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PROTON RELEASE AND FORMATION OF PHOTOINTERMEDIATES AFTER LIGHT-INDUCED PROTON UPTAKE IN BOVINE PHOTORECEPTOR DISC MEMBRANES

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Summary: The hydrogen ion changes resulting from the photolysis of the rod visual pigment, rhodopsin, were investigated at acidic pH (5.2-6.5). After light-induced proton uptake, slow proton release occurred both in the dark and in the light. It was found that the amount of proton release in the dark was not equal to that in the light; about 0.9 proton remained bound to rhodopsin bleached in the dark, while all the bound protons were released in the light. Furthermore, the time course of proton release in the dark is not related to the decay of metarhodopsin II_{380}, but is closely related to the formation of metarhodopsin III_{465}.

Introduction: Absorption of light by rhodopsin in photoreceptor cell membranes initiates excitation of the cell. Light causes the chromophore of rhodopsin, 11-*cis*-retinal, to undergo a *cis* to *trans* isomerization through several photointermediate states (1). The formation of the intermediate photoproducts after illumination of vertebrate rhodopsin involves a distinct series of ionization changes. Such ionization changes result in changes of pH in a solution of vertebrate visual pigment (2-5). During the decay of metarhodopsin I_{478} to form metarhodopsin II_{380}, proton uptake causes the pH of the solution to shift to the alkaline side (6,7). During the subsequent thermal decay of metarhodopsin II_{380} under the same conditions, proton are released slowly (8). However, the actual process of proton release is poorly understood.

In this paper, we report that the proton release process is closely related the formation of metarhodopsin III_{465}, and not to the decay of metarhodopsin II_{380}.

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Materials and Methods: The rod outer segments (ROS) were prepared as described in the previous paper (9). Fresh bovine eyes were obtained from the Tokyo Shibaura Intestine Company. The ROS were isolated from bovine retinas by a sucrose flotation technique. Retinas were suspended in 36 % sucrose solution in Ringer's solution containing 112 mM NaCl, 3 mM KCl, 3 mM $MgCl_2$, 10 mM glucose and 10 mM Tris-HCl (pH 7.4), shaken vigorously, and centrifuged at 10,000 × g for 20 min. The floating ROS thus isolated were suspended in Ringer's solution and centrifuged at 10,000 × g for 20 min to sediment ROS. Discs were prepared by incubating the ROS for 9 hr in 5 % Ficoll-400. The discs were centrifuged at 55,000 × g for 2 hr. The floating discs thus isolated were immediately diluted with Ringer's solution and centrifuged at 10,000 × g for 20 min to sediment discs. After four successive washings in Ringer's solution the disc preparations were stored at 0 °C in Ringer's solution until use. The disc preparations were used immediately after three successive washings with 100 mM NaCl. All operations were carried out under dim red light at 4 °C.

The light-induced pH changes of samples containing 0.12 mg of protein per ml in the presence of 100 mM NaCl were recorded with a Toa HM-5A pH meter and a Hitachi 056 recorder equipped with a Toa prebox unit. Bleaching was achieved by irradiation with a 300-W tungsten lamp (Kondo Co.) or a 300-W xenon lamp (Varian Co.) through a heat-absorbing filter and an interference filter (λ_{max} = 550, 502, 458, 420 or 398 nm). After bleaching, several injections of 10 nmol HCl were made in order to calibrate the signal (responce time of the electrode: 12 ± 3 sec). The number of rhodopsin molecules bleached was determined from the 550 nm absorption (ε = 14,500). Absorption spectra were recorded with a Hitachi 557 spectrophotometer.

Results and Discussion: Fig. 1 shows the light-induced pH change of the disc suspension with time. When a disc suspension containing 100 mM NaCl was illuminated at 20 °C with a 300-W tungsten lamp

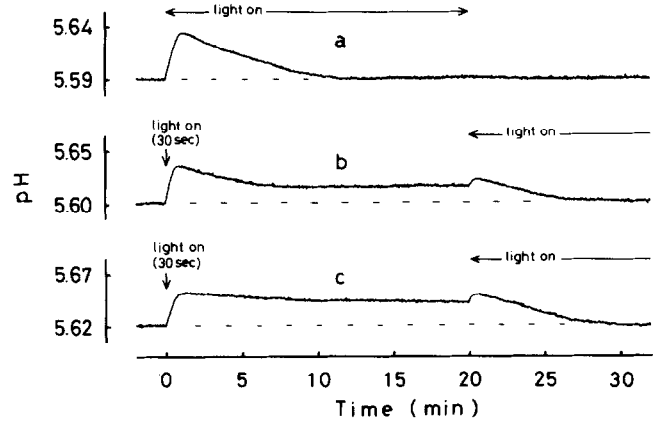

Fig. 1: Tracings of records of the pH changes of disc suspensions containing 100 mM NaCl against time. The sample was illuminated for 20 min at 20 °C (a). Other samples kept in the dark for 20 min after the initial illumination were illuminated again at 20 °C (b), or at 4 °C (c).

through a heat-absorbing filter, rapid proton uptake (pH increase) was observed. After this light-induced pH increase, a slow proton release (pH decrease) occurred, both in the dark and in the light. However, the extent of proton release in the light was greater than that in the dark. In the light, the pH returned to the original level after about 10 min (see a in Fig. 1), whereas this did not occur in the dark. However, when a disc suspension kept in the dark for 20 min after the first illumination was illuminated again, the pH did return to the initial level (see b in Fig. 1). From a and b in Fig. 1, it was estimated that the proton uptake per bleached rhodopsin was 1.7, and 0.9 proton remained bound to rhodopsin bleached in the dark (i.e., the extent of the slow proton release in the dark was 0.8). Moreover, all of the bound protons were released in the light. The proton release in the dark was slower at 4 °C than that at 20 °C (see c in Fig. 1). Sonication of the disc suspension appeared to have no effect to proton release in the dark or in the light.

When the initial pH value of the suspension was raised from 5.2 to 6.5 in the presence of 100 mM NaCl at 20 °C, the extent of

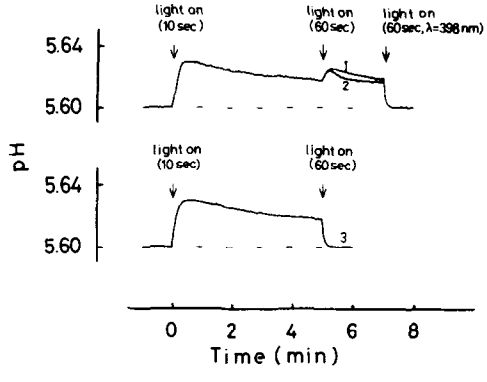

Fig. 2: Tracings of records of the pH changes of disc suspensions containing 100 mM NaCl against time at 20 °C. Samples kept in the dark for 5 min after the initial illumination (λ = 550 nm) were illuminated again for 60 sec. 1, λ = 550 or 502 nm; 2, λ = 458 nm; 3, λ = 420 or 398 nm.

light-induced proton uptake was markedly decreased. The amount of proton uptake per bleached rhodopsin at pH 6.2 was estimated to be 1.0. A similar proton uptake, but not the subsequent slow proton release, was observed by McConnell (4,10) and Bennett (7).

We attempted to correlate the phenomenon of proton release with the formation or decay of various photointermediates. For this purpose, the wavelength dependence of proton release in a disc suspension containing 100 mM NaCl was investigated over the range of wavelength from 398 nm to 550 nm, using interference filters. When disc suspensions were illuminated for 10 sec with a 300-W xenon lamp through a heat-absorbing filter and an interference filter (λ_{max} = 550 nm), proton uptake was observed. Subsequently, when the pH had virtually stabilized, the suspension was illuminated again for 60 sec through an interference filter (λ_{max} = 550, 502, 458, 420 or 398 nm). It was found that the proton release was accelerated by illumination at 398 or 420 nm

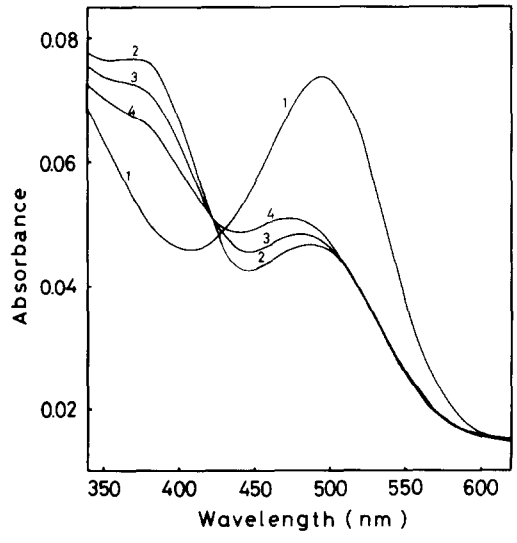

Fig. 3: Spectral changes of a disc suspension containing 100 mM NaCl at 20 °C. The scanning speed was 300 nm/min. Spectrum 1 was taken before illumination. Spectrum 2 was taken after illumination (λ = 550 nm, 10 sec). Spectrum 3 was taken after 5 min in the dark following the illumination. Spectrum 4 was taken after illuminating the sample of spectrum 3 for 20 sec at 398 nm.

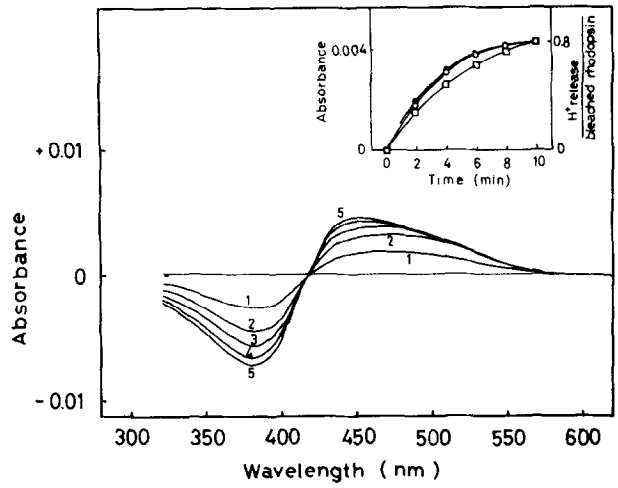

Fig. 4: Spectral changes of a disc suspension containing 100 mM NaCl in the dark after illumination (λ = 550 nm, 10 sec) at 20 °C. The scanning speed was 300 nm/min. Spectrum 1 was taken 2 min after illumination. Spectrum 2, 4 min; Spectrum 3, 6 min; Spectrum 4, 8 min; Spectrum 5, 10 min after the end of the illumination. The inset shows the time courses of absorbance at 465 nm (○), and at 380 nm (□), and that of pH change (●) in the dark at 20 °C.

(see Fig. 3). In the spectrum of the disc suspension after illumination at 398 nm, the absorbance over the range from 350 nm to 420 nm decreased, and the absorbance over the range from 420 nm to 500 nm increased. This suggests that metarhodopsin II_{380} decayed into subsequent intermediates as a result of irradiation at 398 nm. This was confirmed by the time course of spectra in the dark after illumination, as shown in Fig. 4. It can be seen that the absorbance at 465 nm increased first, followed by an increase in the absorbance at 440 nm. This suggests that the decay of metarhodopsin II_{380} in the dark yielded metarhodopsin III_{465} and N-retinylidene-opsin$_{440}$, and it further appeared that the time course of the formation of metarhodopsin III_{465} in the dark was identical with that of proton release (see the inset in Fig. 4).

These results suggest that the proton release process is not related to the decay process of metarhodopsin II_{380}, but is

closely related to the formation process of metarhodopsin III_{465}. This proton release may be related to adaptation.

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