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Retinal Damage by Light: Possible Implication of Singlet Oxygen*

M. Delmelle

Département de Physique, Atomique et Moléculaire, Université de Liège, B-4000 Sart Tilman, Belgium

Abstract. A new hypothesis is proposed in an attempt to explain the mechanism of the irreversible damage which can be induced in the retina by visible light. Upon illumination, retinal generates singlet oxygen and this reactive species can produce lipid peroxidation which in turn may induce membrane instability.

Key words: Retinal damage – Light – Singlet oxygen.

Noell and coworkers (1966) have discovered that the retina can be irreversibly damaged by visible light. Prolonged exposure of rat retinae to diffuse illumination leads to an irreversible reduction in the amplitude of the electroretinogram and to the death of the visual cells associated with pigment epithelium degeneration. Such damage was originally observed in albino rats but has also been reported in pigmented rats as well as in other species such as the mouse and the monkey. In Noell's experiments, the action spectrum of the damaging effect approximates that of visual exciatation as measured by the electroretinogram. More recently however, Ham et al. (1976) studied the effect of eight monochromatic laser beams on monkey retinae and they reported a steep increase in retinal sensitivity with decreasing wavelength (458 and 442 nm). Noell and Albrecht (1971) have shown that vitamin A deficiency protects against light damage although retinol itself does not seem to be the toxic agent. The molecular mechanisms responsible for the deleterious effect of visible light are at present unknown. It is likely that several photochemical pathways might be involved.

The purpose of the present communication is to propose an hypothesis which seems worthwhile to consider in attempting to understand the molecular mechanisms of the light damage. It is well known that light absorption can produce detrimental effects in some living systems (for a review, see: Spikes and Straight 1967). The so-called photodynamic reactions proceed by way of light excitation of an

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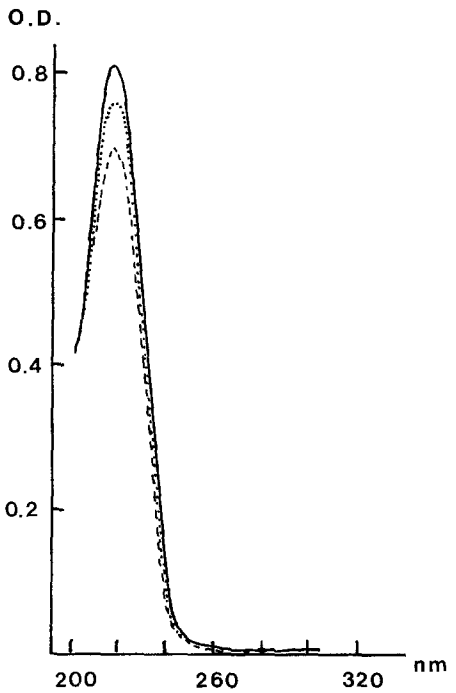

Fig. 1. Difference spectra of 2,5 dimethyfuran (10^{-4} *M*) in retinal solution (ethanol, 2.4 10^{-5} *M*) before (——) and after illumination (1mn., 360 nm. HBO 500 and Schott Jena monochromatic filter) in absence (– – –) and in presence (· · · · ·) of β-carotene. The two cuvettes, sample and reference, contained retinal (——;– – –) or retinal plus β-carotene (· · · · ·); only the sample cuvette contained in addition the dimethylfuran. Both were illuminated in identical conditions

endogeneous or an exogeneous photosensitizer. Two major mechanisms have been distinguished (Foote, 1976): in the first, the trigger is a chemical reaction occurring between the excited sensitizer and a substrate molecule of the medium. Alternatively, the second mechanism relates exclusively to specific interactions between the excited sensitizer and oxygen. The quenching effect of oxygen can be either physical or chemical. The former type of interaction leads to the production of oxygen molecules excited in their singlet states ($^1\Delta_g$) while the chemical quenching implies an electron transfer from the sensitizer to oxygen, yielding a superoxide ion ($O_2^{\cdot-}$). In general, this latter reaction occurs on less than 1% of the deactivating collisions between oxygen and sensitizer triplets (Foote, 1976).

I suggest that bright illumination of the retina could lead to singlet oxygen production and this metastable species could in turn trigger chemical reactions which would be damaging for visual photoreceptors. The rationale for such an hypothesis is based on the following observations. Lion et al. (1976) reported recently on a new method for detecting singlet oxygen and showed that retinal illumination leads to singlet oxygen production. This phenomenon is exemplified again in the following classical experiment: all-trans retinal in ethanol solution (2.4 10^{-5} *M*) is illuminated with monochromatic light (370 nm) in presence of dimethylfuran (DMF). This chemical which is known to be very reactive towards singlet oxygen (Foote, 1976), is

rapidly photooxidized as shown by the disappearance of its 215 nm absorption peak (Figure 1). When singlet oxygen quenchers (β-carotene, sodium azide) are introduced in the solution, the DMF photooxidation rate is markedly reduced, all other conditions being unchanged. Singlet oxygen production depends essentially upon the probability of intersystem crossing between singlet and triplet states. Retinal is known to have a good intersystem crossing yield (Bensasson et al., 1975). For rhodopsin, retinol as well as for protonated and unprotonated retinal Schiff bases, the intersystem crossing efficiency is probably small since, in those cases, no direct observation of the triplet manifold has been reported. However, the triplet states of retinal Schiff bases can be populated via an energy transfer (Alchalel et al., 1975). Moreover singlet oxygen may also be produced by quenching of singlet excited states (Gollnick et al., 1970).

If singlet oxygen production occurs within the retina, this could lead to membrane damage involving oxidation of either the proteins or the lipids or both. Inasmuch as lipids are concerned, singlet oxygen can induce their peroxidation and the following experiment gives a clue on that possibility. Egg lecithin liposomes were loaded with all-trans retinal (molar ratio lipid/retinal: 30–250). Upon illumination, lipid peroxidation occurs as measured by the thiobarbituric acid method (Placer et al., 1966). Singlet oxygen is clearly involved in the phenomenon since it can be enhanced or reduced by manipulating $^1O_2$ lifetime with a deuterated solvent or with specific quenchers. In addition to these results, by using the spin label technique, we have shown that light induced lipid peroxidation increases the fluidity of the model membrane (Delmelle, unpublished).

On the basis of those preliminary observations, the proposed hypothesis appears reasonable. Rod outer segment membranes are made up of highly unsaturated lipids which are known to undergo very rapid oxidation in vitro (Kagan et al., 1973; Farnsworth and Dratz, 1976). Clearly to test this hypothesis, attempts should be made in vivo to manipulate the light damaging effect by using specific quenchers of singlet oxygen. Such experiments may prove to be technically difficult, but more information is needed concerning the retinal sensitivity to oxygen tension as many problems in clinical treatments are thought to be related to this general area.

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