PARAMAGNETIC FLUORESCENCE QUENCHING IN CHLOROPHYLL A CONTAINING VESICLES: EVIDENCE FOR THE LOCALIZATION OF CHLOROPHYLL

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Received August 11, 1977

SUMMARY

Spinprobes (fatty acids, androstane, cholestane) that differ in the location of the paramagnetic centre relative to the polar membrane surface have been incorporated into lipid vesicles containing chlorophyll a. Quenching of the Chl a fluorescence is observed following the Stern-Vollmer-relationship. Quenching is more effective with spinprobes carrying the nitroxide group near the polar head groups of the lipid moiety. Quenching is also observed using a water soluble spinlabel. The porphyrin ring of the Chl a molecules is suggested to be localized in the polar head group region of the bilayer membrane.

INTRODUCTION

Lipid bilayer vesicles containing chlorophyll a can be utilized as model systems to study the organization properties and the localization of chlorophyll in the photosynthetic membrane (1,2). The porphyrin ring is reported to be tilted towards the membrane surface at an angle between 45 and 54^{o} (3). Four possibilities for the position of the porphyrin ring are discussed (9): a) the ring protrudes into the aqueous phase outside the membrane, b) the ring is located within the polar head group region of the bilayer, c) the ring is bent backwards into the hydrophobic region, d) the ring is completely buried in the apolar region between the hydrocarbon chains of the two lipid layers. Oettmeier et al. (4) measured spectral parameters of different spinlabels and changes in the kinetics of the chloro-

ISSN 0006-291X

phyll a mediated photodestruction of these spinprobes. They conclude that the porphyrin ring is located most likely in the polar head group region of the membrane. We have examined the localization of chlorophyll a in lipid bilayer membranes upon looking at the quenching of Chl a fluorescence. Fatty acid spinlabels carrying the nitroxide group at different position in the fatty acid chain were used as quenchers. Additional spinlabels (TEMPOL*, spinlabelled androstane and cholestane) permitted us to probe different regions of the membrane, thus yielding a quenching profile. The fluorescence quenching by paramagnetic probes is based on a reduction of the excited state lifetime of the fluorophore (5,6).

MATERIAL AND METHODS

D-L-α-dimyristoyllecithin from Fluka was checked by thin layer chromatographie and used without further purification. Spinlabels were purchased from Syva, Palo Alto. The pyrene derivatives were synthesized in our own laboratory. Chlorophyll a was obtained by isolating the plant pigments from fresh spinach. Purification was performed on a chromatographic column filled with a suspension of powdered sugar in petrol-ether following the method of Smith and Benitez (7). Vesicles were prepared from a chloroform solution containing lipids, chlorophyll and fatty acid spinlabels. After evaporation with nitrogen the probes were dispersed in a 2 mM solution of CsCl by sonication in the dark and under nitrogen atmosphere for about 10 minutes. The temperature was kept above the phase transition temperature of the lipid. Probes containing cholestane or androstane spinlabel were prepared similarly but the spinlabel was added from a 10^{-2} m methanolic solution according to Bieri et al. (7). TEMPOL was added from an aqueous solution. Fluorescence spectra were taken with a Schoeffel M 460 Photometer equipped with a cooled red sensitive photomultiplier. All measurements were performed at 32° C except the TEMPOL quenching experiments at 12° C. The quenching process is expected to follow the Stern-Vollmer relationship: $(I_o/I) - 1 = k \cdot \tau \cdot c$, where I_o is the fluorescence without quencher, I is the emission intensity in the presence of a quencher, k is the quenching constant, τ the lifetime of the excited state and c the concentration of the quencher.

* Abbrevation used: TEMPOL (2,2,6,6 - tetramethyl - 4 - piperidinol - 1 - oxid)

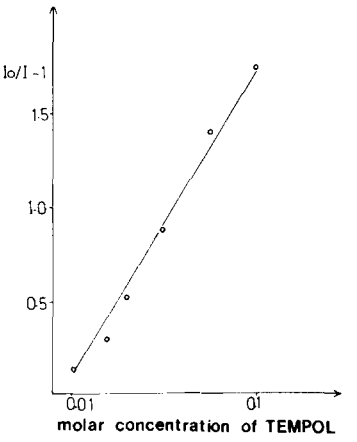

Figure 1. Stern-Vollmer plots of fluorescence quenching with TEMPOL at 12° C in dimyristoyl lecithin bilayers. The chlorophyll concentrations are a) 1 Mole %, b) 3,3 Mole % according to the lecithin. Identical curves are obtained.

RESULTS AND DISCUSSION

a) Quenching of chlorophyll a fluorescence by TEMPOL:

Stern-Vollmer plots indicating the paramagnetic quenching of the chlorophyll a fluorescence caused by TEMPOL are shown in fig. 1. Straight lines are observed for probes containing 1 and 3.3 Mole % chlorophyll relative to the lipid. It is known that molecules like TEMPOL can distribute between the equeous phase and the hydrophic phase of the membrane (8). In order to minimize the label concentration within the membrane our experiments were performed at 12° C that is below the lipid phase transition temperature. According to the EPR-spectra no TEMPOL attached to internally accessable sites was observed at this temperature and the given lipid concentration of 10^{-3} molar. The emission quenching was effective in a concentration range between 10^{-1} and 10^{-2} molar concerning the paramagnetic probes. After reduction of the spinprobes by addition of ascorbic acid the chlorophyll a fluorescence was immediately reestablished

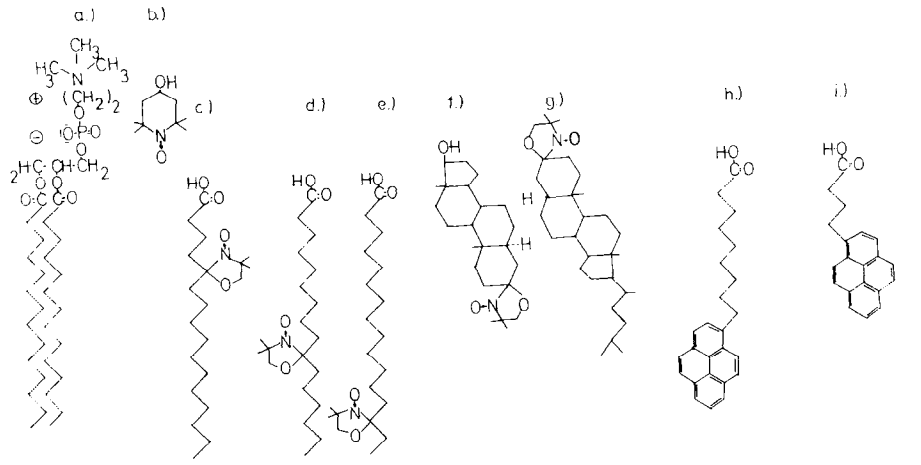

Figure 2. Lipoids used in this study and the relative location of the nitroxide group as well as the chromophore according to the membrane surface indicated by the lecithin molecules. a) dimyristoyl lecithin, b) TEMPOL, c) 5-nitroxide-stearic acid, d) 12-nitroxide-stearic acid, e) 16-nitroxide-stearic acid, f) androstane label, g) cholestane label, h) ω-pyrene butyric acid, i) ω -pyrene-decanoic acid.

to the original value in the absence of any quencher molecule.

b) Quenching caused by fatty acid spinlabels:

Three fatty acid spinlabels differing in the position of the nitroxide groups along the fatty acid chain were used. Their localization in the membrane profile (9) is shown in fig. 2. At the given temperature of 32^{o} C (above the lipid phase transition) the spinlabels are incorporated into the membrane and randomly distributed as can be shown by taking the EPR- spectra of the labels. The Stern-Vollmer plots for 5-nitroxide- stearate, 12-nitroxide-stearate and 16-nitroxide-stearate are shown in fig. 3, c, d and e. Only a small quenching effect is observed using 16-nitroxide-stearate (the paramagnetic group is localized at the apolar end of the fatty acid chain). The quenching effi-

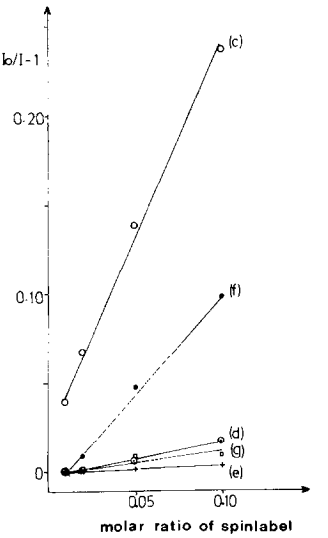

Figure 3. Stern-Vollmer plots of paramagnetic quenching in dimyristoyl lecithin bilayers at 32° C. The spinlabels incorporated into the lipid matrix are c) 5-nitroxide-stearate, d) 12-nitroxide-stearate, e) 16-nitroxide-stearate, f) androstane-label, g) cholestane spinlabel. The chlorophyll a concentration is 1 Mole % according to the lipid.

ciency increases upon shifting the nitroxide group along the chain versus the polar head group. 5-nitroxide-stearate, that is situated near the carbonyl group is a much better quencher than 12-nitroxide-stearate in a medium position. Nevertheless quenching from carbon position 12 is effective. Therefore a localization of the porphyrin ring in the polar headgroup region but not standing out of the membrane surface is favoured by our experiments.

Cholestane spinlabel (fig. 2 g) is suggested to protrude with its nitroxide ring into the membrane surface. It is therefore conceivable that its quenching efficiency is smaller compared to the androstane label. This is revealed by curve g in fig. 3.

Our results confirm a chlorophyll conformation where the porhyrin ring is located near the polar head group region of the membrane. The macrocyclic ring is assumed not to protrude into the water phase but to be bent backwards because:

1. Quenching is most effective using spinlabels with the nitroxide group positioned between carbon atoms five and eight of the lipid fatty acid chains (5-nitroxide-stearic acid and androstane label).
2. Small quenching is observed using spinprobes that are embedded deeply into the hydrophobic part of the membrane (12- and 16-nitroxide-stearic acid).
3. Also small quenching is observed using the cholestane spinlabel with the nitroxide group positioned in the hydrophilic part of the membrane.
4. Using watersoluble TEMPOL quenching is observed only at high label concentrations (10^{-1} molar at a lipid concentration of 10^{-3} molar).

We also checked our method of finding a fluorophore position by stereospecific quenching using ω - pyrene decanoic acid (fig. 2 h) and ω - pyrene butyric acid (fig. 2 i) as fluorophore and the above given spinlabels as quencher. Pyrene decanoic acid with the centre of the aromatic ring at carbon thirteen of the lipid chains is quenched in the following order of efficiency: 12-nitroxide-stearic acid, 5-nitroxide-stearic acid and

and cholestane label, whereas androstane label is the better quencher for both. A detailed description of these experiments will be given elsewhere.

ACKNOWLEDGEMENT

We are indepted to Prof. Sackmann for helpful discussions and to U. Theilen for his excellent technical assistance. J. Luisetti is supported by DAAD (Deutscher Akademischer Austauschdienst).

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