SPIN PROBE STUDIES OF PHOTO-INDUCED STRUCTURAL CHANGES IN PHOSPHOLIPID MULTIBILAYERS CONTAINING LIGHT-SENSITIVE PIGMENTS*

Surendra P. VERMA**, H. SCHNEIDER and Ian C.P. SMITH
Division of Biological Sciences,
National Research Council of Canada,
Ottawa, Canada K1A OR6

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1. Introduction

Biological photochemical processes, for example photosynthesis and vision, are closely associated with membranes [1–3]. These processes are complex, both structurally and functionally, and many model system studies have been carried out in the attempt to understand the nature of the underlying elementary processes. Because lamellar structures are associated with chloroplasts [2, 4] and retinal rod segments [5, 6], a number of photochemical properties of lipid bilayers containing chlorophyll [7] and retinal [8] have been carried out. These were concerned mainly with photo-induced potentials or ionic permeabilities. The present communication indicates that illumination of phospholipid bilayers containing photoactive pigments can cause changes in the structural arrangement of the phospholipids, suggesting that such changes may play a role in biological photochemical processes.

The light-induced structural changes were detected using spin probes intercalated into phospholipid multibilayers. This technique depends on the fact that the shapes and separation of the hyperfine lines in the electron spin resonance (ESR) spectrum of a spin probe are determined by its motion and by its orientation relative to the applied magnetic field [9–11]. A recent review deals with the technique in detail [11].

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2. Materials and methods

The spin probe employed, 3-spiro-[2′-(*N*-oxyl-4′,4′-dimethyloxazolidine)]-cholestane (CSL) was prepared as described by Keana et al. [12]. Egg lecithin was prepared by chromatography on silica gel and alumina and yielded only a single spot by thin-layer chromatography. Chlorophyll a was supplied by Sigma (St. Louis), all-*trans* retinal and retinol by DPI (Rochester, N.Y.), and methylene blue by Anachemia Chemicals (Montreal).

Egg lecithin, the cholestane spin probe (CSL), and the photo-sensitive pigments, chlorophyll a or retinal, were dissolved in chloroform. The concentrations employed were lecithin, 10 mg/ml; CSL, 0.23 mg/ml; retinal or retinol, 2 mg/ml; chlorophyll a, 0.02 mg/ml. The level of cholesterol, when present, was 1.5 mg/ml. The chloroform solutions were then dried in flat quartz ESR cells using a stream of wet nitrogen, followed by exposure to vacuum for 2 hr. Hydration of the lipid films was carried out by wetting with 0.1 N KCl or some other salt solution for 15 min. The ESR spectra were recorded with a Varian E-9 spectrometer after draining the excess electrolyte from the ESR cells. Only spectra with the magnetic field perpendicular to the lamellar plane are reported here. In experiments with methylene blue, films were prepared without pigment, hydrated with 0.1 N KCl and then exposed to 0.1 N KCl containing 10 μM methylene blue. Spectra were obtained after draining the methylene blue solution. Illumination of the films in the cavity of the spectrometer was carried out using either a

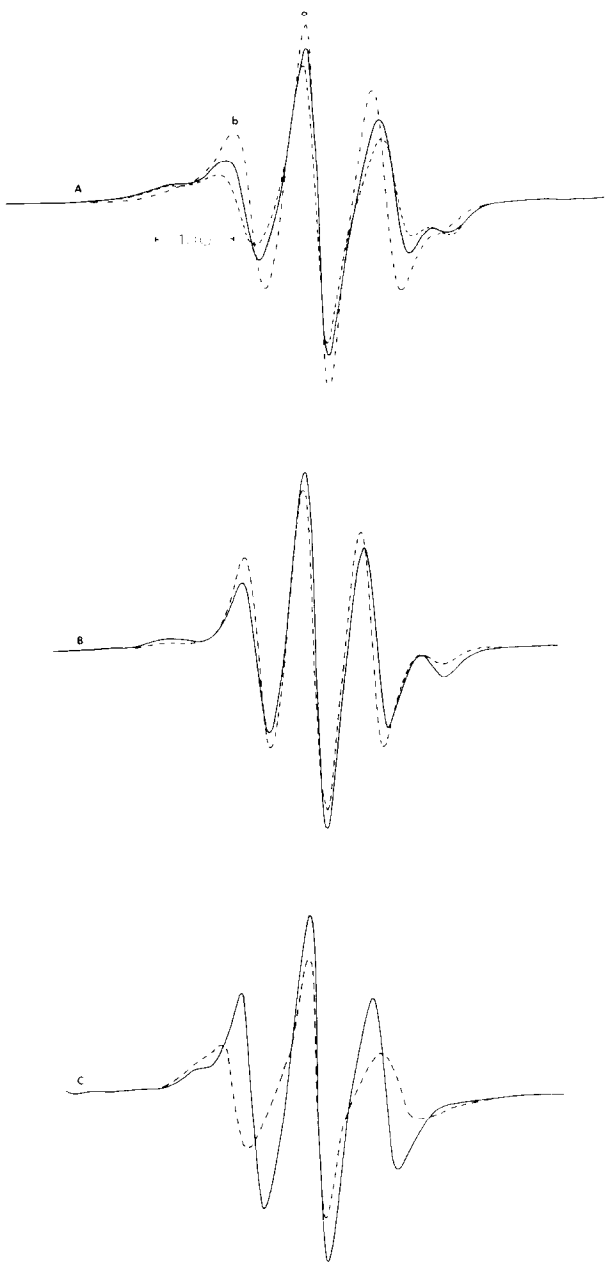

Fig. 1. ESR spectra of CSL in egg lecithin multibilayers containing light-sensitive pigments. A) (–·–) egg lecithin–CSL hydrated with 0.1 N KCl; (—) egg lecithin–CSL hydrated with 10^{-5} M methylene blue and 0.1 N KCl, in dark; (—) egg lecithin–CSL hydrated with 10^{-5} M methylene blue and 0.1 N KCl, in light (tungsten lamp). B) Multibilayers containing egg lecithin, cholesterol and chlorophyll a, and hydrated with 0.1 N KCl; (---) spectrum in dark, (—) spectrum in light (tungsten lamp). C) Multibilayers containing egg lecithin and retinal; (—) spectrum in dark, (---) spectrum in light (tungsten lamp).

760 W Tungsten filament projector lamp or a Bausch and Lomb monochromator (Cat. #33-86-07) and Zenon arc. A 5% $CuSO_4$ solution (10 cm) was used to filter out the infrared radiation.

3. Results and discussion

Illumination of the multibilayer films containing retinal, retinol, or chlorophyll a, or exposed to methylene blue solutions, produced an ESR spectral change (fig. 1). In the absence of pigments, no change in ESR spectra was observed. Photo-induced spectral changes were maximal in the region of absorption bands in the visible. The maximal effect occurred at 670 nm for methylene blue, at 450 nm–650 nm for chlorophyll a, and at 350–375 nm for retinal and retinol. These effects are attributed to pigment molecules incorporated into individual bilayers. The possibility that they are due to pigment molecules in the aqueous interbilayer space is considered unlikely for retinal, retinol and chlorophyll a. These materials are soluble in organic solvent but not in water [13], hence they would partition in favour of the lipid portion in water–lipid lamellar phases, an expectation consistent with results of absorption and fluorescence spectroscopic studies [14–18]. However, methylene blue would be expected to be present in the lipid as well as aqueous layers because it is soluble in both water and organic solvents. Additional evidence suggesting localization of retinol, retinal and chlorophyll a in lipid regions is provided by studies of the effects of their concentration. In the dark retinal and retinol at concentrations less than 2 mg/ml behave like cholesterol [19], inducing greater orientation of the probe. However, at concentrations exceeding 3 mg/ml other dark effects are observed with these materials as well as with chlorophyll a. These dark effects will not be discussed in detail since they are not immediately relevant.

The magnitude of the light-induced ESR spectral changes observed with chlorophyll a depended on the anion in the hydrating solution. Among carboxylate anions spectral changes increased in the order formate > acetate > propionate. Somewhat larger perturbations were caused in the presence of Cl^- and SO_4^{2-} at pH values 2.5 to 5.0 (fig. 2). These anion effects have parallels with light-induced swelling of chloroplasts [20, 21]. The light-induced ESR spec-

Table 1
Spectral parameters of CSL in lecithin multibilayers containing light-sensitive pigments.

Pigments	Hyperfine splitting (G)		b/a (see fig. 1 and text)		Change in θ_0	
	Dark	Light	Dark	Light	Dark	Light
None	8.50	8.50	0.56	0.56	32°	32°
Cholesterol	7.0	7.0	0.76	0.76	20°	20°
Chlorophyll a*	7.25	8.0	0.67	0.50	26°	36°
All-*trans* retinal	8.5	10.5	0.62	0.40	29°	48°
Methylene blue	9.65	10.5	0.44	0.35	42°	55°

* Chlorophyll a data refers to films containing chlolesterol.

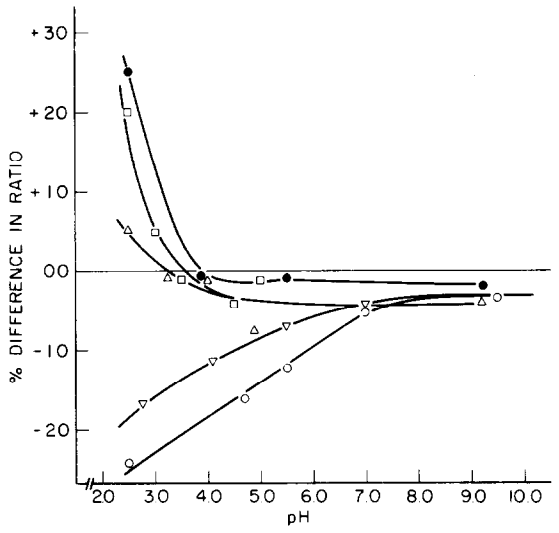

Fig. 2. Effect of anion and pH on the percent difference in a/b ratio between light and dark egg lecithin–chlorophyll a of multibilayers. (○—○—○) KCl; (△—△—△) K_2SO_4; (●—●—●) formate; (□—□—□) acetate; (▲—▲—▲) propionate.

tral shift observed with chlorophyll a was reversible only in the presence of organic anions; it was not reversible with methylene blue, retinal or retinol.

In addition to the spectral shifts, which denote a change in the orientation distribution of the probe, illumination also caused a decrease in spin probe signal intensity. The wavelength dependence of the decay parallelled that of the spectral shifts. The signal decay is presumed to be due to photoproduction of free radicals or electrons which react with the spin probe.

The nature of the ESR spectral shifts, summarized in table 1, can be interpreted in terms of a change in the width of the distribution of spin probe long axes about a perpendicular to the bilayer plane [19]. An increasing degree of order (orientation) is manifest in the ESR spectra by a decrease in the intensity of the extreme low and high field lines and a decreased width and separation of the remaining lines. An arbitrary measure of orientation is the ratios of peaks marked a and b in fig. 1. For perfect order this ratio approaches 1, for no order it approaches zero. In the theoretical treatment the distribution of label orientations is given by $\exp(-2\theta^2/\theta_0^2)$, where θ is the angle between the long axis of the spin label and a perpendicular to the multibilayer plane and θ_0 is the parameter characterizing the width of the distribution. Thus, decreased order is characterized by an increased θ_0 necessary to simulate the experimental spectra. For example, the three spectra of fig. 1A correspond to distribution widths of approx. 32, 42 and 55°.

The spectral shift caused by illumination in the presence of the retinal or retinol could result from *cis–trans* photoisomerization. The all-*trans* form may convert to one or another *cis* isomer(s) on illumination [22]. These latter conformations are bent, and cannot pack as tightly as the linear *trans* isomer in a bilayer. Perturbations in lipid packing would be produced, which would be reflected in the probe orientation distribution. The feasibility of this mechanism was confirmed by spectra obtained in the dark of films incorporating 13-*cis* retinal. These always showed a wider probe distribution than those containing the all *trans* isomer.

An alternative mechanism, which may also be

applicable to methylene blue and chlorophyll a, is that the effect depends on photopotentials across individual bilayers similar to those found in pigment-containing Black Lipid Membranes [7, 8, 23]. The mechanism of producing such photopotentials is still speculative and may depend ultimately on photo-induced free radicals [24–26] and/or electrons [27]. The photopotential could effect probe orientation distribution by changing spacings between lipids, and bilayer dimensions.

Although the present data do not allow us to establish the mechanism of the light-induced changes in the orientation distribution of the probe, hence that of the lipids, the important observation is that such changes occur. Spin probe studies have suggested that changes in orientation distribution of lipids in bilayers can be correlated with changes in membrane functions [11, 28–30]. Therefore, the present results suggest that light-induced changes in the structural arrangement of the lipids in membrane bilayers containing chlorophyll a or retinal could play a role in photosynthesis or vision. The case of methylene blue is of interest because of its solubility in water as well as in nonpolar media. This suggests that amphiphilic cellular components which absorb visible light may induce photosensitivy in naturally-occurring lipid bilayers.

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