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Light- and dark-adaptation of bacteriorhodopsin measured by a photoelectric method

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The process of light- and dark-adaptation of bacteriorhodopsin was studied by measuring the photoelectric response signal corresponding to the M form of the light-adapted photocycle. Two different kinds of sample were used: oriented dried samples and oriented gel samples. It was shown that the quantum efficiency of light-adaptation does not depend on the temperature of the sample, but strongly depends upon the water content, which means that in this process the determining step of the branching is the light excitation (bacteriorhodopsin to bacteriorhodopsin * or bacteriorhodopsin * to the next state), not a later step during the 13-*cis* photocycle. The process of dark-adaptation depends upon both the temperature and the humidity of the sample.

Introduction

In bacteriorhodopsin, the retinal exists in two conformations: 13-*cis* and all-*trans* [1]. The light-adapted bacteriorhodopsin contains only all-*trans*-retinal and has an absorption maximum at 568 nm. During dark-adaptation an equilibrium of both retinal conformations is established and the absorption maximum shifts to 558 nm [2]. Bacteriorhodopsin containing all-*trans*-retinal goes through a photocycle upon light-excitation and pumps a proton across the membrane. This photocycle can be monitored by the M form, which has a characteristic absorption maximum as 410 nm and also is well characterised by its photoelectric signal [3,4]. Bacteriorhodopsin containing 13-*cis*-retinal also undergoes a photocycle [2,5] but does not pump protons and has no M form [7,15].

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The ratio of 13-*cis*- to all-*trans*-retinal in the dark-adapted bacteriorhodopsin was estimated to be 1 [1,2,6,7,20]. Recent measurements improved with a fast retinal extraction method show that this ratio is 2 to 1.8, depending on temperature, measured between 4 and 50 °C [8]. Eisenstein et al. [21] used a ratio of 1.5 in their FTIR measurements.

In light-adapted bacteriorhodopsin, the measured light-induced charge motions correlate well with the photocycle [3]. In the dark-adapted bacteriorhodopsin additional charge motions were observed, which could be ascribed to the bacteriorhodopsin containing 13-*cis*-retinal [9–11]. The first observation of this type of charge motion was made on purple membrane attached to collodion film [9]. According to Ref. 10, the rise of the first negative signal resembles that of the all-*trans* form. In oriented dried samples two more steps were observed [11]. From these electric signals it could be estimated that in the bacteriorhodopsin containing 13-*cis*-retinal the charge does not leave

the membrane, but only undergoes a backwards and forwards motion with respect to the proton transport direction, which is in accordance with the fact that this type of bacteriorhodopsin does not pump protons.

The light-adaption is a light-activated process with an apparent quantum efficiency of 0.035 [5]. When the screening effect of the bacteriorhodopsin containing all-*trans*-retinal is taken into account, the quantum efficiency gives a value of 0.14 [12]. It has not been determined which is the step of the 13-*cis* photocycle at which the branching occurs towards the all-*trans* form of the retinal. Tokunaga et al. [22], using low-temperature measurement data, propose that all the bacteriorhodopsin containing 13-*cis*-retinal going through its photocycle is converted to the all-*trans* form. This proved not to hold at room temperature [2,13]. There were proposed different light-adaptation processes which consider almost all steps of the 13-*cis* photocycle as a possibility for branching [2,5,13], but the authors could not decide which is the real one.

The darl-adaptation of the light-adapted bacteriorhodopsin is a thermally activated process which can be monitored optically [13,14] or electrically [4,7] by measuring the concentration of the M form of the all-*trans* photocycle at a constant moderate excitation intensity. The lifetimes of dark-adaptation were measured at different temperatures [7,15,16] and the activation enthalpy was calculated to be 25 kcal/mol. Kouyama et al. [14] observed that the process of dark-adaptation is nearly independent of the humidity of the sample.

The quantity of 13-*cis*-retinal converted to the all-*trans* form during the light-adaptation depends on the humidity of the sample [4,13]. In these measurements the process of light-adaptation was measured by using a constant light energy, which caused total light-adaptation in the suspension.

In the present work, the process of light- and dark-adaptation on oriented dried samples and oriented gel samples were studied. The very sensitive electric signal measurements provide new information about these processes.

Materials and Methods

The purple membrane was obtained by the standard procedure from *Halobacterium halobium* strain ET 1001 [17]. The preparation of the oriented dried and gel samples has been described in detail elsewhere [18,19]. The gel samples made from the same purple membrane preparation showed a different light-adapted to dark-adapted electric signal amplitude ratio as compared to the data from Refs. 2 and 5 measured in suspension. The observed differences, which are due to the electric properties of the gel sample, do not affect our results, since we measured the changes, not the absolute values.

The measuring system of the photoelectric and optical signals has already been described [4,11]. The water content of the sample was achieved by keeping it for at least 24 h in a closed holder in which the humidity was controlled using various saturated salt solutions [13]. The flash intensity of the exciting Opton dye laser (pulse length 1 μs, at 590 nm) was reduced by neutral density filters to about 10 $\mu J/cm^2$ to avoid light-adaptation. This small excitation intensity ensured that after 100 flashes no light-adaptation could be detected. Characteristic photoelectric signals are shown in Fig. 1. The fast-falling part of the signal corresponds to the bacteriorhodopsin to L transition, while the rising one is connected with the L to M transition. The amplitude of the positive part is proportional to the concentration of the M form [7,11]. In some cases, only for control, the 400 nm absorption change was also measured with a dim monitoring light. In the present paper only the electric signals are discussed. During one measurement, ten signals were averaged, thereby reducing

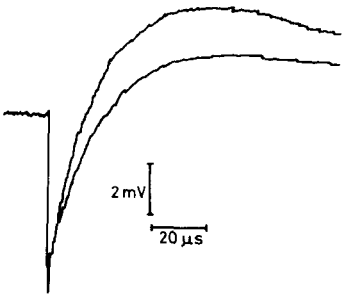

Fig. 1. The photoelectric signal measured on dried sample at 20 ° C and 0.06 g H_2O/g bacteriorhodopsin water content with 1 mΩ resistance parallel to the sample. Lower curve: signal from the dark-adapted sample; upper curve: after light-adaptation.

the error in signal amplitude to less than 5%. The repetition rate of the signals was about 5 s. As a control of the system, an unoriented dried sample was measured under the same conditions and no electric signal could be detected.

The capacitance of the oriented sample was greater then 100 pF and slightly increased with increasing humidity. The resistance of the sample usually was around 10^{11} Ω. The measuring resistance was 1 MΩ, so the time constant (RC) of the electric circuit was over 100 μs. At high water content of the sample, its resistance fell to a value of the order of magnitude of the measuring resistance. This could cause some decrease in the signal amplitude. During the calculations mainly the ratios of the signals measured at the same humidity were used, so this effect over the amplitudes was diminished.

Total dark-adaptation of the samples was assured by keeping them overnight in the dark. Light-adaptation was accomplished by using a mercury lamp (HBO 200, Carl Zeiss, Jena) with heat and green light glass filters (average light intensity 50 mW/cm^2). The different degrees of light-adaptation were achieved by using neutral density filters during the 0.5–2 min illumination. The irradiance was measured with a photometer (Alphametrics, model dc 1010).

The data collection and analyses were done using a personal computer (Sinclair QL).

Results

Light-adaptation

The amplitude ratios of light-adapted to dark-adapted signal as a function of water content (Fig. 2) were measured for high energy density (about 10 J/cm^2). The shape of the upper curve, which is the ratio of the M form existent in the light- and dark-adapted bacteriorhodopsin measured under the same conditions is similar to that described in Refs. 4 and 13. This ratio is equal to the ratio of the all-*trans*-retinal content in the light- and dark-adapted bacteriorhodopsin. From the M form amplitude measurement at high water content it was estimated that the dark-adapted bacteriorhodopsin contains about 55% 13-*cis*- and 45% all-*trans*-retinal. This ratio is independent of the humidity of the sample, as the signal amplitude of the

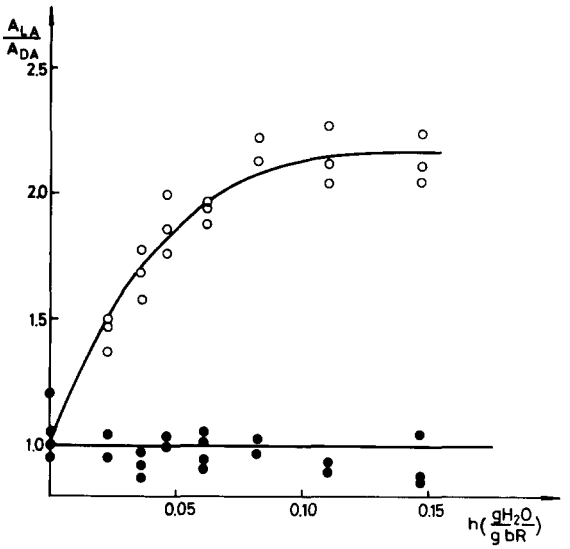

Fig. 2. The dependence of the amplitude ratio of the light-adapted to the dark-adapted signal in the case of the maximal radiant energy density (about 10 J/cm^2) as a function of the water content of the dried sample at 20 °C. The lower curve corresponds to the fast negative signal, the upper curve to the signal of the M form.

dark-adapted form is approximately constant for all water contents. The first fast negative photoelectric signal is independent of the light adaptation at every water content (Fig. 2, lower curve), in good agreement with the results in Ref. 10.

To gain a better understanding of the dependence of light-adaptation on the water content of the sample measurements were made at different radiant energies (Fig. 3) at 20 °C. The change of the amplitude of the L–M electric signal at high water content for the highest energy was considered to correspond to 100% light adaptation. Further increase in light-adaptation energy did not cause any change in the signal amplitude. This showed that the sample possibly had only all-*trans*-retinal. Fig. 3 shows that a given radiant energy makes a more complete light-adaptation at high sample water content. The extent of light-adaptation plotted as a function of radiant energy density (Fig. 4) implies that even the highest energies for light-adaptation saturate only those samples containing a large quantity of water. The temperature dependence of the light adaptation at different humidities could not be measured due to

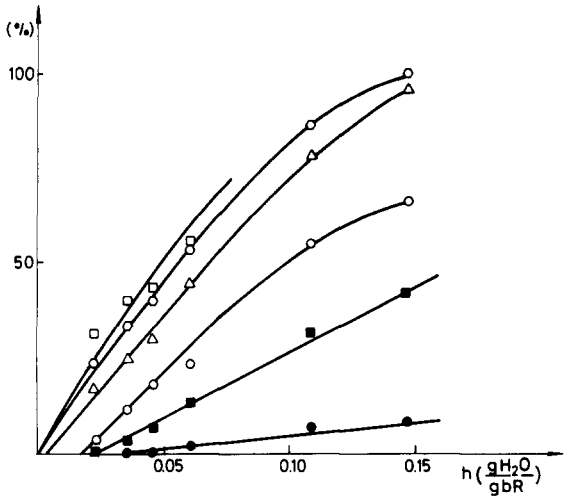

Fig. 3. The change in light-adaptation as a function of the water content of the dried sample at different irradiated light energies (J/cm^2): ●, 0.011; ■, 0.056; ○, 0.179; ▽, 0.54; ○, 1.9; □, 4.6. The errors are less than 10%.

the fact that by changing the temperature of the sample its water content is also changed.

The oriented gel sample has a light-adaptation curve very similar to the one with high humidity (Fig. 4), but shifted to lower energies. As the water content of the sample increases, less radiant energy is needed to convert the 13-*cis*-retinal to the all-*trans* form. The light-adaptation of the oriented gel sample was investigated in a wide energy range at different temperature (Fig. 5). In the gel sample 100% light-adaptation was considered separately for every temperature. Surprisingly, the

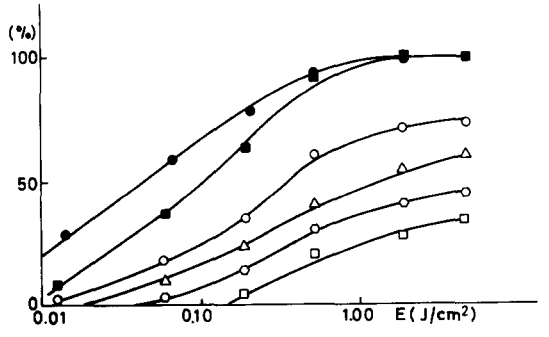

Fig. 4. The change in the light-adaptation as a function of the radiant energy measured at 20 °C for different water contents of the dried sample: (g H_2O/g bacteriorhodopsin): □, 0.023; ○, 0.036; △, 0.046; ○, 0.061; ■, 0.11. ●, Oriented gel sample. The errors are less than 10%.

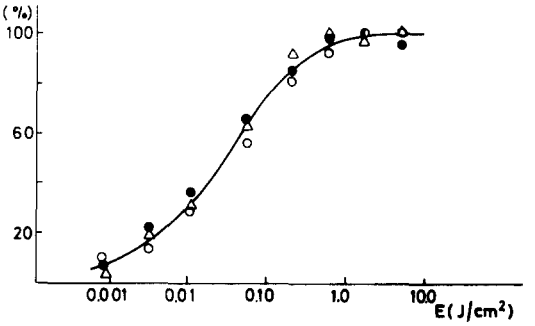

Fig. 5. The change of the light-adaptation in function of the radiant energy in the oriented gel sample at different temperatures: ●, 5 °C; ○, 20 °C; △, 35 °C. The errors are less than 10%.

process of light-adaptation does not depend on temperature.

Dark-adaptation

The process of dark-adaptation was also studied as a function of the water content of the oriented dried sample. To determine the lifetime of dark-adaptation, the procedure described for the determination of the M concentration was used every 5–30 min over a long time interval in which the sample was kept in the dark. The measured amplitude changes could be fitted with two exponentials (Fig. 6) possessing two almost identical amplitudes. The one exponential fit was strongly different from the measured kinetics. There is a pronounced dependence of the dark-adaptation lifetime upon the water content. This contradicts the observation made by Kouyama et al. [14], who found no humidity dependence. The contradiction can be due to the fact that they did the measurement over a very short time interval (the time of observation was that in which the amplitude of the signal decreased from 1 to 9/10), where the size of the signal can be strongly affected by the drying effect of the illuminating light if the sample is not well thermostated.

For the oriented gel the same method gave a single exponential with lifetime of 30 min at 35 °C, in good agreement with data from the literature [7,15,16]. From the temperature dependence of the lifetime of dark-adaptation, the Arrhenius parameters of the energy barrier were calculated. The activation enthalpy is 99 kJ/mol (24 kcal/mol), in

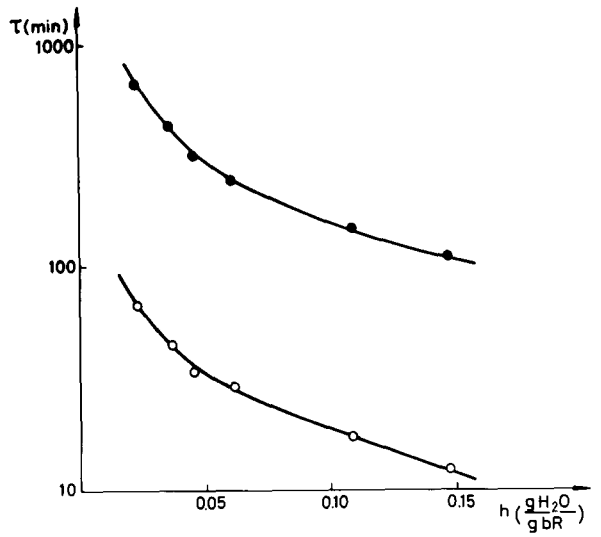

Fig. 6. The dependence of the dark-adaptation lifetime from the water content of the dried sample, when the process of dark-adaptation is fitted with two exponentials. The amplitude ratio of the two components is around 1 over the whole humidity range. The errors are less than 5%.

good agreement with Ohno et al. [15] and Dencher et al. [16]. The frequency factor is $4 \cdot 10^{13}$ s^{-1}.

Discussion

The first negative photoelectric signal amplitude (Fig. 1) does not depend upon the light-adaptation state of the bacteriorhodopsin and the humidity (Fig. 2). This charge motion seems to be similar for the all-*trans* and 13-*cis* conformation of the retinal [10].

On the rising part of the signal (Fig. 1) the dark-adapted one contains an additional component, as was shown in Ref. 11. The maxima of both signals are almost at the same place and this is considered to be characteristic only for the all-*trans* photocycle [7,9,11]. From the earlier studies of the electric signals on oriented bacteriorhodopsin samples, it is evident that this amplitude is proportional to the concentration of the M form [3,4,7,9].

The dependence of light-adaptation upon the hydration of the sample and the radiant energy (Figs. 3, 4) indicates that the quantum efficiency of the process depends on the water content. Fig. 4 shows that higher light energies could convert more 13-*cis* into the all-*trans* form at low sample humidity. The dependence of the light-adaptation upon the hydration of the sample, published earlier [4,13] and in this paper, is valid only for a constant moderate irradiation of the sample. These measurements do not show that at high water content the sample considered 100% light-adapted contains only all-*trans*-retinal. It can be stated only that it contains more all-*trans*-retinal than the dark-adapted one and that further light-adaptation does not change this ratio. From this fact it was concluded that the bacteriorhodopsin already contains only all-*trans*-retinal. If this does not hold, then in the dark-adapted form the 13-*cis*-retinal content should be even greater than 55%.

The independence of the light-adaptation from the temperature of the sample (Fig. 5) means that this process is not determined by the thermally activated energy barriers. While the temperature change of the sample causes changes in the transition probabilities through the low energy barriers of the bacteriorhodopsin, which are in the ground state, the hydration can make disturbances in the energy levels of the excited state by the big permanent dipole moment of the water molecule. The relative position of the 13-*cis* and all-*trans* excited state to the 13-*cis* ground state can be changed by changing the water molecules. So the probability of transfer from the 13-*cis* ground state to 13-*cis* or all-*trans* excited state can be influenced by the water content of the sample. The quantum efficiency of the light-adaptation is determined by the position of the excited states relative to the 13-*cis* ground state.

The dependence of the final amplitude of light-adaptation upon the temperature [8] shows that the equilibrium between the 13-*cis* and all-*trans* conformation of the retinal stabilised during the dark-adaptation depends on the temperature, which means that the two ground states are not exactly at the same energy level.

Concerning the dark-adaptation, it was shown that this is strongly dependent on the water content of the sample. Further studies to clarify what is the role of the water and what are the Arrhenius parameters describing this phenomenon are now in progress.

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