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Low temperature spectrophotometry on the photoreaction cycle of sensory rhodopsin

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The photoreaction of sensory rhodopsin (sR) was studied by using low temperature spectrophotometry. Upon irradiation of sR_{590} at temperatures ranging from −30 to −60°C, sR_{373}, a photointermediate was produced without any preceding photoproducts. The formation of sR_{373} was temperature-dependent, and an Arrhenius plot gave an activation energy of 15.7 kcal/mol, suggesting that there might be a potential barrier in the excited state of sR_{590}.

Sensory rhodopsin; Photointermediate; Photoreaction; Potential barrier; Activation energy; Low-temperature spectrophotometry; (*Halobacterium halobium*)

1. INTRODUCTION

The cell membrane of *Halobacterium halobium* contains two light-driven electrogenic pumps, bacteriorhodopsin and halorhodopsin, both of which are retinal proteins [1]. The former is a light-driven proton pump which actively transports protons from inside the membrane to outside while the latter is an inwardly directed light-driven chloride pump [2].

Sensory rhodopsin (sR) is the third retinal protein found in the cell membrane [3–10]. Upon illumination at room temperature, sR_{590} is converted into sR_{680}, a bathochromic photoproduct, which is then thermally transformed into sR_{373}, a long-lived photointermediate [4].

From measurements of the phototactic behavior of *H. halobium* cells, it is concluded that sR_{590} is the photoreceptor for the long-wavelength attractant response while sR_{373} is the one for the short-wavelength repellent response [8].

Although photointermediates of sR have been detected by the flash photolysis method [4], exact pathways in the photochemical reaction of sR are uncertain.

The present study focuses on the photochemical process of sR, which is measured by low temperature spectrophotometry.

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Abbreviation: Mops, 3-(*N*-morpholino)propanesulfonic acid

2. EXPERIMENTAL

2.1. *Materials*

The carotenoid-deficient strain of *Flx3* (bR^-,hR^-,sR^+) [3] was grown in 16 l peptone medium [11]. The cells were washed three times with 4 M NaCl by centrifugation and suspended in about 40 ml of 4 M NaCl on ice. The membrane vesicles were prepared by sonication (Heat Systems model W-220, 20% duty cycle, 15 s, four times). The vesicles were incubated at 20°C with DNase for 2 h. The DNase-treated vesicles were then incubated with 3% Tween-20 on ice for 1.5 h. From the membrane thus obtained, the excess detergent was removed by washing twice with 4 M NaCl and

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the membrane was finally suspended in 4 M NaCl-10 mM Mops, pH 7.2.

2.2. *Low temperature spectrophotometry*

The difference spectra of sR before and after photochemical reactions were recorded on a Hitachi model 330 UV-visible spectrophotometer equipped with a specially designed glass cryostat [12]. The sR membrane in 66% glycerol was placed into a silicon rubber ring (9 mm diameter), sandwiched between a quartz and an opal plate. The light path was either 2 or 5 mm. The temperature of the samples was monitored with a copper-constantan thermocouple connected to the cell holder. The light source for irradiation of the samples was a 1 kW tungsten-halogen lamp on a slide projector (Sanko). The data from the spectrophotometer were transferred to a NEC model 9801F computer and treated by a signal-averaging program.

3. RESULTS

Fig.1 shows difference spectra of sR before and after irradiation with orange light (>580 nm). At −40°C the difference absorbance at around 600 nm decreased, accompanied by an increase of absorbance at about 380 nm, as the time of irradiation was prolonged (fig.1A). No measurable time-dependent changes due to the thermal reaction were detected at this temperature. The light-induced absorption changes correspond to the formation of sR_{373} from the original pigment (sR_{590}) as previously reported [4,9].

As shown in fig.1, the efficiency of photoconversion was temperature dependent. After 85 min irradiation at −60°C (curve 5 in fig.1B), the amplitude of absorption change at 600 nm was less than one third that caused by 42 min illumination at −40°C (curve 6 in fig.1A). Similarly to the photoreaction at room temperature, sR_{373} thermally reverted to sR_{590} when the temperature of the sample was raised as illustrated in fig.2. No photointermediates or photoproducts other than sR_{373} were detected during the step of irradiation with orange light at a wavelength longer than 560 nm or green light at 540 nm at various temperatures ranging from −40 to −190°C (not shown).

As fig.3 shows, the formation of sR_{373} was

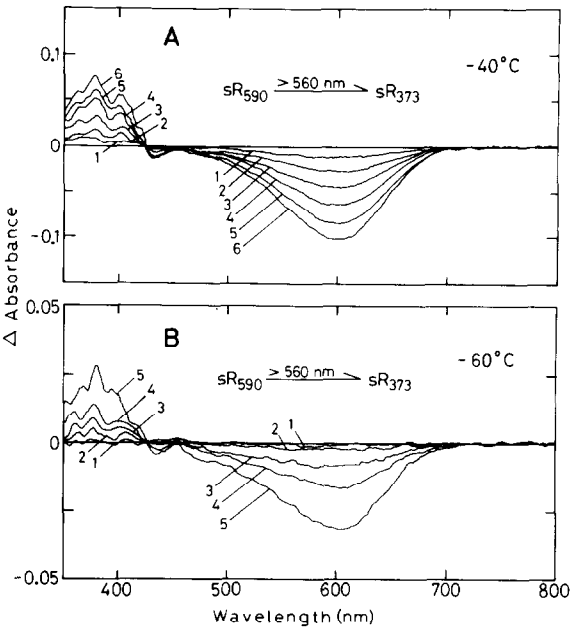

Fig.1. The spectral changes through red light illumination. The sR membrane was illuminated through a 580 nm high-pass filter by a tungsten lamp at −40°C (A) or −60°C (B). Irradiation times: panel A, curve 1, 40 s; 2, 2 min 40 s; 3, 5 min 20 s; 4, 10 min 40 s; 5, 21 min 20 s; 6, 42 min 40 s; panel B, curve 1, 20 s; 2, 1 min 20 s; 3, 21 min 20 s; 4, 42 min 40 s; 5, 85 min 20 s.

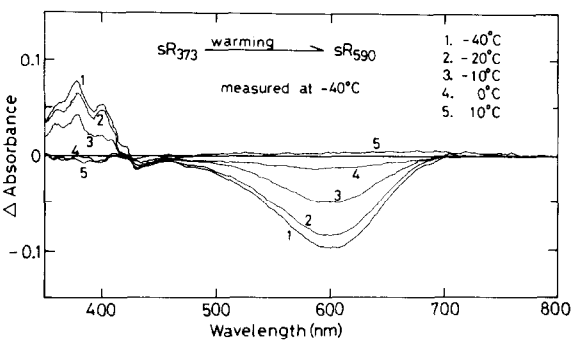

Fig.2. The thermal transition from sR_{373} to sR_{590}. The sR_{373} was formed by illuminating sR membrane at −30°C for 15 min with a high-pass filtered light (580 nm). The temperature of the sample was then rapidly dropped to −40°C by liquid nitrogen. The temperature of the sample was dropped immediately after it reached the temperature shown in the figure.

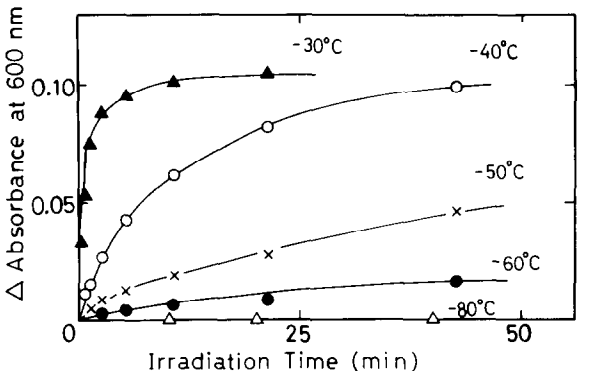

Fig.3. The time course of absorption changes at different temperatures during red light illumination. The irradiation light was through a 580 nm high-pass filter.

greatly dependent on temperature. At −80°C, the absorption change at 600 nm could hardly be seen even after 40 min irradiation. Over the temperature range between −30 and −60°C the amplitude of the difference absorbance at 600 nm increased according to a first-order reaction with a correlation coefficient of more than 0.99. As illustrated in fig.4, the Arrhenius plot was linear, giving an activation energy of 15.7 kcal/mol.

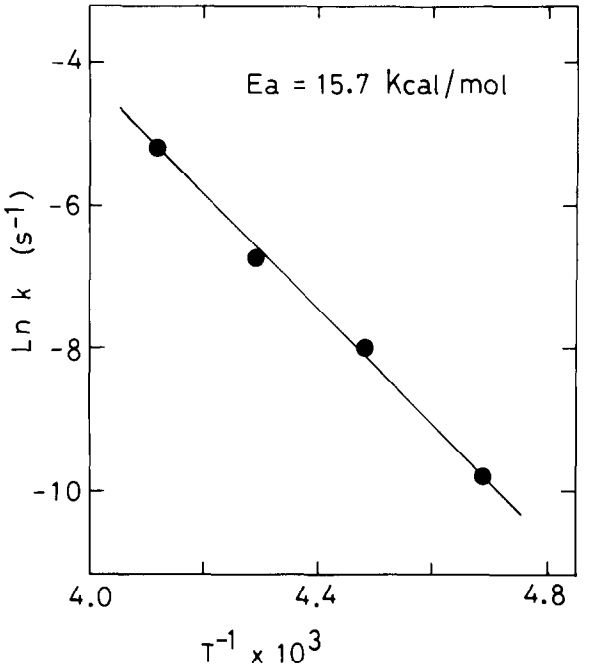

Fig.4. The Arrhenius plot of the first-order rate constants of photoconversion from sR_{590} to sR_{373}. The rate constants were obtained from the data in fig.3 by using an exponential regression program.

4. DISCUSSION

The photoconversion of sR_{590} to sR_{373} showed a striking temperature dependency below −30°C (figs 1 and 3). The photoreactions of other retinal proteins, such as rhodopsin or bacteriorhodopsin are reported to be independent of temperature [13]. The temperature-dependent formation of sR_{373} and the lack of precursors of sR_{373} suggest that there might be a high energy potential barrier prior to the formation of photoproducts, presumably in the excited state, as shown in the reaction scheme below at low temperature.

$$sR_{590} \xrightarrow{h\nu} [sR_{590}]^* \overset{E_a}{\rightsquigarrow} [sR_{590}]^*_{eq.} \rightsquigarrow sR_{373}$$

Since other photoproducts have been found at room temperature by flash photolysis [14], we might also consider another possibility that the observed activation energy, 15.7 kcal/mol, may be for the conversion of other intermediates, not detected at low temperature, to sR_{373}, which may be accompanied by retinal isomerization or some other structural changes of the protein. Recently, a potential barrier (E_a = 1.4 kcal/mol) in the excited state has been reported in the photochemical reaction of phytochrome [15]. The value for sR, 15.7 kcal/mol, seems to be rather large for the activation energy of the excited state in the photochemical reaction. Unlike other retinal proteins, the retinal molecule in sR may be very tightly fixed by the surrounding amino acid residues which, upon excitation, would offer resistance against retinal isomerization. Further experiments such as flash photolysis at low temperature will be necessary to exclude the involvement of intermediates other than sR_{373} in the photochemical reaction of sR at low temperature.

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