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LIGHT-INDUCED FREE RADICAL OXIDATION OF MEMBRANE LIPIDS IN PHOTORECEPTORS OF FROG RETINA

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SUMMARY

Exposure to light of frog retinae and rod outer segments results in accumulation of free radical oxidation products (hydroperoxides) in the lipid phase of photoreceptors. The action spectrum of this process is similar to the absorption spectrum of the visual pigment rhodopsin and the spectral sensitivity curve of frog retina, indicating that the photosensitive pigment participates in the induction of lipid photooxidation.

INTRODUCTION

Photoreceptor membranes are metabolically active fluid membranes, characterized by a high level of polyunsaturated fatty acids (especially docosohexaenoic – more than 30%)[1], which appear to have functional significance. Lack of essential fatty acids in the diet leads to a negligible decrease in the content of polyene fatty acids in the major phospholipids of retina photoreceptor membranes, while distinctive features of vitamin F deficiency develop in other tissues[2]. The location of part of the photopigment molecule (rhodopsin) in the lipid hydrocarbon layer of the retinal receptor disc membrane and the sinking of the molecule into the lipid layer on bleaching[3,4] provide evidence in a possible immediate participation of the lipids surrounding the rhodopsin molecule in the mechanisms of the visual process.

It is also well known that polyenoic lipids, including phospholipids of various cell membrane structures, are able to undergo free radical oxidation. In the initial stages of this process polar hydroperoxide groups occur in hydrophobic fatty acid residues[5–8]. The chain of metabolic conversions of phospholipid acyls thus started brings about the formation of lysophosphatides, alterations in the structure of membrane units and decreases in activity of membrane-bound enzymes, as is observed after treatment with phospholipases and detergents[9]. These considerations, together with the reported chlorophyll-sensitized photooxidation of fatty acids, induced by low-energy quanta of visible light[10,11], encouraged us to study free radical oxidation of membrane photoreceptor lipids of the retina and to investigate the role of the rhodopsin complex in this process of photooxidation.

EXPERIMENTAL

Experiments were run using frogs (*Rana temporaria*) which had been dark adapted for 2 h. After 30 min of light (or dark) exposure, lipids were extracted by a chlorophorm–methanol mixture (2:1, v/v)[12] from the whole retinae or from isolated rod outer segments, the latter being sedimented (CLR-centrifuge, swing-out rotor RK-25 5000 $\times g$ for 30 min) in a sucrose density gradient (45%, 35%, 28%) according to Kimura *et al.*[13]. The primary products of free radical oxidation (hydroperoxides) were analysed immediately. Hydroperoxide content was determined by means of the absorbance at 230 nm, which is the absorption maximum characteristic of conjugated diene groups in polyunsaturated lipids (double-beam spectrophotometer SF-8)[14] and by means of polarographic assay with mercury-dropping electrodes in a system of organic solvents (LP-7 polarograph)[15]. To avoid lipoperoxidation *in vitro*, all operations during lipid extraction and analysis were performed in an inert atmosphere at ice-cold temperature. All results are statistically significant ($P \leqslant 0.01$), when Student's t-test is applied.

The lipid hydroperoxide content in illuminated retinae increases over an intensity range from 900 to 1600 lux, with a maximum at 1100 lux (Table I), while in

TABLE I

LIPID HYDROPEROXIDE CONTENT OF RETINA AND BRAIN

Spectrophotometric data; ultraviolet absorption of lipids soluted in ethanol–hexane (2:1, v/v) at $\lambda_{max} = 230$ nm (1 cm optical path; absorbance of solution containing 1 mg of lipids in 1 ml).

No.	*Tissue*	*Lipid hydroperoxides*			
		Dark adapted	*Illuminated*		
			900 lux	*1100 lux*	*1600 lux*
1	Retina	2.0 ± 0.1	2.1 ± 0.3	3.2 ± 0.3	2.9 ± 0.3
2	Brain	1.2 ± 0.2	—	—	1.3 ± 0.3

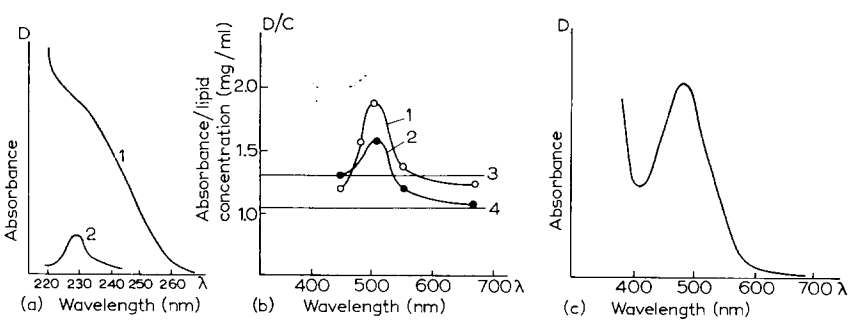

Fig. 1. (a) Ultraviolet absorption spectra of lipids from rod outer segments of frog retina. 1, lipids from illuminated segments; 2, difference spectrum (light–darkness). (b) Action spectra of photochemical lipid oxidation in retinae and rod outer segments (spectrophotometric data). 1, light-exposed rod outer segments; 2, light-exposed retinae; 3, dark-adapted rod outer segments; 4, dark-adapted retinae. (c) Absorption spectrum of rhodopsin extract from rod outer segments (2% Triton X-100 in 0.05 M Tris–HCl buffer, pH 7.4).

frog brain homogenate, which has a similar lipid composition[16], exposure to light in the same dose range does not result in accumulation of lipoperoxidation products.

To elucidate the role of the rhodopsin complex in lipid-free radical oxidation the action spectrum of this process was studied using retinae and rod outer segments (Figs 1 and 2). After illumination by equivalent light pencils ($6.5 \cdot 10^{14}$ quanta/second · cm^2) of different wavelengths (obtained by means of a set of interference filters with transmittance maxima at 455, 494, 508, 539, 551 and 668 nm) the maximum concentration of lipid hydroperoxides was found to occur at 508 nm (light-exposure at 508 nm leads to a 95% bleaching of the rhodopsin in rod outer segments), gradually decreasing to the level of non-illuminated controls at shorter (455 nm) as well as longer (668 nm) wavelengths. Attention is drawn to the following facts: the magnitude of lipid free radical oxidation is higher in rod outer segments than in whole retinae, indicating that lipoperoxidation is preferentially located in the membranes of photoreceptor

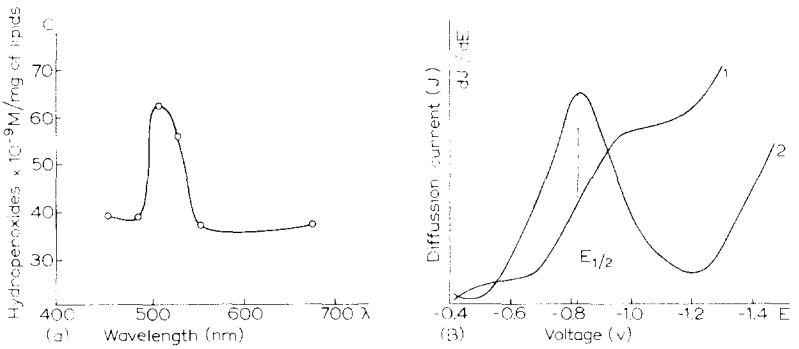

Fig. 2. (a) Action spectrum of photochemical lipid oxidation in rod outer segments (polarographic data). (b) Polarogram of lipids from rod outer segments of frog retina ($E_{1/2} = -0.78 +$ − 0.83 V). 1, integral polarogram; 2, derivative polarogram.

TABLE II

LIPID HYDROPEROXIDE ACCUMULATION IN ROD OUTER SEGMENTS

		Rod outer segments	*Rod outer segments + tocopherol ($5 \cdot 10^{-4}$ M)*
Spectrophotometry *	Dark	1.37 ± 0.14	1.42
	Light	1.90 ± 0.19	1.37
Polarography **	Dark	45.4 ± 3.7	43.2
	Light	67.2 ± 2.2	42.0
Craft polymerisation ***	Dark	2.99 ± 0.39	
	Light	4.08 ± 0.40	

* Spectrophotometric data; ultraviolet absorption of lipids soluted in ethanol hexane (2:1, v/v) at $\lambda_{max} = 230$ nm (1 cm optical path; absorbance of solution containing 1 mg of lipids in 1 ml).

** Polarographic data; $\times 10^{-9}$ M hydroperoxides/mg lipids.

*** Dried pellet of rod outer segments suspension after incubation with ^{14}C-labelled acrylamide-monomer was Soluene treated and put into SL-40 Liquid Scintillation Spectrometer in toluene scintillation mixture (cpm/mg dry weight $\times 10^3$).

cells. The action spectrum of photochemical lipid oxidation is similar to the absorption spectrum of visual pigment rhodopsin and the spectral sensitivity curve of the frog retina, where rods are predominant (Figs 1 and 2). This is evidence for a participation of rhodopsin in photooxidation. At the same time no accumulation of lipid hydroperoxides was observed when rod outer segments were exposed to light in the presence of the natural inhibitor of free radical reactions, α-(+)-tocopherol ($5 \cdot 10^{-4}$ M) (Table II). It should also be noted that in an attempt to detect intermediate free radical centres using the method of Craft polymerisation of ^{14}C-labelled acrylamide monomers[17,18] we observed an increased incorporation of the label (copolymerisation to free radical centres) in the illuminated (508 nm) rod outer segments as compared to dark-adapted controls (Table II, detailed data will be published elsewhere).

The experimental results demonstrate the induction of free radical oxidation reactions in the lipid phase of retina photoreceptor membranes by light mediated by the visual pigment rhodopsin. The question whether rhodopsin is a direct sensitizer of photooxidation of associated phospholipids or participates in this process causing generalised light-induced changes of photoreceptor membrane conformation[3,19] forms the subject of subsequent research.

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