

## THE DIRECTION OF LIGHT-INDUCED pH CHANGES IN PURPLE MEMBRANE SUSPENSIONS

### Influence of pH and temperature

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Received 11 July 1977

#### 1. Introduction

It is well established that bacteriorhodopsin (bR) located in the purple membrane of the halophilic bacterium *Halobacterium halobium* acts as a light-driven proton-pump. Upon illumination this protein translocates protons electrogenically from the cell interior to the external medium [1–3]. Fragments of purple membrane, in which bacteriorhodopsin is the only protein present, are routinely isolated. These fragments retain the ability to pump protons, since reconstituted proteoliposomes in which they are incorporated show light-induced proton uptake [4–7]. Illuminating purple fragments causes a small acidification [8–10], which presumably is the net result of simultaneous proton release and uptake occurring on opposite sides of the membrane (both sides, of course, being exposed to the same medium) [9].

In this communication the influence of pH and temperature on the light-induced pH changes observed in purple fragments were studied. It is shown that external conditions not only effect the extent and initial rate of the pH change, but may even reverse its direction. It is suggested that pH and temperature influence the protonation and deprotonation processes in different ways.

#### 2. Materials and methods

*Halobacterium halobium* was grown as described by Danon and Stoeckenius [11], purple membrane

fragments were prepared according to the method of Oesterhelt and Stoeckenius [12], and soy-bean lecithin proteoliposomes were made as described by Racker [4]. The light-induced pH changes were followed by a combined glass electrode as described previously [13].

#### 3. Results

Figure 1 shows the extents and initial rates of the light-induced pH changes observed in purple membrane fragments at various pH values. For high pH values the membrane acidifies its medium as reported previously [1,8,14–17]. Reducing the pH by addition of small amounts of HCl decreased the acidification to zero, and a further reduction of the pH caused light-induced alkalization. The changes in extent and initial rate were reversible.

Thus, increasing the pH of a suspension at pH 4 by adding small amounts of KOH resulted in a similar pH profile, with a small but significant shift to alkaline pH values. This shift may stem from irreversible changes occurring in the membrane on exposure to low pH values, or from a decrease in ionic strength due to the addition of acid and base. Preliminary studies indicated that reducing the KCl concentration below 1 M reduced the extent as well. Kinetic analyses of the light-induced pH changes were not performed, since the first-order behaviour reported previously [8,10] was observed only in the neighbourhood of pH 7 (elsewhere the kinetics seemed to be more com-

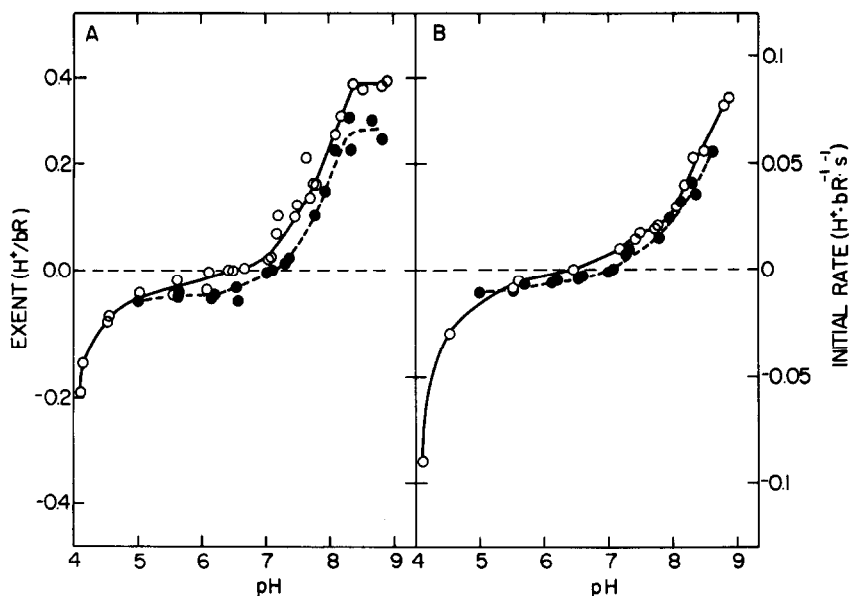


Fig.1. Effect of pH on light-induced pH changes. Purple membrane was suspended in 1 M KCl to a final concentration of 13  $\mu$ M at 14°C. Light-induced pH changes were followed as described under Materials and methods, and the pH scale was calibrated by adding 10  $\mu$ l aliquots of 1 mM HCl solution. The initial pH of 8.8 was reduced to 4.2 by adding HCl (open symbols) and then increased again by adding KOH (closed symbols). (A) Extent. (B) Initial rate.

plex). It should be emphasized that the results shown in fig.1 are representative only; with other preparations the cross-over point could vary from pH 5 to pH 7.

Figure 2 shows the effect of temperature on the extent and initial rate measured at pH 7.6. Increasing the temperature from 7°C to 27°C reduced the extent of the light-induced acidification from 0.2 H<sup>+</sup>/bR to zero. Further heating yielded light-induced alkalization which changed only slightly with increasing temperature. When a suspension at 30°C was cooled back to 7°C full reversibility of the change in extent was obtained, but heating above 30°C caused an irreversible decrease in the extent (not shown). As in the previous case, a first-order kinetic analysis could not be performed at all temperatures. At temperatures at which the light-induced pH change was first-order, an increase in rate constant with increasing temperature was observed.

The effect of temperature on the light-induced pH change was studied at various pH values. Figure 3 shows the extents observed for several pH values at three selected temperatures. It is seen that in all cases a decrease in the acidification, or an increase in the

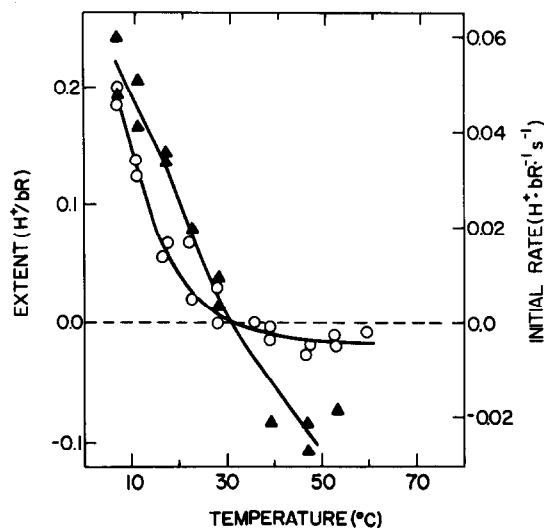


Fig.2. Effect of temperature on light-induced pH changes. Purple membrane (different preparation than in fig.1) was suspended in 1 M KCl to a final concentration of 11  $\mu$ M, at pH 7.6. Light-induced pH changes were measured at different temperatures as described under Materials and methods and the legend to fig.1. The suspension was incubated for 30 min at each temperature before performing the measurement. (○) Extent. (Δ) Initial rate.

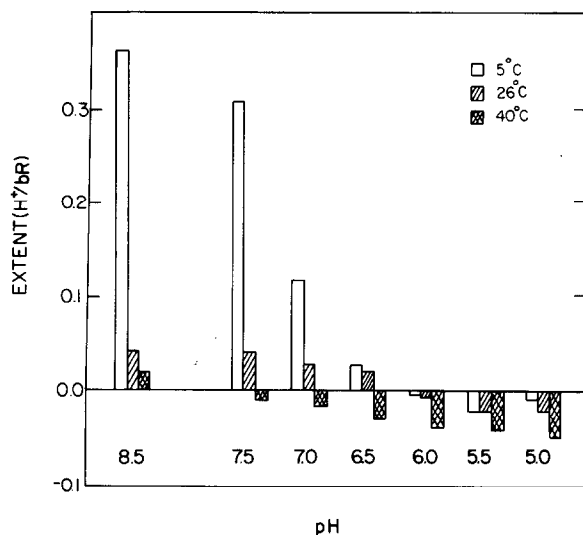


Fig.3. Effect of pH on the extent measured at different temperatures. The experiment described in the legend to fig.1 was repeated at three temperatures: 5°C, 26°C and 40°C. At each temperature a fresh sample of the same suspension was used.

alkalization, was obtained upon heating. The largest changes in extent were measured at high pH values where the acidification at 5°C was correspondingly large. From these data it can also be seen that the dependence of extent on pH, shown in fig.1, varies with temperature. Heating the suspension causes a shift in pH such that zero extent is observed at more alkaline values.

In order to see whether purple membrane preparations which alkalinize the medium upon illumination operate in an opposite sense in oriented systems, proteoliposomes were made at pH 5 with fragments exhibiting light-induced alkalization at that pH. These particles pumped protons inward as usual.

#### 4. Discussion

A model which has been suggested for the mechanism of proton translocation by bacteriorhodopsin is the following: Upon illumination, the chromophore, retinal, undergoes a cyclic photoreaction which causes

conformational changes in the protein [8,17–21]. These changes in turn cause pK shifts in several groups [22] and as a result association and dissociation of protons occur [10,23]. The small net acidification usually observed in fragments of purple membrane is due to differences in the extent of proton uptake and release which take place on opposite sides of the suspended fragments, and probably reflects differences in the pK values of the two surfaces [10]. Thus, it should not be surprising that by altering the pH one can increase the alkalization over the acidification and so reverse the direction of the net pH changes. This result in fact lends support to the model. Other possible explanations such as the effects of pH on the photocycle, or on the conformational changes taking place in the protein, are less likely. We can also exclude the possibility of a reversal in the direction of operation of bacteriorhodopsin since no reversal was obtained in proteoliposomes.

The light-induced pK shifts assumed in the model may involve either changes in groups which had been at equilibrium with the medium in the dark, or changes in the number of groups exposed to the medium. The observation that increasing the pH causes an increase in deprotonation over protonation suggests the second possibility as the more probable one, although the first one cannot be excluded.

The dependence of light-induced acidification on pH has been reported previously [8,14]. In these studies the extent approached zero at low pH values, but light-induced alkalization was not observed.

Temperature may affect each step of the conversion of light energy to a proton gradient, but the sensitivity of the temperature effect to pH suggests that the changes observed on heating occur in the association–dissociation steps and not in the photocycle. In principle, changes in the extent on heating might be explained by changes in pK values with temperature, but it seems to us that the temperature dependence of the pK values is too small to account for the large decrease in acidification measured at high pH values. We suggest that heating may change the ratio between the number of groups binding protons and those releasing them by affecting the light-induced conformational changes occurring in the protein. The observation that the change in extent levels off at about 30°C, which is a phase transition temperature of the purple membrane [24] and is

irreversible on heating the system above this temperature, supports our view. If this is really so, the effect of increasing temperature would be to permit conformational changes which below the phase transition are limited by the highly viscous environment within the membrane [15,25]. This argument appears to contradict the observations of Oesterhelt and Hess [8] that the rate constant of the light-induced acidification is temperature-independent. As discussed above, in our experiments the process could not be analyzed as a first-order reaction at all temperatures, although when the analysis could be performed a clear increase in rate constant with increasing temperature was obtained. A possible explanation of the contradiction may be the different medium used by Oesterhelt and Hess [8]. Bakker and Caplan [14] also measured the extent of the light-induced pH changes at different temperatures. According to their results the acidification approaches zero at 25°C, but unfortunately further heating was not performed.

Since the observed pH change with purple membrane fragments is the difference between two processes, the kinetics of the net process is essentially unpredictable. The fact that in a restricted temperature range the reaction seems to be monophasic and first order indicates, in our opinion, that both the dissociation and association reactions may have a common, rate-limiting step. Another explanation may be that where monophasic first-order acidification is observed, the alkalization reaction is inhibited and vice versa.

We conclude by pointing out that the protonation and deprotonation processes occurring on opposite sides of the purple membrane are influenced differently by the external conditions, probably because of differences in the chemical nature of the two processes. Actually there is evidence in the literature that pH and temperature may affect association and dissociation in opposite ways. According to Lozier et al. [9], who studied light-induced pH changes in an aqueous suspension of purple membrane at 21°C and pH 7.85, the faster process is the dissociation. Dencher and Wilms [20] carried out a very similar study, at 10°C and pH 5, and got the opposite result. The simplest explanation of this is that the rate constants of proton uptake and release are affected differently by pH and/or temperature, so that under different conditions apparently conflicting results can be obtained.

## Acknowledgements

The authors are grateful to Mrs Charlotte Weissman for preparing the purple-membrane fragments. This study was supported by grants from the US-Israel Binational Science Foundation (BSF), Jerusalem and from the National Council for Research and Development, Israel and the KFA Jülich, FRG. One of us (M.E.) is indebted to The Bat-Sheva de Rothschild Foundation for the Advancement of Science in Israel for a research grant.

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