0	8.5	24.2	9.1	20.4	9.6
chlorophyll *a*	29.4	8.6	27.2	8.6	20.5	9.5

like those of phytol (Fig. 1C). More pronounced changes in the esr signal are observed when chlorophyll *a* is incorporated (Fig. 1D). As compared to propionic acid phytol ester, $T_{||}'$ are increased by 1.4 (5-Doxyl) and 3.0 (12-Doxyl) (Table I). The spectral parameters of 16-Doxyl, however, are not altered (Fig. 1D). These results lead us to conclude that the porphyrin ring must be located near the polar head group region of the lipid. Thus, possibilities a) and d) for the site of chlorophyll in the liposomes can be ruled out. If the porphyrin ring indeed protrudes into the water phase, the pronounced changes observed in spectral parameters of the 5- and 12-Doxyl as compared to those observed with phytol or phytol propionate cannot be expected. Furthermore, if the porphyrin ring is buried between the hydrocarbon chains of the lipid bilayer, the rotational behavior of 16-Doxyl has to be affected, and it is

not. The results presented here, however, do not allow us to distinguish between possibilities b) and c).

This can be achieved by applying the photodestruction technique to the spin labels (13). The kinetics of the photodestruction of the three spin labels in the presence of chlorophyll a (as judged by the decrease in signal height) are shown in Fig. 2. No reduction in intensity of the spin label signal is observed if chlorophyll a is not present in the liposome. As can be seen from Fig. 2, 5-Doxyl is destroyed at a faster rate than 12- and 16-Doxyl, which undergo decomposition only slowly. Spin label photodestruction should occur at the fastest rate where the donor (chlorophyll a) and the nitroxyl radical are closest together. We conclude, therefore, that the chlorophyll macrocyclic ring is most likely in the polar head group region of the liposome (possibility b)). Should the macrocylic

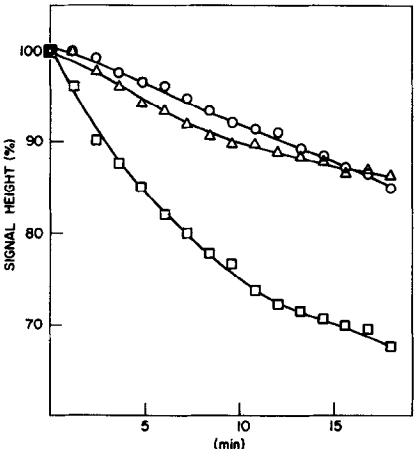

Figure 2. Kinetics of the photodestruction of Doxyl spin labels incorporated into liposomes in the presence of chlorophyll a at 20°. Conditions as in Fig. 1, except that the concentration of spin labels was 2 mM. The magnetic field was adjusted to the center peak of the spin label resonance in traces D, Fig. 1. □ - □ 5-Doxyl, O-O 12-Doxyl, Δ-Δ 16-Doxyl.

ring be bent backwards (possibility c)), faster destruction of 12-Doxyl as compared to 16-Doxyl is to be expected, and this is not observed.

The main amount of chlorophyll *in vivo* appears to occur in an oligomeric form (14), whereas the state of chlorophyll in the liposomes *in vitro* is essentially monomeric (2). It seems to be reasonable however, to assume similar locations of the macrocyclic ring of chlorophyll within the polar head group region of the lipid bilayer *in vivo* for those chlorophyll molecules that are associated with the lipid region of the thylakoid membrane.

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