

## Flash Photolysis of Rhodopsin

## I. Measurements on Bovine Rod Outer Segments

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Reactions of the bovine visual pigment rhodopsin up to the formation of metarhodopsin II were measured kinetically by means of flash light photolysis<sup>1-3</sup>. Rod outer segments as well as fragments of rod outer segments sedimenting between 4000 g and 30 000 g were used as test material. The fragments were produced according to the following method: Isolated rod outer segments<sup>3</sup> were treated with buffered (pH 7) 0,5% digitonin solution for 30 min. and then centrifuged at 4000 g. The supernatant was separated from the precipitate and then centrifuged at 30 000 g. The resulting precipitate consisted of fragments of rod outer segments sedimenting between 4000 g and 30 000 g\*\*\*.

The amount of fragments obtained from a certain quantity of rods after digitonin treatment varies with the temperature at which the rods were previously frozen and stored. Rods frozen and stored at  $-195^{\circ}\text{C}$  in liquid nitrogen yield more fragments and less

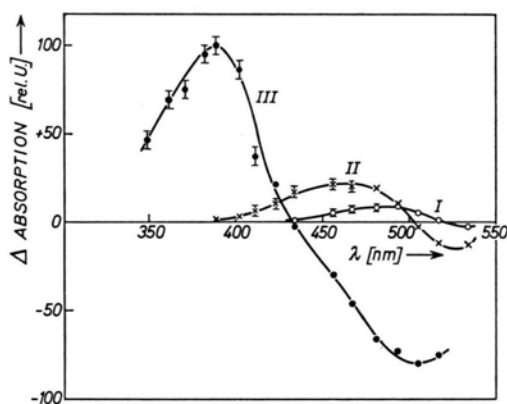


Fig. 1. Separated difference spectra measured on rod outer segments after a flash. I. Rhodopsin—lumirhodopsin; II. lumirhodopsin—metarhodopsin I; III. metarhodopsin I — metarhodopsin II.

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\*\*\* The supernatants resulting from centrifugation are rhodopsin digitonin solutions. Measurements on these solutions will be reported in a further paper.

<sup>1</sup> G. PORTER, Proc. Roy. Soc., Ser. A [London] **200**, 284 [1950].

<sup>2</sup> H. T. WITT, Fast Reactions and Primary Processes in Chemical Kinetics, Interscience Publishers, p. 81, 1967.

rhodopsin solution whereas rods frozen and stored at  $-15^{\circ}\text{C}$  yield more rhodopsin solution and less fragments.

The reactions occurring after a flash were characterized by difference absorption spectra and activation energies<sup>3</sup>.

Fig. 1 shows three difference absorption spectra measured on suspensions of rod outer segments. The same spectra also occur in fragments of rod outer segments treated with digitonin. These difference absorption spectra correspond with extinction spectra having the following extinction maxima:

I:  $\lambda_{\text{max}} = (498 \pm 4)$  nm, II:  $\lambda_{\text{max}} = (485 \pm 4)$  nm, III:  $\lambda_{\text{max}} = (386 \pm 6)$  nm.

The reactions associated with these difference spectra are most probably rhodopsin—lumirhodopsin, lumirhodopsin—metarhodopsin I, and metarhodopsin I—metarhodopsin II<sup>4-9</sup>.

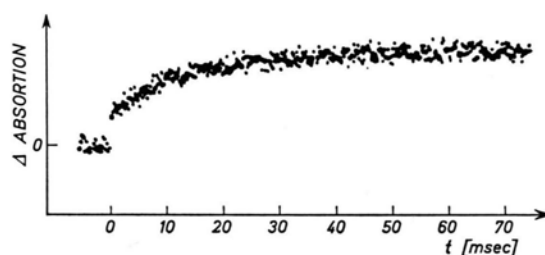


Fig. 2. Time course of the absorption changes at 434 nm measured on rod suspensions. Suspension medium: modified Ringe's solution; temperature  $0^{\circ}\text{C}$ ; flash at  $t=0$ .

Fig. 2 shows the time course of the absorption change at 434 nm measured on rod suspensions. The fast, temporally not resolved rise of the absorption includes all reactions up to the formation of lumirhodopsin. (Even at  $-25^{\circ}\text{C}$  the rise could not be resolved with a time resolution of 100  $\mu\text{sec}$ .) The further rise (temporally resolved) can be attributed to the reaction lumirhodopsin—metarhodopsin I and is characterized by the difference absorption spectrum II. Up to a temperature of  $20^{\circ}\text{C}$  this reaction could be observed in rod suspensions as well as in rod fragments treated with digitonin. At higher temperatures the signal to noise ratio was smaller than 1 and did not allow a definite statement. According to these measurements

<sup>3</sup> G. V. SENGBUSCH, Thesis, Technische Hochschule Aachen, Germany, 1970.

<sup>4</sup> R. A. CONE, Exp. Eye Res. **8**, 246 [1969].

<sup>5</sup> R. A. CONE, Processing of Optical Data by Organisms and by Machines, p. 187, Academic Press, 1969.

<sup>6</sup> W. A. HAGINS, Nature [London] **177**, 989 [1956].

<sup>7</sup> E. W. ABRAHAMSON and S. E. OSTROY, Progress in Biophysics and Molecular Biology, p. 181, Pergamon Press 17, 1967.

<sup>8</sup> V. J. WULFF, H. LINSCHITZ, and E. W. ABRAHAMSON, Ann. N. Y. Acad. Sci. **74**, 281 [1958].

<sup>9</sup> R. G. MATTHEWS, R. HUBBARD, P. K. BROWN, and G. WALD, J. gen. Physiol. **47**, 215 [1963].

the reaction lumirhodopsin—metarhodopsin I can be observed in rods at temperatures above 0 °C. This agrees with new measurements by CONE<sup>10</sup>. The half life time of the reactions is  $\tau_{1/2} = (100 \pm 50) \mu\text{sec}$  (extrapolated to 37 °C).

Fig. 3 shows the temperature dependence of the reciprocal relaxation time for the reactions lumirhodopsin—metarhodopsin I and metarhodopsin I—metarhodopsin II in rod suspensions. The values measured on rod fragments treated with digitonin do not differ from those given for rod suspensions within limits of error.

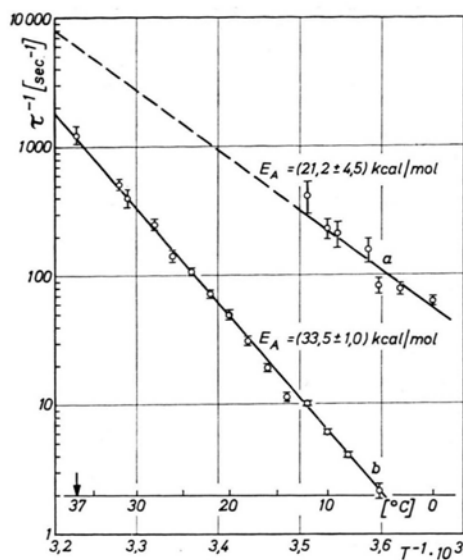


Fig. 3. Temperature dependence of the reciprocal relaxation time for the reactions lumirhodopsin—metarhodopsin I and metarhodopsin I—metarhodopsin II in rod suspensions. Suspension medium is a modified R i n g e r's solution.

<sup>10</sup> R. A. CONE, personal communication.

Fig. 1.  $d_a$ -fibrils of a rod outer segment. Outer segment disrupted by addition of distilled water, fixed with 1% osmium tetroxide solution and negatively stained with PTA and bovine serum albumin. 182000 : 1.

Fig. 2.  $d_b$ -fibrils after drop preparation. Negatively stained with PTA and glycerol solution. 290000 : 1.

Fig. 3. Twin fibrils in twisted array. Drop preparation, fixed with 1% osmium tetroxide solution and negatively stained with PTA and glycerol solution. 274000 : 1.

Figs. 4 and 5. Twin fibrils in parallel array. Preparation as in Fig. 3. 182000 : 1.