BIOCHEMISTRY

Photoreactions of Retinol and Derivatives sensitized by Flavins

CRYSTALLINE riboflavin is present in the eyes of several fishes and mammals (for example, *Galago crassicaudatus*)¹. Its role in vision, however, is obscure. One possible function may be that of a transducer to convert blue light (efficiently absorbed by the flavin but not by rhodopsin) into energy that can be utilized by rhodopsin producing the chemical signal. This conversion may be achieved by the trivial process of re-emission (the wavelength of fluorescence of riboflavin is at 530 mμ, which is favourable for absorption by rhodopsin)² or by direct energy transfer either of a singlet-singlet or of a triplet-triplet type.

In our studies we have chosen all-*trans*-retinol and the 13-*cis*-derivative as models for the photosensitive part of the visual pigment rhodopsin. This choice was primarily governed by the consideration that the absorption spectra of these compounds involve transitions of higher energy than the longest wavelength absorption of riboflavin. This, then, excludes the possibility of the trivial process or of singlet-singlet energy transfer in our system.

When an anaerobic solution of all-*trans*-retinol (1.6×10^{-5} molar) and lumiflavin (2×10^{-5} molar) in 98 per cent methanol/water is illuminated (using light composed of wavelengths above 400 mμ) a gradual decrease in the peak height of retinol at 328 mμ is observed (Fig. 1). No reaction is found for either of the reactants alone under these conditions. Lumiflavin can be replaced by riboflavin or flavin mononucleotide (FMN) and all-*trans*-retinol by 13-*cis*-retinol. In the dark no interaction is observed between retinol and lumiflavin either by spectrophotometry or by fluorescence methods.

The photoreaction is inhibited by 10^{-2} molar nickel sulphate, oxygen and potassium iodide (Fig. 1). Potassium iodide also quenches the fluorescence of lumiflavin but less efficiently than it quenches the photoreaction (Fig. 2).

Thin-layer chromatography of the reaction mixtures of 13-*cis*-retinol and lumiflavin has shown qualitatively the formation of all-*trans*-retinol. The reverse of this reaction was also observed although in this case the analytical procedure did not distinguish the 13-*cis*- from the 11-*cis*-isomer. The thin-layer chromatography was essentially as already described³.

Thus the flavins can sensitize a reaction of retinol similar to that observed in the ultra-violet illumination of retinol or retinal alone⁴. The *cis-trans* isomerization is undoubtedly accompanied by some destruction of the compound. We have no direct evidence for the mechanism

Fig. 1. Sensitized photoreaction of all-*trans*-retinol. ●, Retinol and lumiflavin; ■, retinol, lumiflavin and 10^{-2} molar nickel sulphate; □, retinol, lumiflavin and oxygen.

Fig. 2. Inhibition of sensitized photoreaction against fluorescence quenching by potassium iodide (concentrations shown in brackets).

of the photosensitization, but the quenching experiments suggest that the flavin triplet is involved as in other photoreactions of flavins⁵. Triplet-triplet energy transfer has been suggested for the lumichrome-sensitized isomerization of stilbene-4-carboxylic acid⁶ and the process described here may involve a similar mechanism. Alternatively the triplet may react directly with retinol to form a covalent labile intermediate similar to that formed in the reaction of phenylacetic acid with 3-methyl-lumiflavin⁷.

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ANN GORDON WALKER
G. K. RADDA

Department of Biochemistry,
University of Oxford.

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Localization of Lactic Dehydrogenase Isozymes in Lysosomal Fraction of the Neutrophil of Normal Human Blood

THE lysosomal fraction of the leucocyte is the principal source of peroxidase and hydrolases¹, and our unpublished results show that it also contains lactic dehydrogenase (LDH) usually found in the soluble compartment of cells. Vesell and Bearn² and Vesell and Osterland *et al.*³ have shown that whole homogenates and sonicates of the leucocyte contain four isozymes of lactic dehydrogenase.