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THE THERMAL DECAY OF METARHODOPSIN II_{380} IN THE FROG RETINA

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SUMMARY

The kinetics of the thermal decay of metarhodopsin II_{380} have been investigated in the excised frog (*Rana pipiens*) retina. Two first order processes have been separated and the rate constants and activation parameters for these processes have been determined. Another faster process is also suggested by the data.

The activation parameters of the slowest process are consistent with values obtained for the decay of metarhodopsin II_{380} in the retina of the albino rat and solutions of cattle rhodopsin. The reaction shows no pH dependence.

The faster process has somewhat different activation values and is pH dependent. The results suggest a reaction involving a major disordering of the molecule or solvent.

INTRODUCTION

The thermal decay of rhodopsin intermediates in extracted solution and retina has been of interest for elucidating the conformational changes of the rhodopsin molecule and in relating these changes to the visual process[1]. DONNER AND REUTER[2,3] had suggested that metarhodopsin II_{380} was a mediator of an adaptation process and more recently, W. A. H. RUSHTON and M. ALPERN (unpublished observations) have presented data which implicate rhodopsin intermediates in this process. Also the thermal decay of metarhodopsin II_{380} may be of some direct relevance to the receptor process since the metarhodopsin I_{478} to II_{380} reaction has been shown to correspond on a time scale to the early receptor potential[4–6].

In the albino rat retina and solutions of extracted rhodopsin, workers have studied the thermal decay of metarhodopsin II_{380} by direct photometric methods[7,8] and in the former case by photoreversal potentials[9–11]. In these studies the activation parameters for metarhodopsin II_{380} thermal decay have been calculated. Though single temperature studies of the photoproducts of rhodopsin have been reported in other species of frog retina[12–14], no temperature kinetic studies have been presented. We should like to report on such a study of the thermal decay of metarhodopsin II_{380} in the excised retina of the frog (*Rana pipiens*).

MATERIALS AND METHODS

Leopard frogs (*R. pipiens*) were dark adapted for at least 12 h and their retinas excised under dim red light. The retinas were then mounted in a specially designed

chamber containing frog Ringer's solution[15] with quartz windows[6] and placed in a Cary-14 recording spectrophotometer. For the pH study the Ringer's solution was modified to permit regulation with phosphate buffer[6]. An ERG was observed at higher pH's. Temperature was regulated with a Lauda K 2/R constant temperature bath and monitored with a Yellow Spring Model No. 42SC Tele-thermometer. The retina was flashed with a Honeywell 600 strobonar focused through a light pipe (American Optical LG 5 inch × 12 inch) and filtered by a heat filter and yellow cut off filter ($\lambda > 460$ nm, Corning 3-71).

The absorbance at 380 nm was followed on the chart of the Cary 14 with initial artifact eliminated by covering the photomultiplier with a narrow pass 380-nm interference filter (Baird-Atomic, Type 3).

RESULTS

A representative plot of the rate data for the thermal decay of metarhodopsin II_{380} is presented in Fig. 1. Using the assumption of concurrent first-order processes and the analysis procedure employed previously[16] the slowest process (k_1) is subtracted from the early portion and another first-order process determined (k_2). Two processes are resolved. Another faster reaction is also suggested because the k_2 process does not intersect the absorbance measured at 7 msec.

The rate constants of the two processes were then measured over a range of temperatures. An Arrhenius plot of the data indicating the properties of the two reactions is presented in Fig. 2. Both processes show Arrhenius behavior over the temperature range, with the faster process having a somewhat larger energy of activation.

Absolute rate theory[17] was also applied to the data and the rate constants, enthalpy and entropy of activation of the two processes are presented in Table I.

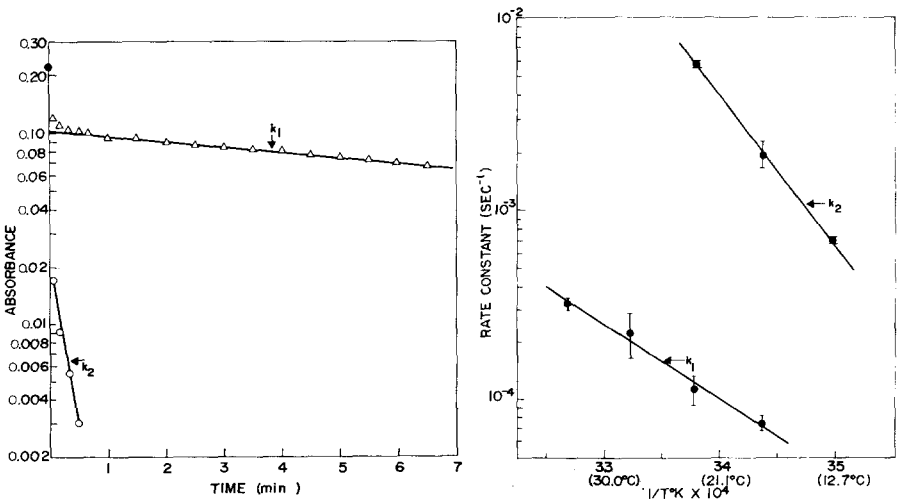

Fig. 1. Rate data for the thermal decay of metarhodopsin II_{380}. Temp., 23°; pH 6.1. Point at 7 msec determined by oscilloscope monotoring of Cary-14 driver amplifier[6].

Fig. 2. Arrhenius plot of metarhodopsin II_{380} thermal decay. pH 6.1.

TABLE I

RATE CONSTANTS AND ACTIVATION PARAMETERS FOR THE THERMAL DECAY OF METARHODOPSIN II_{380} (pH 6.1)

(a) *Slowest process* (k_1)

Temp.	k_{r1} (sec^{-1}) × 10^4
18°	7.6 ± 0.6
23°	11.4 ± 2.1
28°	22.5 ± 5.9
33°	32.4 ± 1.9

Q_{10} = 2.5
$\Delta H^{\ddagger}$ = 17 kcal/mole
$\Delta S^{\ddagger}$ (23°C) = −15 e.u.

(b) *Second process* (k_2)

Temp.	k_{r2} (sec^{-1}) × 10^3
13°	7.1 ± 0.1
18°	19.6 ± 3.3
23°	58.6 ± 0.0

Q_{10} = 3.1
$\Delta H^{\ddagger}$ = 35 kcal/mole
$\Delta S^{\ddagger}$ (23°C) = +54 e.u.

TABLE II

pH DEPENDENCE OF THE THERMAL DECAY OF METARHODOPSIN II_{380} (23°)

pH	k_{r1} (sec^{-1}) × 10^4	k_{r2} (sec^{-1}) × 10^3
5.15	10.4	86.6
6.10	11.4	58.6
7.20	10.1	26.7
7.95	10.4	25.7

The slowest process (k_1) has a smaller enthalpy of activation than the faster process and a negative entropy of activation. The faster process, on the other hand, has a decidedly positive entropy of activation. The results suggest two somewhat different reactions with the slowest process involving an ordering of the rhodopsin (or solvent) and the faster process involving a substantial disordering.

The effect of changes in pH of the Ringer's solution on the metarhodopsin II_{380} thermal decay was also measured. The results are presented in Table II and indicate a pH dependence of the faster reaction (k_2) but no such effect on the slowest proces (k_1).

DISCUSSION

The activation parameters for the slowest reaction in the decay of metarhodopsin II_{380} in the frog retina seem quite consistent with those obtained in both

the albino rat retina and solutions of cattle rhodopsin (Table III). The results suggest that the reaction represents a similar conformation change in the three systems. Since the activation values are the same whether the reaction is monitored by the decay of metarhodopsin II_{380}[9,11] or buildup of metarhodopsin III_{465} (ref. 10), it also suggests that the thermal decay of metarhodopsin II_{380} to metarhodopsin III_{465} is the process being observed. The negative entropy of activation is consistent with this idea since the process probably involves reprotonation of the Schiff base binding site[7].

TABLE III

STUDIES OF THE THERMAL DECAY OF METARHODOPSIN II_{330}

Investigators	*Source*	*Technique*	$\Delta H^{\ddagger}$ (*kcal/mole*)	$\Delta S^{\ddagger}$ (*e.u.*)
CONE AND COBBS[9]	Albino rat	Spectra and PP* (metarhodopsin II_{380})	18.8**	2.4**
EBREY[10]	Albino rat	PP* (metarhodopsin III_{465})	16 (± 5)	−10 (± 10)
HOSOYA[11]	Albino rat	PP* (metarhodopsin II_{380})	20.7	0.0
GEDNEY AND OSTROY	Frog (*R. pipiens*)	Spectra (k_1)	17	−15
OSTROY *et al.*[7]	Bovine soln.	Spectra (pH 5.1)	7.5	−50
HUBBARD *et al.*[8]	Bovine soln.	Spectra	19	−7

* PP (photoreversal potential).
** Estimated from original data.

The faster process that we have measured in the thermal decay of metarhodopsin II_{380} (k_2) appears to represent a somewhat different process. The larger enthalpy of activation and large positive entropy of activation indicate more bond breakage and a substantial disordering of the rhodopsin or solvent. The activation parameters suggest a reaction such as the direct decay of metarhodopsin II_{380} to retinal, but the present data are not sufficient to provide this information.

Finally the results help to further pinpoint the stages in the bleaching of rhodopsin which may be related to the receptor process. The two resolved reactions of the thermal decay of metarhodopsin II_{380} would appear to be too slow to be involved in the initial receptor event and are on a time scale consistent with the process of adaptation[2,3]. Since the metarhodopsin I_{478} to II_{380} reaction has been shown to correspond on a time scale to the early receptor potential[4–6], it suggests that the late receptor potential is a result of: a delayed chemical event resulting from the metarhodopsin I_{478} to II_{380} reaction[6] or an earlier reaction, or a result of the presence of metarhodopsin II_{380} (ref. 18), or the thermal decay of its fastest form.

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