PHOTOSENSITIZATION AND QUENCHING OF SINGLET OXYGEN BY PIGMENTS AND LIPIDS OF PHOTORECEPTOR CELLS OF THE RETINA

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1. Introduction

Studies of $^1O_2$ generation and quenching in solutions of pigments and lipids isolated from photoreceptor cells are important for understanding the molecular mechanisms of photodamage of the retina [1–5]. The first works in this field were published by Delmelle ([4] and references therein), who attempted to demonstrate the ability of retinal to produce $^1O_2$, using indirect 'chemical' methods of $^1O_2$ detection. Here we have employed a more accurate method, i.e., registration of oxygen luminescence observed in air-saturated solutions of various pigments ([5,6] and references therein). With the aid of this emission the following questions were studied: is retinal able to generate $^1O_2$ and what is the efficiency of $^1O_2$ interaction with retinal and photoreceptor lipids?

2. Materials and methods

The luminescence was measured in an apparatus supplied with a photomultiplier and a mechanical phosphoroscope as in [6]. CCl_4 of spectral grade was used as a solvent. The retinals were synthesized in the M. V. Lomonosov Institute of Fine Chemical Technology. Egg lecithin, phosphatidylcholine and phosphatidylethanolamine from frog retina were isolated according to [7] and purified by thin-layer chromatography.

3. Results and discussion

3.1. *Generation of $^1O_2$ by retinal*

Illumination of air-saturated solutions of 11-*cis*, 13-*cis* and all-*trans* retinals results in a delayed luminescence in the region of $^1O_2$ emission from $^1\Delta_g$ state (1270 nm). The excitation spectra coincide with the absorption spectra of the pigments (fig.1.). The lifetime of the luminescence (τ) is equal to 28 ± 3 ms, which corresponds to τ $^1O_2$ in CCl_4 [6]. The $^1O_2$ quenchers, β-carotene, α-tocopherol, 1,3-diphenylisobenzofurane (DPIBP) and 1,4-diazabicyclo(2,2,2)-octane (DABCO) quench the luminescence. The rate constants of quenching (k_q) coincide with those for $^1O_2$ determined by other methods (table 1).

The data obtained suggest that the luminescence results from emission of $^1O_2$ formed due to energy transfer from the photoexcited molecules of retinals. The quantum yield of $^1O_2$ generation (γ_g) was evaluated by comparing the intensity of $^1O_2$ emission in CCl_4 solutions of retinals and porphyrins (protoporphyrin and tetraphenylporphyrin, γ_g = 0.8 [6]). The obtained value of γ_g for the retinals is equal to 0.55 ± 0.1, which exceeds the value reported by Delmelle for retinal in ethanol (0.09) [4].

3.2. *Quenching of $^1O_2$ luminescence*

This was studied in solutions of protoporphyrin (1.5×10^{-7} M) upon illumination by red light at 627 nm. The retinals, retinyl acetate and lipids were found to quench $^1O_2$ in accordance with the Stern–Volmer equation (fig.1). The values of k_q calculated from this equation suggest that these compounds are relatively weak quenchers of $^1O_2$ (table 2). Amongst the retinals the all-*trans* isomer has the highest and 13-*cis* has the lowest activity. The quenching activity of the phospholipids studied was sufficiently lower and did not practically depend on their molecular structure.

In order to elucidate the mechanism of quenching

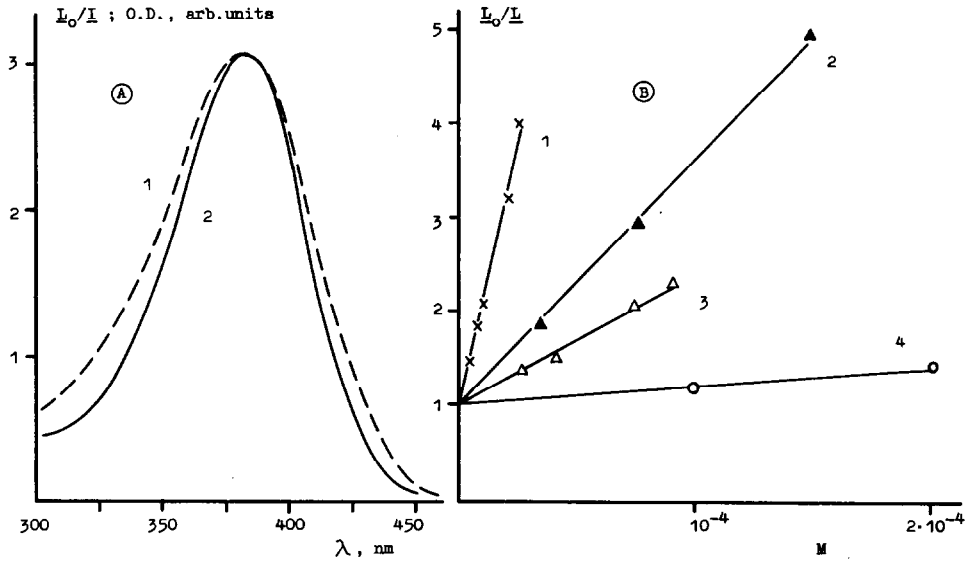

Fig.1. (A) Comparison of the absorption spectrum (1) with the excitation spectrum (2) of the $^1O_2$ luminescence in solutions of 11-*cis* retinal in CCl_4. (B) Quenching of $^1O_2$ luminescence by all-*trans* (1), 11-*cis* (2) and 13-*cis* (3) retinals and ROS phosphatidylcholine (4). L, L_0, intensities of the luminescence in the presence and in the absence of the quencher; I, intensity of the exciting light; OD, absorbance.

the reaction between $^1O_2$ and all-*trans* retinal was studied in more detail [5]. It was shown that $^1O_2$ quenching results in a decrease of the absorption bands corresponding to the original pigment and an appearance of new bands at 325 and 340 nm. This process was inhibited by β-carotene, the inhibition rate constant being equal to that for $^1O_2$ quenching. The dependence of the reciprocal value of the quantum yield of bleaching ($1/\psi_{ox}$) on the reverse concentration of retinal ($1/C$) appeared to be linear (fig.2). The intersection of the straight line with the ordinate ($1/C = 0$) corresponds apparently to the maximal value of ψ_{ox}, i.e., $1/\psi_{ox}^{max}$. Such dependence is known to be characteristic of the photosensitization process involving $^1O_2$. In this case $1/\psi_{ox}^{max} = k_q/\gamma_g \times k_{ox}$, where k_{ox} is the rate constant of retinal oxidation by $^1O_2$ (see also [5,6] and references therein). Hence, the value of k_{ox} can be determined from the following equation:

$$k_{ox} = \frac{\psi_{ox}^{max} \times k_q}{\gamma_g}$$

Table 1
Values of k_q ($M^{-1}\ s^{-1}$) for quenching of $^1O_2$ and the luminescence sensitized by all-*trans* retinal

Quencher	Luminescence (own data)	$^1O_2$ (literature data)
DABCO	6.6×10^6	7.3×10^6 [8]
DPIBP	8.0×10^8	7.0×10^8 [9]
β-Carotene	8.0×10^9	1.1×10^{10} [10]
α-Tocopherol	10^8	1.7×10^8 [11]

Table 2
Values of k_q ($M^{-1}\ s^{-1}$) for retinals and lipids (errors ±10%)

Quencher	k_q	Quencher	k_q
All-*trans* retinal	3.7×10^6	Egg lecithin	5×10^4
13-*cis* retinal	3.6×10^5	ROS phosphatidylcholine	10^5
11-*cis* retinal	10^6	ROS phosphatidylethanol-	
Retinyl acetate	7×10^5	amine	10^5

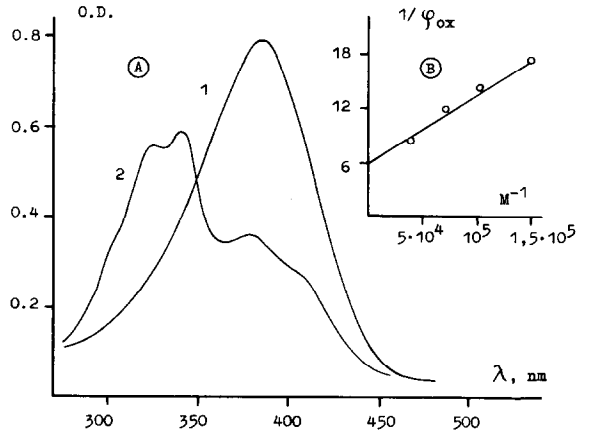

Fig.2. Protoporphyrin-sensitized photooxidation of all-*trans* retinal in CCl_4. (A) Absorption spectra of retinal before (1) and after (2) photooxidation. (B) Dependence of quantum yield of photooxidation on retinal concentration. Porphyrin was 1.5×10^{-7} M, the wavelength of the actinic light is 545 nm.

The values of k_q and γ_g are given above. The value of ψ_{ox}^{max} was determined by comparing the efficiency of protoporphyrin-sensitized photobleaching of retinal and naphtacene under the same illumination conditions. The absolute value of ψ_{ox}^{max} for naphtacene was established in [5]. In our experimental conditions it was 0.8. For retinal we have obtained $\psi_{ox}^{max} = 0.16$. Thus, $k_{ox} = 0.2 \times k_q = 7.5 \times 10^5\ M^{-1}\ s^{-1}$. It may be concluded therefore that retinals are mainly physical quenchers of $^1O_2$; however, the relative efficiency of the chemical quenching is rather high. We have not attempted to establish the role of chemical quenching in general quenching effect of lipids. However, a comparison with the data in [12] demonstrates that the obtained values of k_q exceed those of k_{ox} for methyl esters of oleic, linoleic and linolenic acids by $\leqslant$ 3-fold.

Thus, these data support the assumption that free retinal which is known to be always present in the photoreceptor cells might be an effective generator of $^1O_2$ in the retina. The results of experiments on luminescence quenching show that $^1O_2$ can attack at least three components of the photoreceptor membranes, i.e., phospholipids, retinal and α-tocopherol. The molar ratio of these components in the membranes is equal to 1000:<<10:1 [13], assuming that the amount of free retinal in the membrane is much lower than that of rhodopsin. Taking into account the values of k_q, we have to assume that the proportion between the rates of $^1O_2$ interaction with the above components corresponds to 10:<<1:10. This means that $^1O_2$ interacts mainly with α-tocopherol and phospholipids, whereas the destruction rate of the retinal is extremely low. Hence α-tocopherol and phospholipids play the role of a protector of the retinal in the photoreceptor membrane. In this connection it is noteworthy that illumination of high intensity results in accumulation of lipid peroxides in the retinal photoreceptors, the effect being enhanced in vitamin E-deficient animals [2,3].

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References

[1] Noell, W. K., Walker, V. S., Kang, B. S. and Berman, S. (1966) Invest. Ophthalmol. 5, 450–471.
[2] Kagan, V. E., Shvedova, A. A., Novikov, K. N. and Kozlov, Yu. P. (1973) Biochim. Biophys. Acta 330, 76–79.
[3] Kagan, V. E., Barybina, G. V. and Novikov, K. N. (1977) Bull. Exptl. Biol. Med. (Russian) 4, 411–413.
[4] Delmelle, M. (1978) Photochem. Photobiol. 27, 731–734.
[5] Krasnovsky, A. A., jr and Kagan, V. E. (1978) Dokl. Acad. Nauk SSSR (Russian) 242, 229–232.
[6] Krasnovsky, A. A., jr (1979) Photochem. Photobiol. 29, 29–36.
[7] Folch, J., Lees, M., Stanley, A. H. S., Meath, S. A. and Le Bason, F. N. (1951) J. Biol. Chem. 191, 883–891.
[8] Young, R. N. and Martin, R. L. (1972) J. Am. Chem. Soc. 94, 5183–5185.
[9] Stevens, B., Perez, S. R. and Ors, J. A. (1974) J. Am. Chem. Soc. 96, 6846–6850.
[10] Farmilo, A. and Wilkinson, F. (1973) Photochem. Photobiol. 18, 447–450.
[11] Stevens, B., Small, R. D., jr and Perez, S. K. (1974) Photochem. Photobiol. 20, 505–512.
[12] Matsushita, S., Terao, J. and Yamauchi, R. (1978) in: Tocopherol, oxygen and biomembranes (de Duve, C. and Hayaishi, O. eds) pp. 23–39, Elsevier/North-Holland, Amsterdam, New York.
[13] Daemen, F. J. M. (1973) Biochim. Biophys. Acta 300, 255–288.