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Light-Induced Conductivity Changes in Purple Membrane Suspensions

M. A. Slifkin[1], H. Garty[2]*, W. V. Sherman[3], M. F. P. Vincent[4], and S. R. Caplan[2]

[1] Department of Pure and Applied Physics, University of Salford, Salford M5 4WT, U.K.,
[2] Department of Membrane Research, The Weizmann Institute of Science, Rehovot, Israel,
[3] Department of Science, Chicago State University, USA, and
[4] Department of Physics, University of Queensland, St. Lucia, Australia

Abstract. Small light-induced changes in the conductivity of light-adapted purple membrane suspended in strong electrolyte solutions were detected. The method used involved modulated light and a phase sensitive detector and it allowed us to detect accurately changes as small as 0.0001% in the conductivity of the suspension. The light-induced conductivity changes turned out to be composed of at least two different events: a small fast increase in conductivity ($\tau \sim 2$ ms) followed by a slower and larger decrease in this parameter ($\tau = 70$ ms–80 ms).

The effects of pH and temperature on these changes were studied. Both events reached maximal values around neutral pH and approached zero at both high and low pH's. Heating the suspension decreased the photoconductivity change and Arrhenius plots of the data showed breaks around 31° C.

It is suggested that the conductivity changes reflect changes in the surface charge of the membrane and can be used to follow the kinetics of the conformational changes occuring in the system.

Key words: Bacteriorhodopsin – Conductivity – Modulation excitation – Purple membrane.

Introduction

The purple membrane of the halophilic bacterium *Halobacterium halobium* contains a single protein – bacteriorhodopsin, to which a retinal molecule is attached. This system operates as a light-driven proton pump. Absorption of light by the retinal drives a cyclic photoreaction which brings about uptake of protons from the inner side of the membrane and release of protons at the outer side [for review, see Stoeckenius et al. (1979)]. According to several reports, conformational changes in the protein-lipid complex are involved in the process (Oesterhelt and Hess, 1973;

* To whom requests for reprints should be addressed

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Trissl and Montal, 1977; Caplan et al., 1977; Eisenbach et al., 1978; Bogomolni et al., 1978; Caplan et al., 1978).

Light-induced conductivity changes have been measured by conventional means in suspensions of rhodopsin (Hara, 1963) and rod outer segments (Falk and Fatt, 1968) at low ionic strength. In both cases the observed change was due to more than one event, interpreted as proton uptake, conformational change (which induces a change in the surface potential of the membrane), or heating of the suspension.

In this commmunication we describe measurements of light-induced changes in the conductivity of 4 M NaCl solutions containing purple membrane fragments (open sheets 0.5 μm in diameter) by a modulation-excitation technique. This technique was necessitated by the high salt concentration, in which the relative light-induced conductivity changes were expected to be very small. The conductivity changes are related to changes in the surface charges of the light-adapted protein-lipid complex resulting from the different conformations in the light and in the dark.

Methods and Materials

Halobacterium halobium was grown as described by Danon and Stoeckenius (1974) and purple membrane fragments were prepared according to the method of Oesterhelt and Stoeckenius (1974).

The conductivity measurements were performed on light-adapted samples as shown in Figure 1. The sample was placed in a Radiometer CDC 324 conductivity cell [1][1]. This is a water-jacketed pipette cell holding 1 ml, with three concentric bright platinum electrodes, the two outer ones being earthed. (Owing to the danger of adsorption of purple membrane fragments, the use of platinized platinum was felt to be inadvisable). The cell is connected to the input of a low noise 100 dB amplifier (Brookdeal LA350) [2] and the base emitter current of the input transistor flows through it. With 4 M NaCl the cell has a resistance of about 30 Ohm, and thus a minute voltage is generated across it. It is illuminated with light modulated by a mechanical chopper. The light source is a 500 W high pressure DC mercury arc with light and heat filters [3]. It gives a radiation of 577 nm, the light intensity being

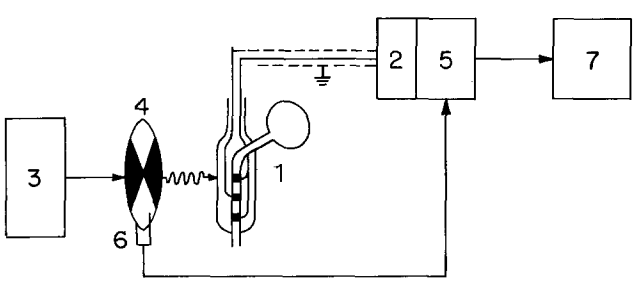

Fig. 1. Experimental arrangement for light-modulated conductivity measurements. For details, see text

1 The numbers in square brackets refer to annotations in Figure 1

340 W/m^2. The chopper [4] is driven by a servo-controlled DC motor giving a range of modulation frequencies from 2.9 to 780 Hz. The frequencies are read to an accuracy of 0.1 Hz on a Catronics digital frequency meter. The modulation frequencies as recorded on the meter and examined on a cathode ray oscilloscope are stable to better than 0.1 Hz.

The output from the low noise amplifier is fed to a Brookdeal PM 322A phase sensitive detector [5]. The reference signal for this is obtained from a small pea bulb and phototransistor mounted on the periphery of the chopper and which can be moved around it with a micrometer screw, thus allowing for adjustments of phase [6]. The rectified output from the phase sensitive detector is displayed on a Telsec 700 pen recorder [7].

If absorption of light by the sample should cause a conductivity change, the conductivity of the suspension will be modulated at the same frequency as the chopped light, and as a result minute fluctuations in the DC current will occur. The phase-sensitive detector will separate the appropriately modulated fluctuations from the constant level current, and the output of the detector will be proportional to their amplitude. This measurement should not be affected by electrode polarization. Additionally, it should be remembered that very low currents (ca. 0.1 microamp.) and voltages (ca. 1 microvolt) are used.

The sign of the signal is established in the following manner. All light and heat filters are removed and pure electrolyte is illuminated. A small signal is observed due to the heating effect of the source. The conductivity of these electrolytes is well known to increase with increasing temperature, so that the observed signal establishes the direction for increasing signal.

The life-times of the processes giving rise to the observed light-induced signal are derived thus. It was shown (Labhart, 1964) that for a simple first order decay

$$S = K\tau/(1 + \omega^2\tau^2)\,,$$

where S is the observed signal, K is some instrumental constant, ω is the modulation circular frequency, and τ is the lifetime (i.e., inverse rate constant) for the process. Thus

$$S^{-1} = 1/K\tau + \tau\omega^2/K$$

and a plot of the inverse signal against the square of the circular frequency should give a straight line for a single process and more complex behaviour for a combination of first-order processes of higher order processes. The lifetime of a single first-order process is obtained from the ratio of the slope and intercept of the straight line.

Results

Figure 2 shows the light-induced conductivity changes measured in a suspension of purple membrane fragments at different chopping frequencies of the exciting beam. At high frequencies the modulated illumination causes an increase of conductivity.

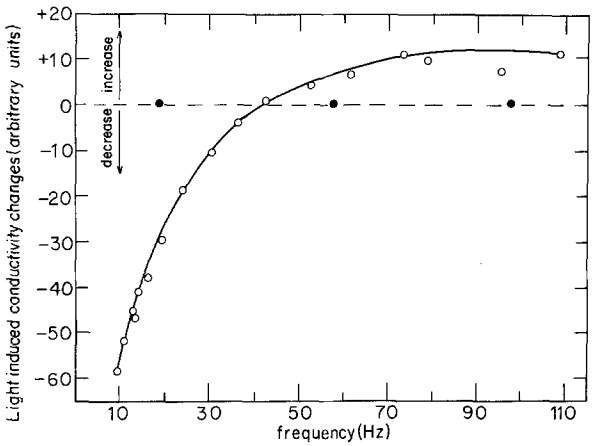

Fig. 2. Light-induced conductivity changes at various frequencies: purple membrane fragments were suspended in 4 M NaCl to an optical density of 0.45 at 565 nm. The pH was brought to 6.7 in the absence of buffer. 1 ml suspension was placed in the conductivity cell, the phase was set to maximalize the signal at 10 Hz, and the signals were measured at various frequencies as described under Methods and Materials. The temperature was 18° C. O——O, purple membrane fragments; ●——●, pure electrolyte

But at low modulation frequencies a decrease in the conductivity is obtained. High frequency events are those which occur shortly after illumination. The cross-over point of about 45 Hz corresponds to a time about 10 ms after illumination[2]. When the membrane fragments had been replaced by pure electrolyte no light-induced conductivity change could be observed under the experimental conditions.

Analysis of the data up to 30 Hz was performed as described under Methods and Materials and the results are shown in Figure 3. As can be seen from that figure the experimental points up to a chopping frequency of 20 Hz can be fitted very well to a straight line, the correlation coefficient being 0.997, but for higher frequencies the line appears to curve. The data above 30 Hz were not analysed since the low signals gave rise to high experimental errors in S^{-1}. The lifetime of the process under 20 Hz, calculated as described under Methods and Materials, resulted in values of 70–80 ms, depending to some extent on the preparation of the fragments. Thus we can conclude that the light-induced conductivity changes in purple membrane fragments suspended in 4 M NaCl are composed of at least two different effects in opposite directions, but most of the change is brought about by first order loss of conductivity characterised by life-times of 70–80 ms. The lifetime of the fast increase in conductivity could not be measured precisely but it is less than about 2 ms.

In order to measure the activation energy required for the conductivity change we looked at the effect of temperature on the photoconductivity. The experiments

2 The plot of S vs. frequency gives the inverse Laplace transform of the effect of a single pulse of light with high frequencies corresponding to earlier events and lower frequencies to later events. Consequently examination of such plots enables one to monitor the events caused by illumination as a function of time

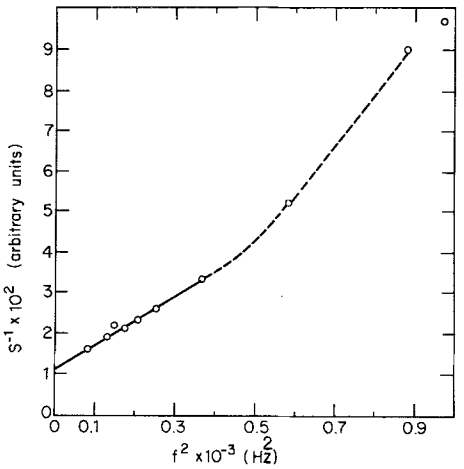

Fig. 3. Kinetic analysis of the conductivity changes: the data shown in Figure 2 were analysed as described under Methods and Materials, points being taken up to 30 Hz only

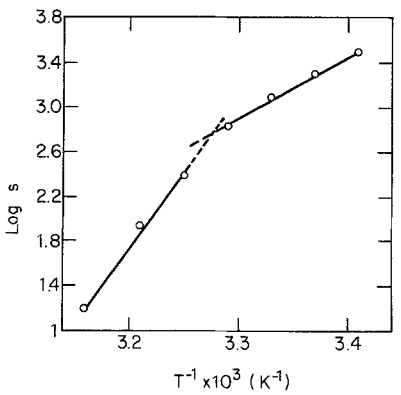

Fig. 4. Arrhenius plot of the light-induced conductivity changes. The conductivity changes obtained using a chopping frequency of 10 Hz were measured under the same conditions as in Figure 2 at various temperatures. The suspension was equilibrated for 30 min at each temperature

were performed at 10 Hz so that only the slow first-order loss of conductivity was detected. Heating the suspension from 20° C to 43° C caused a 10-fold decrease in the observed signal, and the Arrhenius plot of the data is shown in Figure 4. As can be seen from the figure, two straight lines were obtained intersecting at 31° C. A transition point in this region has been reported by several authors (Korenstein et al., 1976; Lanyi and Hilliker, 1976; Degani et al., 1978). The decrease in photoconductivity could be reversed by cooling the system back to 20° C, but the Arrhenius plot did not exhibit the break at 31° C (not shown). The activation energies for the conductivity changes, calculated from the slopes in Figure 4, are 10.9 kcal/mol and 26.4 kcal/mol for low and high temperatures, respectively.

In order to see whether the conductivity changes are sensitive to pH and whether the different processes contributing to that change exhibit similar pH dependence, we repeated the experiment described in the legend to Figure 2 at different pH values. The signals measured at 10 Hz and 90 Hz, which presumably reflect the slow and the fast processes, were plotted against the pH as shown in Figure 5. It turned out that both the fast increase in conductivity and the relatively slow loss of conductivity show similar dependence on the pH. They reach maximal values around neutral pH and approach zero at high and low pH values.

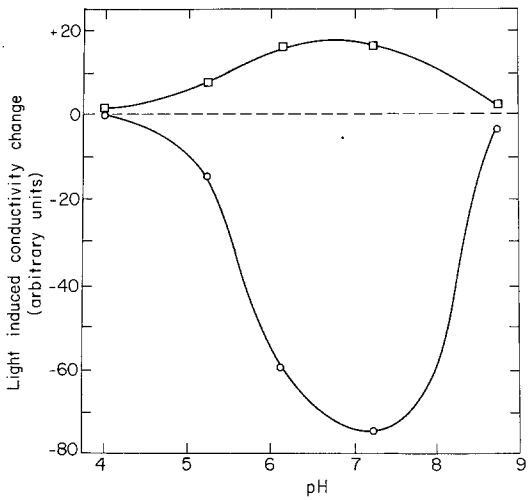

Fig. 5. Effect of pH on the light-induced conductivity changes. The experiment described in the legend to Figure 2 was carried out using several aliquots which were brought to different pH values in the absence of buffer. 10 Hz: O——O, 90 Hz: □——□

Discussion

The key question in this study is to establish which of the processes known to occur in purple membrane upon illumination are responsible for the photoconductivity.

The relative conductivity changes measured of the order of $1-2 \times 10^{-6}$, probably reflect changes in the number of current carriers in the suspension, and thus it is natural to try to correlate them with the light-induced protonation and deprotonation processes[3]. However, one can immediately see that the first-order loss of conductivity, which is the dominant conductivity change, cannot be due to those processes. Its lifetime is much too long to result from the deprotonation of the Schiff base (Dencher and Wilms, 1975; Lozier et al., 1976) and too short to result from pH changes measured in purple membrane suspensions on a time scale of seconds as a consequency (Garty et al., 1977). Furthermor acidification is expected to increase the conductivity, and in our case a net decrease in conductivity was observed under all conditions.

A possible artifact that could conceivably give rise to a light-induced conductivity change is modulated heating of the suspension (either direct heating of the electrolyte by the modulated beam or indirect heating by thermal dissipation of absorbed light). We would like to stress that such an artifact cannot account for our results for the following reasons:

1. Heating can increase the conductivity but not reduce it.

2. Heating is not expected to be affected by pH or temperature in the way that the observed signal was affected.

3. When pure electrolyte or dye solutions were illuminated no light-induced conductivity change was observed.

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3 From the optical density the concentration of bacteriorhodopsin in these experiments is about 8×10^{-6} M, while the total ionic concentration is about 8 M. Thus the observed light-induced conductivity changes would correspond to the association or dissociation of 1–2 ions per molecule of bacteriorhodopsin

We suggest that the loss of conductivity having a life-time of 70–80 ms is due to binding of ions, other than protons, to the membrane. This must be a result of light-induced changes in surface charge occuring on this time scale. It has already been established that upon illumination the protein-lipid complex undergoes conformational changes (Trissl and Montal, 1977; Bogomolni et al., 1978). If, as a result of these changes, charged groups on the membrane are exposed to the external medium, small mobile ions may be attracted to them and the conductivity of the suspension decreased. Part or even all of the charged groups exposed may be those involved in the protonation-deprotonation processes. Assuming that the association and dissociation reactions are not the rate-limiting step the above interpretation of the experimental data would indicate a light-induced conformational change characterised by a life-time of 70–80 ms and leading to a net exposure of charged groups to the external medium.

The above interpretation of the experimental observation is tentative, but predicts that the light-induced conductivity change should be sensitive to the ion composition and concentration. Preliminary experiments indicate that this is actually the case. Replacing 4 M NaCl by 2 M $MgCl_2$ or 2 M $K_2C_2O_4$ seems to double the conductivity change, and reducing the salt concentration strongly diminishes the signal. This observation indicates that both cations and anions are involved in the observed conductivity change.

The fast increase in conductivity measured may result either from protonation of the Schiff base (known to occur on this time scale), or from release of ions to the medium due to fast conformational changes (which cause a change in the number of surface charges on the membrane). The fact that both types of conductivity changes have similar dependence on the pH may suggest that the first is essential for the second to occur, and determines its magnitude.

The measured temperature dependence lends support to the model in so far as it indicates that the process is sensitive to the physical state of the membrane. Furthermore, the Arrhenius plot observed is very similar to the one obtained for the microviscosity of the purple membrane (Korenstein et al., 1976). In both cases the break in the plot was observed around 31° C and the activation energies above and below the transition temperature differ by a factor of ca. 2.

The measurements of the light-induced conductivity changes reported in this work demonstrate the high accuracy of the modulation excitation technique. In these experiments we could detect changes as small as 10^{-8} mho superimposed on a background conductivity of more than 0.03 mho, i.e., changes of the order of 3×10^{-7}. The power of the method comes of course from the fact that by using a phase sensitive detector we can eliminate completely the high DC background and measure precisely the small AC signal which has the same frequency as the chopped exciting beam.

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