CHANGES IN pH OF RHODOPSIN SOLUTION ON EXPOSURE TO A DIM GREEN LIGHT

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It has been reported by many authors that the pH of rhodopsin solution changed following illumination. According to RADDING and WALD¹⁾, exposure to flashes of intense light induced not only slow acid-base changes of rhodopsin solution, but also an immediate rise of pH. This immediate rise of pH was observed between pH 2 and 8, and was followed by slow changes which were always in the direction of neutrality. OKUBO²⁾ also reported that pH of rhodopsin solution changed toward neutrality on exposure to strong light (100 lux).

These two reports show almost the same results except for the following two points: First, according to RADDING and WALD, at a range of pH 6 and 7, pH showed little change following illumination, while OKUBO emphasized that pH changed following illumination toward 7 in the same pH range. Secondly, OKUBO reported no immediate rise of pH. So the present study was made to clarify the status and significance of pH changes of rhodopsin solution following illumination.

METHODS

In each experiment, 30 bull frogs' (*Rana catesbiana*) eyes were employed. Their outer segments were isolated by KIMURA's floatation technique³⁾, and rhodopsin was extracted with 1% digitonin solution (3 ml) and the extract was diluted with 3 ml of distilled water and put into a little vessel of 15 ml capacity. Then pH of rhodopsin solution was adjusted by the addition of 0.004 N HCl or 0.004 N NaOH.

pH meter. pH of rhodopsin solution was calibrated by a pH meter (H-5 type pH meter of HITACHI INSTRUMENTS INC.) under a dim red light.

Vacuum apparatus. The molecular oxygen contained in rhodopsin solution was let out with a vacuum apparatus (FIG. 1) which was composed of a main tube (B), a side arm (A), cocks (C, D and E) and a shringe (F), and was connected with a vacuum pump through cock C. A mixture of rhodopsin solution (6 ml) and distilled water (8 ml) was put into the side arm (A), then cocks D and E were closed while cock C remained open. Then the air contained in the main tube and the side arm was let out by aspira-

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FIG. 1. Vacuum apparatus.

tion with the vacuum pump. Following aspiration, most of the diluted rhodopsin solution (14 ml) bubbled from the side arm (A) and overflowed into main tube (B). Then the air contained in rhodopsin solution was let out for 30 successive minutes, so that the oxygen molecules combined with rhodopsin molecules⁵⁾ were reduced.

Then, cock C was closed and the vacuum pump was disconnected, immediately followed by the opening of cock E, so that the liquid paraffin contained in the shringe (F) was poured into the main tube (B) covering the rhodopsin solution.

The procedures described above were carried out in a dark room under a dim red light.

RESULTS

Rhodopsin solution was scarcely bleached following illumination of a dim green light. Even if rhodopsin solution was exposed to a dim green light (1.5 lux) at 19°C for 40 min, 96% of rhodopsin remained unbleached (TABLE 1).

TABLE 1.
Bleaching of rhodopsin on exposure to green light (1.5 lux) for 40 minutes.

Temperature	bleached rhodopsin	unbleached rhodopsin
19°C	3.8%	96.2%
22°C	4.1%	95.9%
28°C	11.2%	88.8%

Changes in pH of the rhodopsin solution following illumination were various depending upon temperature and pH employed in experiments. Under certain experimental conditions, changes in pH following illumination failed to be observed. For example, at 33°C with initial pH 3.9, acid-base changes following illumination were scarcely observed. Where pH remained unchanged in spite of illumination is called an isoionic point, being represented by P_0 , which is subjected to temperature changes. At 6°, 9°, 19°, 27°, 30° and 32°C, P_0 was

FIG. 2. P_0 -curve and the area in which element R was demonstrated.

FIG. 3. Element D_1 .

found at pH 6.2, 6.1, 5.7, 5.0, 4.6 and 4.2 respectively (FIG. 2), showing a P_0 -curve.

When initial pH of a rhodopsin solution was below P_0 , a rise of pH was observed during illumination (FIG. 3), being represented by element D_1 .

On the alkaline side of P_0 -curve, a trend of decrease in pH was gradually induced following illumination (FIG. 4). This trend is represented by D_2 . Be-

FIG. 4. Elements R and D₂.

tween 19°C and 30°C, however, an initial rise of pH (element R) was evoked before D₂ was induced (FIG. 4) and in 4 or 5 min it began to decrease. Now, R was demonstrated in the shaded area shown in FIG. 2.

Aetiologically, the present author maintained that element R might be evoked through a photo-release of oxygen from rhodopsin solution^{4,5}). In removing this element, rhodopsin solution was put into the vacuum apparatus (FIG. 1), and aspirated by a vacuum pump for 30 min, liquid paraffin was then poured on the surface of the solution. Then, the rhodopsin solution was put into a little vessel of 15 ml capacity. With scanty oxygen, R failed to be

FIG. 5. With scanty oxygen, element R failed to be observed following illumination.

found following illumination (FIG. 5B). From this finding it may be clear that R was evoked through photo-release of oxygen from rhodopsin solution.

For the purpose of inhibiting elements D_1 and D_2 , 4 ml of sucrose solution (specific gravity was 1.20) was mixed with 4 ml of rhodopsin solution. At 33°C, the changes in pH, on exposure of the mixture (8 ml) to light, were composed of either of D_1 or enfeebled D_2 (FIG. 6B). Thus, element D_2 was apparently enfeebled by sucrose at 32°C. Inhibition of D_2 by the addition of sucrose caused a shift of P_0 to the alkaline side (FIG. 6).

FIG. 6. Inhibition of element D_2 by the addition of sucrose.

DISCUSSION

According to RADDING and WALD, there were found few obvious relations between pH changes of rhodopsin solution and bleaching of rhodopsin, because pH changes involve principally opsin, spectral changes principally the prosthetic group of rhodopsin. And their finding might be confirmed in the present study as follows. It was demonstrated that alterations in pH of rhodopsin solution were observed without color changes. It is considered that changes in pH were possibly evoked on the surface of opsin.

The reactions brought about on the surface of opsin following illumination might be chemical ones, because elements D_1 , D_2 and R were apparently affected by temperature (FIG. 3 and 4). Thus, it may be concluded that some kind of ions appeared on the surface of rhodopsin molecules from the inside as a result of exposure to light, causing acid-base alterations such as elements D_1 and D_2 .

Element R depressed in low O_2 concentration (FIG. 5), so that this element might refer to a photo-release of oxygen from rhodopsin solution⁴.

Presently it was demonstrated that, at 28°C element R was induced following illumination within range of pH 5.9 and 7.6. And, it was reported in the previous paper⁴) that an optimum pH range for the release of oxygen molecules from rhodopsin was between 6 and 7.5 at 28°C. Thus, an optimum pH range for the release of oxygen molecules following illumination and that

for the provocation of R were very much alike. Therefore it may be considered that the photo-release of oxygen induced immediate changes of pH⁰ continuously during illumination, and the summation of these immediate changes of pH formed element R.

With reference to R, it leaves a question unclarified that, why R was observed only between 19°C and 30°C, not on both sides of this range (FIG. 4 and 2). A probable answer about this question may be as follows: First, all chemical reactions are reduced at lower temperatures, so that D₁, D₂ and R were all enfeebled. Secondly, oxygen concentrations in rhodopsin solution might be reduced at higher temperatures, so that R was apparently inhibited at higher temperatures.

D₁ was found on the acid side of P₀-curve and D₂ on the alkaline side. Active chemical groups concerning each of these two elements might be different individually, because the addition of sucrose was able to inhibit element D₂ but no D₁.

P₀ was variable under different temperatures, and the higher the temperature the smaller the P₀ (FIG. 2). Relations between P₀ and temperature (t°C) may be expressed as

$$(41-t)(7-P_0) \doteq 28,$$

where 7 means the neutral point of pH, and 41 and 28 are constants which were determined by analytical geometry. TABLE 2 shows the values of P₀ which were calculated from this equation against the experimentally determined values.

TABLE 2.
Values of isoionic point (P₀) for different temperatures.

Temperature t°C	Experimentally determined value of P ₀	$P_0 = 7 - \frac{28}{41-t}$
6	6.2	6.20
9	6.1	6.13
19	5.7	5.73
27	5.0	5.00
30	4.6	4.46
32	4.2	3.89

SUMMARY

1. Changes in pH of rhodopsin solution was induced following illumination with the least color changes.
2. Changes in pH of rhodopsin solution on exposure to a dim green light might be clarified into 3 components, D₁, D₂ and R.

3. Isoionic point (P_0) might be affected with temperature. At lower temperatures (6°C, 19°C) P_0 was found in a neutral range, but the higher the temperature (19°C-30°C) the smaller the P_0 . Relations between temperature ($t^\circ\text{C}$) and P_0 may be expressed as

$$(41-t)(7-P_0) \doteq 28.$$

4. At 19°C to 30°C, element R was observed on the alkaline side of P_0 -curve.
5. Element R might be the summation of immediate changes in pH evoked by successive illumination.
6. Origin of element R might be a photo-release of oxygen from rhodopsin solution following illumination.

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