The Active Site of the Visual Protein, Rhodopsin

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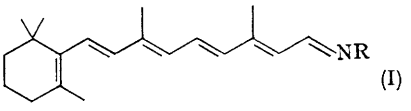
RHODOPSIN, the light-sensitive pigment of bovine retina, consists of an apoprotein, opsin, and a polyene aldehyde, 11-*cis*-retinal.[1] In this Communication we report a convenient procedure for the introduction of tritium label into rhodopsin and describe experiments which have led to the conclusion that the polyene aldehyde in rhodopsin is attached to an ϵ-amino-group of lysine.

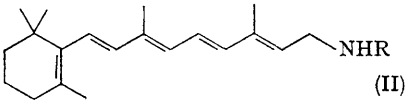
The presence of a Schiff base linkage between 11-*cis*-retinal and opsin was originally suggested by Morton and co-workers.[2] Convincing evidence in support of this hypothesis was provided by our previous work[3] and also that of Wald *et al.*[4] It was shown that the bleaching of rhodopsin in the presence of sodium borohydride resulted in the irreversible bonding of the retinyl moiety with opsin, and the overall reaction was considered to involve the conversion of an imino-linkage into a dihydro-form as shown below by the equation (Ia → IIa).

For reasons described in detail elsewhere,[3] the reduced derivative was named as dihydrometarhodopsin (IIa). We have now taken advantage of the stability of the bond which exists in dihydrometarhodopsin (IIa) to determine the active site of the visual protein.

11-*cis*-Retinal was reduced with tritiated sodium borohydride (6·7c/mmole) and the resulting tritiated 11-*cis*-retinol was oxidized with MnO_2.[5] Radioactive 11-*cis*-retinal thus obtained was incubated with opsin at 20° and pH 6·4 for 2 hr. to give a solution of rhodopsin. The excess 11-*cis*-retinal in the solution was converted into the corresponding oxime by addition of hydroxylamine, which does not attack the retinyl moiety linked at the active site.



(I)



(II)

a; NR=protein, b; NR=ϵ-lysyl,
c; NR=α-lysyl

This solution of labelled rhodopsin was treated with sodium borohydride in the presence of light,

and the reaction mixture containing dihydrometarhodopsin (IIa) was then freeze-dried. The residue was washed with methanol–ether to remove retinyl oxime. The resulting solid (A) gave 24×10^6 counts/min. and an absorption maximum at 335 mμ with a total extinction equivalent to 2 μmole of the retinyl moiety. Chromatography of the solid revealed a minimum of 92% of the radioactivity in the protein fraction, the remaining activity corresponding to a nonpolar material, located at the solvent front in most amino-acid solvent systems. Solid (A) was used for analytical work without any further purification.

Preliminary degradative work indicated the involvement of a lysine residue at the active site of rhodopsin and this led to the development of the final experimental procedure which is described below.

The solid (A) containing dihydrometarhodopsin (IIa) (0·5 μmole equivalent of the retinyl moiety; 6×10^6 counts/min.) was mixed with carrier ε-*N*-retinyl-lysine (IIb); 8 μmole) and the solution then hydrolyzed with 5 N-NaOH at 110° for 24 hr. The neutralized hydrolysate was freeze-dried and the retinyl-amino-acid extracted with 20% chloroformic methanol. This extract contained 50% of the original radioactivity. On chromatography in five different amino-acid solvent systems, 40—55% of the activity in the extract was always associated with the ε-*N*-retinyl-lysine fraction (IIb), whilst the only other significant activity (15—25%) was located at the solvent front.

The extract was reduced catalytically with hydrogen in the presence of palladium on charcoal, and the resulting solution, on chromatography, was again found to contain 40—55% of the activity in the ε-*N*-perhydroretinyl-lysine fraction (IIb totally reduced). The activity at the solvent front was also unchanged. In the solvent systems employed, we were unable to separate ε-*N*-perhydroretinyl-lysine from α-*N*-perhydroretinyl-lysine (IIc totally reduced). For the complete identification of the active site, it was therefore essential to differentiate an ε-derivative from an α-derivative.

When ε-*N*-perhydroretinyl-lysine was treated with dinitrofluorobenzene in the presence of sodium hydrogen carbonate,[6] a di-DNP derivative with R_F† of 0·5 was obtained, whereas α-*N*-perhydroretinyl-lysine, in a parallel experiment, gave a mixture of a mono-DNP derivative with R_F 0·2 and a di-DNP derivative with R_F 0·7. The totally reduced extract of the protein hydrolysate was purified to isolate the active component, and a sample (10^6 counts/min.) was treated with dinitrofluorobenzene under identical conditions. The majority of the activity ($0{\cdot}85 \times 10^6$ counts/min.) was located at the position of the di-DNP derivative of ε-*N*-perhydroretinyl-lysine. No activity could be detected in the regions corresponding to the DNP derivatives of α-*N*-perhydroretinyl-lysine. These results prove that the derivative of rhodopsin which is reduced with sodium borohydride contains the retinyl moiety attached to an ε-amino-group of lysine.

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† Chloroform–methyl alcohol–glacial acetic acid, 95 : 5 : 1.

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