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EFFECT OF WATER ON THE STRUCTURE OF BACTERIORHODOPSIN AND PHOTOCHEMICAL PROCESSES IN PURPLE MEMBRANES

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Summary

Visible and infrared spectra of bacteriorhodopsin films under different humidities at room and low temperatures are investigated. On dehydration of purple membranes at room temperatures an additional chromophore state with the absorption band at 506 nm is revealed. The photocycle of purple membranes in the dry state is devoid of the 550 nm intermediate and involves the long-lived intermediate at 412 nm. As water is removed, the 550 nm intermediate becomes undetectable. The analysis of the infrared spectra shows that dehydration does not affect the ordering of the main network of the interpeptide hydrogen bonds which stabilizes the α-helical conformation (slightly distorted in the initial humid dark- and light-adapted state); light adaptation (*cis-trans* isomerization) of bacteriorhodopsin results in an increase of sorbed water in purple membranes. Dehydration of purple membranes decreases the reaction rate of *cis-trans* isomerization.

Introduction

Bacteriorhodopsin is a retinylidene protein localized in the cell membrane of the extreme halophile *Halobacterium halobium* [1]. In purple membranes light energy is converted into an electrochemical proton gradient across the membrane [2—5]. Retinal is responsible for the intensive absorption band of purple membranes with the maximum at 560 nm. On illumination bacteriorhodopsin undergoes cyclic photoconversion which can be followed by changes of the absorption band [6—9]. Retinal in the dark-adapted bacteriorhodopsin

Abbreviation: BR, bacteriorhodopsin.

consists of the equimolar mixture of the 13-*cis* and *all-trans* forms. In light-adapted specimens only *all-trans* retinal is found [10]. In purple membranes the protein molecules are spaced in the hexagonal array, with three molecules clustered around a 3-fold axis [11,12]. The protein contains seven α-helical segments which form a superhelical 'coiled-coil' type grouping aligned perpendicular to the membrane plane [13]. Photoconversions of bacteriorhodopsin can be observed in both purple membrane suspensions [6—8] and the solid state [14—16]. Spectroscopic studies of films are undoubtedly of particular interest in elucidating the role of water in the photochemical events. The spectra of films at room temperature suggest that there is a well-pronounced hydration effect [15,16]. Our paper presents further experimental evidence of the influence of water on the structure of bacteriorhodopsin and photochemical processes in purple membranes at room and low temperature.

Materials and Methods

Purple membranes were isolated from *H. halobium* (RL_1), according to the procedure of Oesterhelt and Stoeckenius [1]. The samples were prepared in films dried from the concentrated ($4 \cdot 10^{-4}$ M) water suspensions (pH $\approx$ 7) and deposited on fluorite or quartz plates. The film thickness was about 3—5 μm. Absorption spectra in the visible were determined on a spectrophotometer SP-800 (Cambridge, U.K.). Infrared spectra were measured with a spectrophotometer UR-20 (Karl Zeiss, Jena, G.D.R.). To avoid the effect of visible light in the infrared spectrophotometer, Si or Ge plates transparent for infrared radiation only were placed on the exit window of the source (globar). Kinetic measurements were carried out using a spectrophotometer designed in our laboratory (Fig. 1) on the basis of the double-grating monochromator DFS-12 (LOMO, Leningrad, U.S.S.R.). It permitted to pass the light through the sample from two sources simultaneously. The actinic beam λ_1 of a selected (by an interference filter) wavelength was passed through a photo-shutter and fell onto the sample. The measuring beam λ_2 reflected from the transparent quartz plate was passed through the sample and the monochromator and was detected by a photomultiplier-measuring circuit. A variable temperature cell (Fig. 2) permitted measurements over a range from —196 °C to +200 °C. Cooling was effected by liquid nitrogen. Heating was achieved electrically. With the

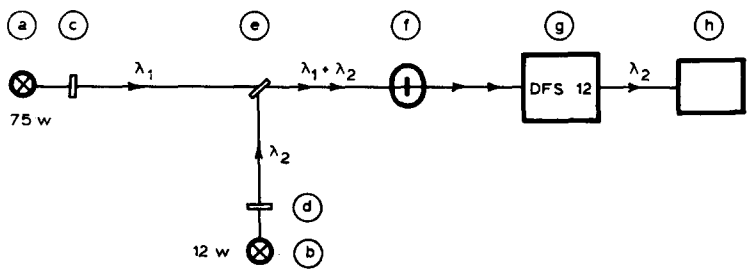


Fig. 1. Diagram of the equipment for studying kinetic processes. (a and b) light sources, filament lamps; (c and d) interference filters; (e) quartz plate; (f) variable temperature cell; (g) monochromator DES-12 and (h) measuring circuit.

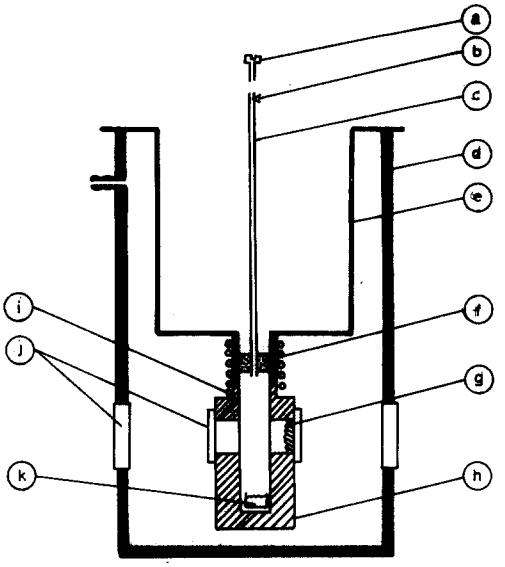


Fig. 2. Variable temperature cell. (a) a screw for regulation of nitrogen gas flow; (b) exit hole with screw; (c) tube for the removing of nitrogen gas from the cell; (d) external wall, and (e) inner wall of the Dewar vessel; (f) felt wad; (g) sample; (h) cooper cell; (i) electric heater; (j) fluorite windows, and (k) salt solution.

open exit tube (b), liquid nitrogen entered the cell leaking through a felt wad (f). With the closed tube (b), the pressure of nitrogen gas prevented the entering of liquid nitrogen into the cell. The cell enabled one to easily vary the cooling and heating regime and to keep a selected temperature for a long time. The required humidity in the cell was maintained by the conventional procedure [15,17]. Purple membrane films deposited on the window (Fig. 2) were incubated at room temperature for 24 h in an atmosphere of saturated salt solutions in the hermetic cell sealed with an air-tight stopper (instead of the felt wad (f)). Either H_2O or ^2H_2O atmosphere was used. In dry films water was removed by vacuum pumping for 3—4 h (in this case purple membranes were deposited on the outer side of cell window). The fixation of water content in the sample at low temperature was achieved by a fast cooling of films in a given hydrated state. For dark adaptation purple membranes were kept in dark at room temperature for 24 h. Special attention was paid to eliminate the action of the measuring light. Under our experimental conditions the action was negligibly small and did not induce noticeable changes in absorbance spectra.

Results

Visible spectra

Dark-adapted purple membranes in films at 25 °C and 100% relative humidity give an absorption band which coincides with that for an aqueous suspension. Dehydration of the films from 100% to 15% relative humidity induces small changes in the spectra. At lower humidities they become markedly pronounced. The shape of the band changes. Its maximum shifts towards the

high-frequency region (Fig. 3). In the difference spectrum a peak near 470 nm appears. These changes are reversible. The isosbestic point at $\lambda = 526$ nm in the spectra is observed. Using the experimental procedure put forward by Fok [18,19], the observed changes in the spectra can be presented as result of a band superposition. The diagrams plotted according to Fok (a ratio of absorbance determined at two different humidities $A_2(\lambda)/A_1(\lambda)$ vs. wavelength λ) show two distinct plateaus and a line segment (Fig. 4). They indicate that at least two overlapping components $\varphi_1(\lambda)$ and $\varphi_2(\lambda)$ are present in the spectra, which are changed on dehydration in the intensity and remain approximately constant in the position and shape:

$$A_1(\lambda) = \varphi_1(\lambda) + \varphi_2(\lambda); A_2(\lambda) = a_1\varphi_1(\lambda) + a_2\varphi_2(\lambda) \quad (1)$$

The plateaus correspond to the spectral region where the components are not overlapped. The plateau ordinates in the Fok's diagrams determine the factors a_1 and a_2 in Eqn. 1. If A_1, A_2, a_1 and a_2 are known it should be possible to calculate the separate components $\varphi_1(\lambda)$ and $\varphi_2(\lambda)$ from the expressions (Eqn. 1). The second line segment observed in the diagrams in the 400—450 nm region (Fig. 4) is due to overlapping of the φ_2 and the weak β-band remaining constant upon dehydratation. The β-band of the chromophore may be considered as a baseline. The low-frequency shoulder of the β-band is calculated on assumption that after the excluding of the β-band the Fok's diagram in the region of 400—450 nm is transformed into the plateau with the a_1 ordinate. In the region of 450—700 nm the baseline is found from the spectra of bleached bacteriorhodopsin films treated with C_2H_5OH/H_2O mixture and dehydrated after illumination (Fig. 5). The dehydration of purple membranes gives rise to an additional component in the absorption spectra of bacteriorhodopsin with the maximum at 506 nm (Fig. 4). The intensity of the peaks at 506 nm and 560 nm depends on relative humidity in a complicated manner (Fig. 6). At humidities from 100% to 55% (relative humidity) an increase of the peak at 506 nm and decrease of the peak at 560 nm occur. Further

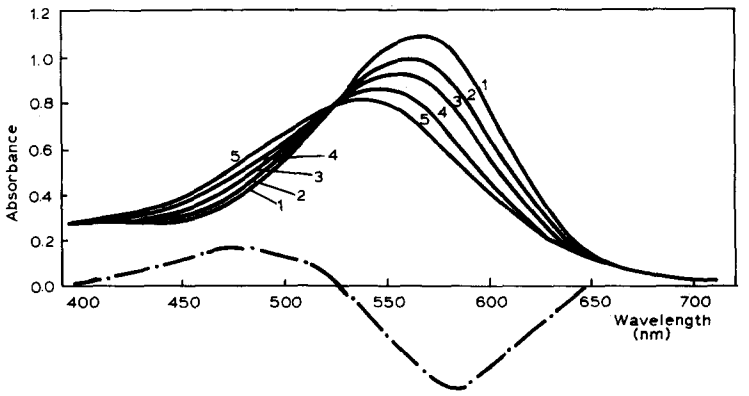


Fig. 3. The 560 nm absorption band of the dark-adapted purple membranes upon dehydration of the film. (1) The wet film, 75% relative humudity. (2—5) The film spectra at humidities below 15% relative humidity (different stages of dehydration after 10 min, 20 min, 30 min, 40 min of vacuum pumping. At the bottom of the figure the differential spectrum is given.

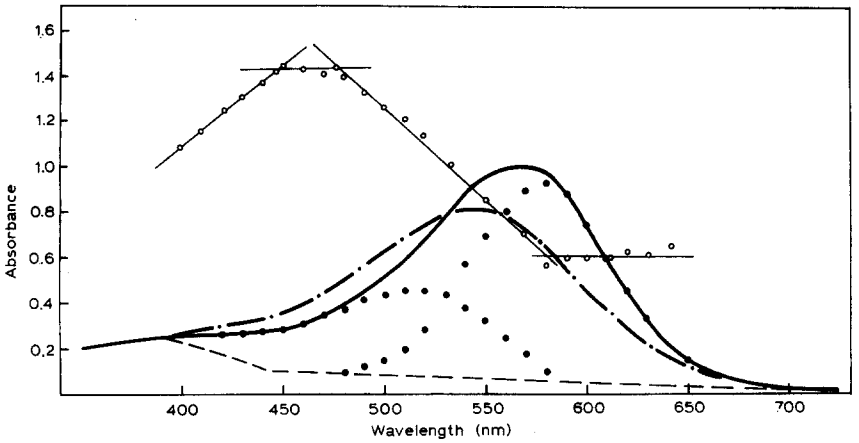


Fig. 4. Decomposition of the absorption band by Fok [18,19]. ———, **the wet film, 75% relative humidity,** $A_1(\lambda)$; · — · — ·, **the dry film, 30 min of vacuum pumping,** $A_2(\lambda)$; - - - - -, **the baseline (the β-band of the chromophore plus the scattering light);** ○○○, **the ratio** $A_2(\lambda)/A_1(\lambda)$; ●●●, **the components obtained by the procedure of Fok.**

dehydration up to 20% relative humidity is accompanied by a reverse intensity redistribution from the peak at 506 nm to the peak at 560 nm. Full dehydration induces the intensity of the peak at 506 nm to rise sharply again and that at 560 nm to fall. However, even with prolonged vacuum pumping at room temperature, about 50% of bacteriorhodopsin molecules are found to remain in the form characterized by the absorption peak at 560 nm. Probably due to the strong overlapping of the components the 506 nm peak was not detected earlier. Dehydration of light-adapted films is followed by substantial spectral changes (much more distinct than that of dark-adapted ones) described in detail earlier [16]. The spectra of light- and dark-adapted purple membranes in the dry state coincide. The changes of the shape on dehydratation can be also

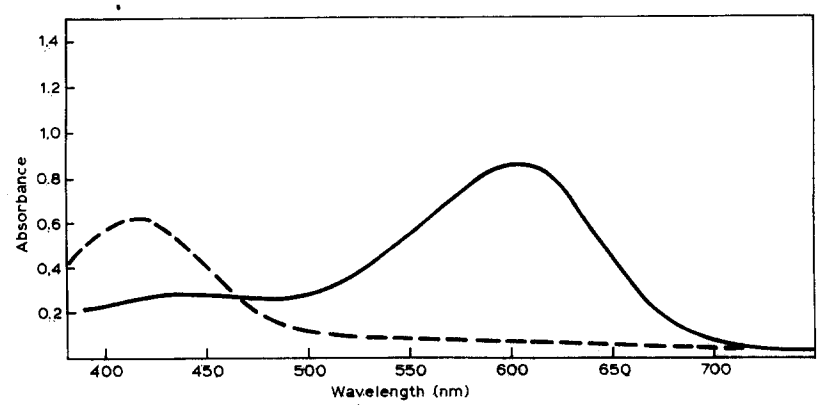


Fig. 5. The spectrum of the dry film after bleaching of purple membranes. ———, **the wet film in the light-adapted state (75% relative humidity);** - - - - -, **the dry film (30 min of vacuum pumping) after treating with** C_2H_5OH/H_2O **mixture (50%, v/v). The absorbance in the region of 530—700 nm is used as a baseline (see Fig. 4).**

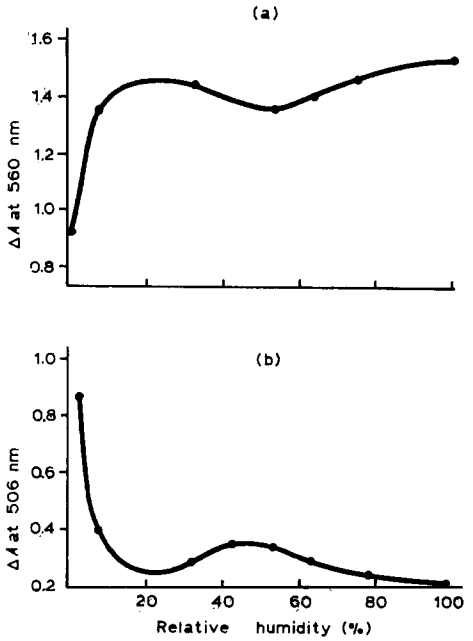


Fig. 6. Changes in intensity of the components in the spectra of dark-adapted purple membranes depending on relative humidity (T = 20°C). The absorbance at the band maximum in the humid state (100% relative humidity) is 1.6 absorbance units.

presented as a result of band superposition. The position and shape of the dehydration-induced peak at 506 nm are found to be independent on light adaptation of purple membranes. Dehydration of light-adapted purple membranes (similar to that of dark-adapted ones) is followed by the conversion of about 50% of bacteriorhodopsin molecules into the 506 nm form.

Infrared spectra

Dark- and light-adapted purple membranes have well-pronounced infrared spectra typical of lipoprotein complexes (Fig. 7) [20—22]. Some of the charac-

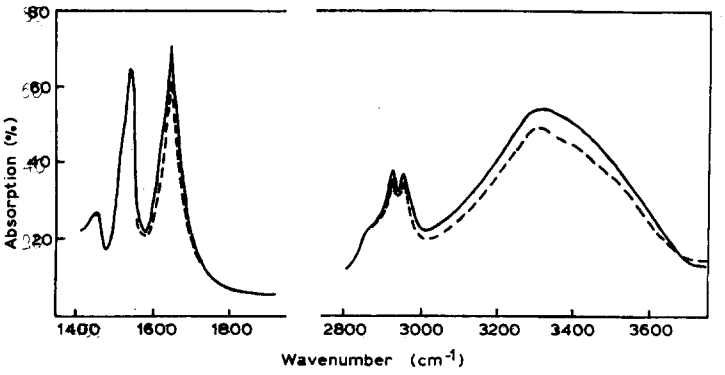


Fig. 7. Infrared spectrum of the purple membrane film at 100% relative humidity and temperature +25°C. - - - - - -, dark-adapted specimen; ———, light-adapted specimen illuminated with the light of λ = 560 nm.

teristic absorption bands can be attributed to respective vibrations of the protein polypeptide chain. In the spectra there are an amide A band of NH-stretching vibrations (3315 cm^{-1}), an amide I band of CO-stretching vibrations (1662 cm^{-1}), and an amide II band (1548 cm^{-1}). These bands are sensitive to the conformation of polypeptide chains and are widely used for the conformational analysis of proteins [23—25]. The shapes and the maximum positions of the amide I band (and hence the α-helical polypeptide conformation [24,25]) for the saturated atmosphere of ^2H_2O and for the ^2H_2O suspension are alike [26]. The amide I contour does not change on light adaptation. The half-width of the amide I band amounts to $\Delta\nu_{1/2}$ = 43 cm^{-1}. This value is rather high, taking into account that in purple membranes we deal with the protein of the high α-helical content and the small contribution of random-coil conformation [11—13]. Such high values of $\Delta\nu_{1/2}$ are typical of polypeptides and proteins with the distorted α-helical structure [27,28]. Decomposition of the amide I contour by the conventional procedure [24,27] and analysis of the α-helical component lead to the same conclusion (unpublished results). The maximum of the amide I band at 1662 cm^{-1} in bacteriorhodopsin is disposed at frequencies substantially higher (approx. 10 cm^{-1}) than those found in α-helical polypeptides and proteins [23,28]. The main network of interpeptide hydrogen bonds in the initial humid dark-adapted and light-adapted state of bacteriorhodopsin seems to be disturbed, possibly due to the specific packing of the bacteriorhodopsin polypeptide chain in purple membranes [13]. Dehydration of purple membranes does not induce any noticeable changes in the parameters of amide bands. The shape of amide I band and the half-width of the 'α-helical' component are retained. The half-width of the 'residual' amide A band, measured in saturated atmosphere of ^2H_2O (the parameter often used for indicating conformational changes in polypeptides and proteins [29,30]) does not alter as ^2H_2O vapours are removed.

Photoactivity at room temperature

Irradiation of a dehydrated film with light of a wavelength from 430 to 550 nm gives rise to the absorption band of the 412 nm intermediate (called M_{412} [6]). After turning off the light, the band at 412 nm gradually disappears and the chromophore returns to the initial state. The formation of M_{412} occurs rapidly, beyond the time resolution of the spectrophotometer used (0.5 s). The decay of M_{412}, on the contrary, proceeds slowly over a measurable time. On illumination of purple membranes under conditions far from light saturation the photostationary concentration of the long-lived M_{412} is determined by the absorbance of only the 506 nm component rather than the whole band. The M_{412} amplitude is negligibly small on excitation at 600—700 nm and maximal on excitation at 500—520 nm. The results is supported by more detailed studies at low temperatures (see below). The photostationary concentration of the 412 nm intermediate (ΔA) and its half-time ($\tau_{1/2}$) depend on water content in the film (Fig. 8). The most pronounced changes in ΔA and $\tau_{1/2}$ occur at a relative humidity of below 10%. The decay kinetics of M_{412} are complicated. The kinetic curves can be represented at least by a sum of three exponentials. This result is in agreement with the data of Korenstein and Hess [15]. The half-life time of M_{412} is not only humidity but also temperature dependent. As

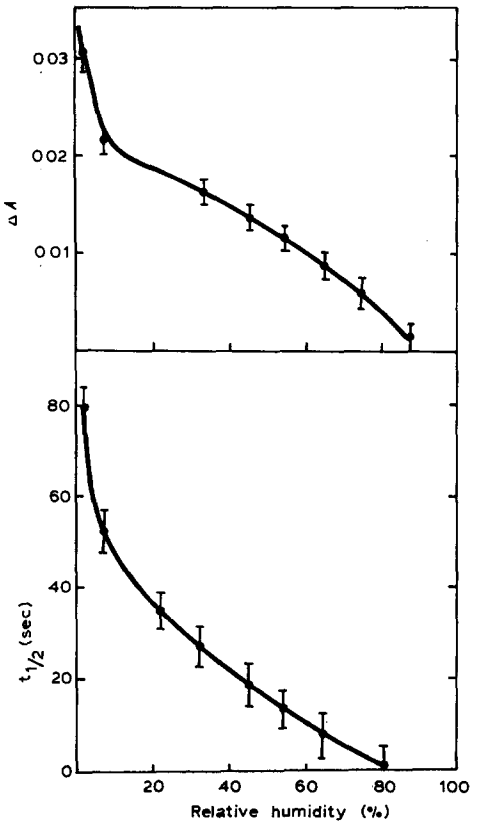


Fig. 8. Changes in the M_{412} photostationary amplitude (ΔA) and half-life period ($\tau_{1/2}$) depending on relative humidity (T = 20°C). The wavelength of acting light is 510 nm. The absorbance at the band maximum in the humid state (100% relative humidity) is 1.5 absorbance units.

temperature decreases, the half-life time increases. In dehydrated films at temperatures below −10°C the 412 nm photoproduct shows no decay at all. When analyzing the spectrum of an entirely dehydrated film at temperature −8°C to −10°C, we can easily see that only one of two components (506 and 560 nm) in the original spectrum undergoes some changes. Under these conditions the formation of the 412 nm intermediate due to illumination results in a decrease of intensity of only the 506 nm component (Fig. 9). Obviously, it is the 506 nm photocycle that involves the long-lived 412 nm intermediate. On hydration of the purple membrane the half-life time of the 412 nm intermediate shortens up to milliseconds [15].

In a saturated atmosphere of water vapours the illumination of a wet film in a wavelength range from 500 to 600 nm causes a red shift of the absorption band from 560 nm to 568 nm. The spectral changes induced by light adaptation are entirely the same as in aqueous suspensions of purple membranes [1,8]. After turning off the light the initial state of the chromophore is restored. In a saturated water atmosphere the half-time of the reverse process is about 30 min. As the film is dehydrated at room temperature (20°C) the 'red' shift decreases (Fig. 10). The decay kinetics measured at λ = 580 nm are

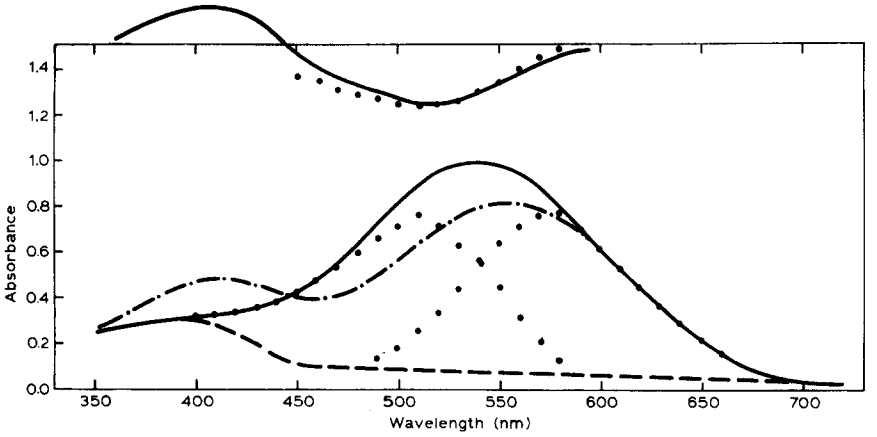


Fig. 9. Photoactivity of purple membranes in the dry state at the temperature −10°C. ———, the spectrum of the film dehydrated by vacuum pumping in dark; · — · — ·, the spectrum of the film illuminated at λ_{act} = 510 nm; - - - - - -, the baseline (see Figs. 4 and 5), and ● ● ●, the components obtained by the procedure of Fok. At the top of the figure the differential spectrum is given. The correlation between the negative peak in the differential spectrum and the 506 nm component shows that at this temperature the $M_{412} \rightarrow BR_{506}$ transition takes place.

distinctly biphasic (Fig. 11). Both the rate constants k_1 and k_2 are humidity dependent (Fig. 12). We have found that the rate constant k_2 relates to changes of the background line in the spectrum. On illumination of a wet film in the visible spectrum along with the 'red' shift a jump of the background line is observed. In dark the background line is restored to its original form. Similar changes of the background line occur also in hydration of the film in the absence of the actinic light. Evidently the photoisomerization of the retinal changes the sorbing properties of purple membranes. This suggestion is supported by infrared studies of purple membrane films in the dark- and light-adapted states. The experimental conditions are described above. The temperature control showed that the illumination (12 W filament lamp with an interference filter λ = 560 nm) does not result in heating of the sample. The *cis-trans* isomerization reaction is found to stimulate the increase of infrared absorption (Fig. 7). The spectral changes are well seen particularly in a range

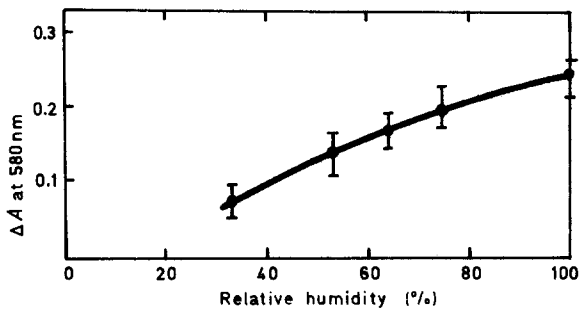


Fig. 10. The difference in absorbance at 580 nm between the light- and dark-adapted films of purple membranes depending on relative humidity (T = 20°C). Absorbance at the band maximum in the humid state (100% relative humidity) is 1.5 absorbance units.

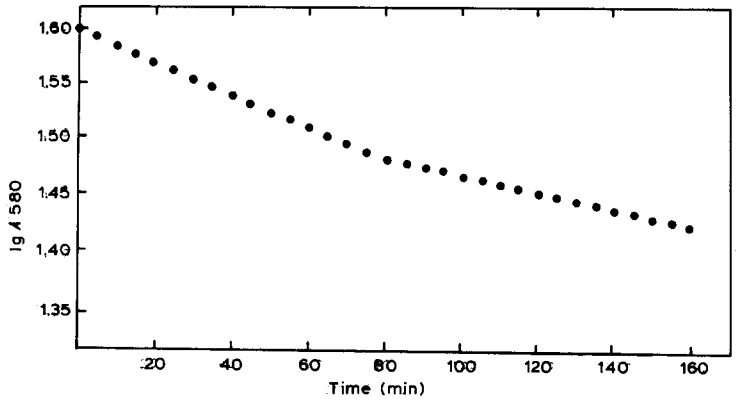
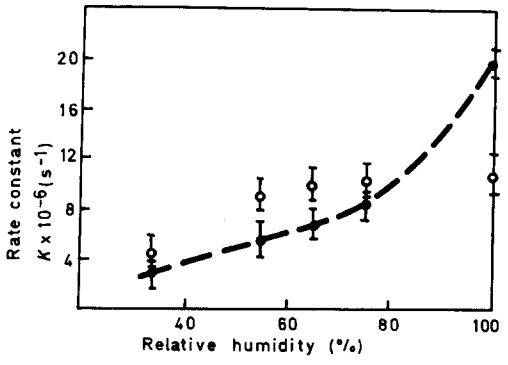


Fig. 11. Semilogarithmic plots of the 568 nm decay (54% relative humidity), $T = 20°C$.

3400—3500 cm^{-1} and 1650 cm^{-1} for the specimens exposed to H_2O vapours and 2500—2600 cm^{-1} and 1210 cm^{-1} for the specimens exposed to ^2H_2O vapours. The analysis of the differential spectra shows that the light-induced infrared bands coincide with the spectrum of sorbed water. There is a straight correlation between the sorption isotherm and the rate constant k_2 (Fig. 12). Thus, illuminating purple membranes causes increased sorption of moisture on them (about 200 molecules of water per one molecule of bacteriorhodopsin). It should be possible to estimate this value using the molar absorptivity of the 3450 cm^{-1} OH-stretching band for H_2O in the infrared region and that of the 568 nm band in the visible region for bacteriorhodopsin molecules [8]. In dark, purple membranes give up the added moisture and return to the initial state. Simultaneously, the 'red' shift in the spectrum disappears and the back-



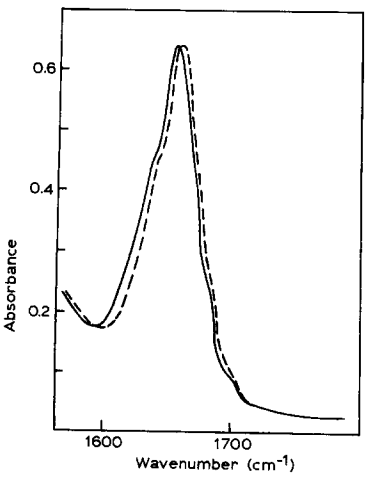


Fig. 12. Dependence of rate constants k_1 (○) and k_2 (●) of the 'red shift' decay at $\lambda = 580$ nm on relative humidity; - - - - -, isotherm sorption measured by the infrared method.

Fig. 13. Amide I contour in the dark-adapted (- - - - -) and light-adapted (———) state under conditions of the saturated ^2H_2O atmosphere ($T = 20°C$).

ground line is restored. The transition from the dark- to the light-adapted state does not induce noticeable changes in the shape of the amide bands in the infrared spectra (provided the overlapping effect of water absorption (^2H_2O or H_2O) is taken into account and eliminated). An upshift of the amide I frequency (about 5 cm^{-1}) is observed (Fig. 13). The frequency shift depends on humidity and is absent in dry films.

Photoactivity at low temperature

Cooling dry and wet purple membranes to —196°C is accompanied by pronounced changes in the absorption spectra. The absorption band gets narrower and shifts to a long-wavelength region. A distinct fine structure of the band emerges (Figs. 14 and 15). The difference in the spectra between dry and wet films becomes less pronounced. In dry films at —196°C the maximum of the band is shifted to short wavelengths as compared to wet films, the absorbance is decreased, and the half-width is broadened a little. However, additional components similar to that observed in dry films at room temperature are not detected by the method of Fok. Illumination of both wet and dry specimens with a 'short' wavelength light (λ = 480—560 nm) induces a shift of the band to a long wavelength (Figs. 14 and 15) due to the K_{610} formation [6,7]. The primary photoproduct is stable at —196°C. The reaction is reversible by 'long-wavelength' light (λ = 580—700 nm). The primary transition seems to be hydration independent. The influence of water becomes distinct in subsequent photochemical events initiated by the rising temperature. In the dehydrated state, at temperatures higher than —120°C, a direct conversion of K_{610} to M_{412} complex is observed (Fig. 14). The detailed analysis of the low-temperature spectra shows that the 550 nm intermediate (L_{550}) does not occur in dry films by slow raising of the temperature from —196°C to +20°C. At temperatures above —40°C the $M_{412} \rightarrow BR_{568}$ transition takes place with the distinct isosbestic point in the spectra at λ = 452 nm (Fig. 16).

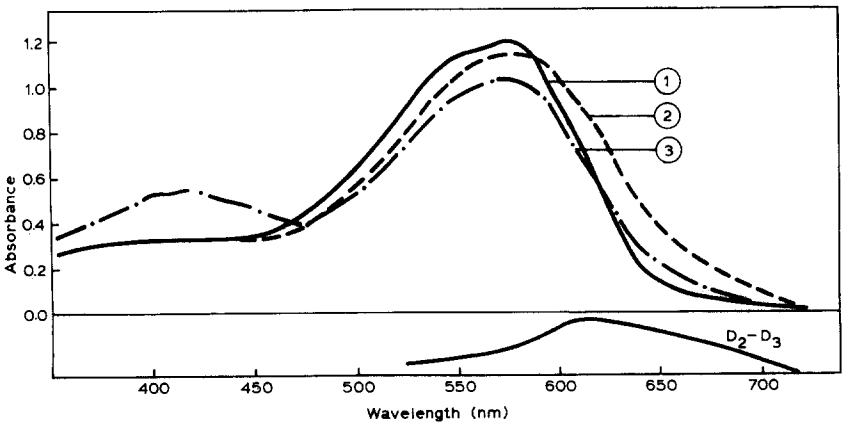


Fig. 14. Dehydrated (0% relative humidity) film of purple membranes at low temperature. (1) Dark-adapted specimen (T = —196°C); (2) specimen illuminated with the light at λ = 520 nm (T = —196°C); (3) illuminated specimen heated to —90°C and cooled sharply again to —196°C. Below is given the spectrum of the 610 nm photoproduct obtained as a difference of spectra (2) and (3).

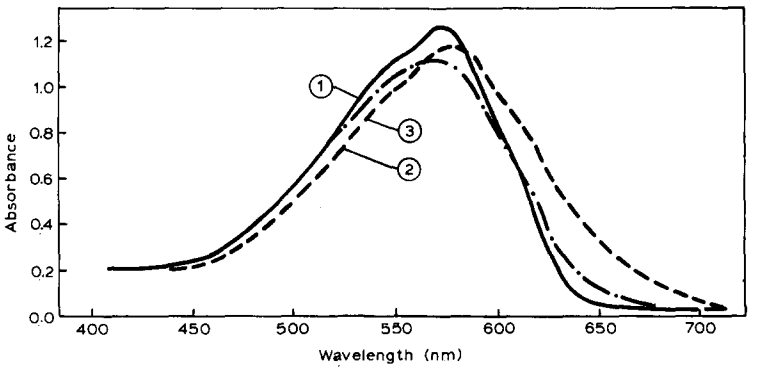


Fig. 15. Humid (100% relative humidity) film of purple membranes at low temperatures. (1) Dark-adapted specimen; (2) specimen illuminated with the light of λ = 520 nm (T = —196°C); (3) illuminated specimen heated to —90°C.

The M_{412} intermediate decays completely only on warming above —10°C. At these temperatures the $M_{412} \rightarrow BR_{506}$ transition is found to occur with the isosbestic point emerging at λ = 440 nm (Fig. 16). As was shown above, the $M_{412} \rightarrow BR_{506}$ transition corresponds to the long-lived M_{412} intermediate observed at room temperatures. Thus using low-temperature spectroscopy two pathways of the M_{412} decaying can be distinguished, which correspond probably to different photochemical cycles in the dry state:

$$BR \xrightarrow{h\nu} K_{610} \xrightarrow[kT]{-120^\circ C} M_{412} \xrightarrow[kT]{-40^\circ C} BR_{568} \qquad (A)$$

$$BR \xrightarrow{h\nu} K_{610} \xrightarrow[kT]{-120^\circ C} M_{412} \xrightarrow[kT]{-10^\circ C} BR_{506} \qquad (B)$$

Low-temperature studies of humid films (75—100% relative humidity) fixed according to the above-mentioned technique give the results completely similar

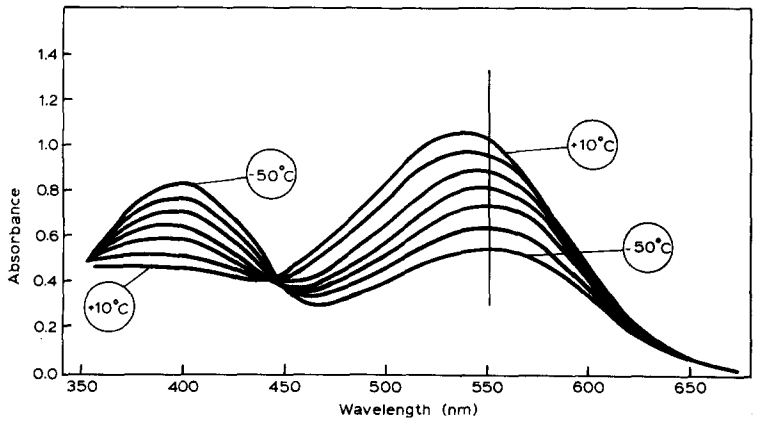


Fig. 16. The decaying of M_{412} in dark by raising temperature from —50°C to +10°C. The difference in the temperature between successive spectra amounts to 10 K. The light-adapted films are dehydrated at 20°C and irradiated with the acting light at λ = 510 nm after cooling until T = —80°C.

to those obtained earlier by Stoeckenius and coworkers [2,7] for water/glycerol suspensions. For hydrated purple membranes, the following intermediates can be distinguished by spectroscopy:

$$BR_{570} \xrightarrow{h\nu} K_{610} \xrightarrow[kT]{-120^\circ C} L_{550} \xrightarrow[kT]{-90^\circ C} M_{412} \xrightarrow[kT]{-40^\circ C} BR_{568}$$

In humid specimens the 550 nm intermediate is clearly defined. On water removing the concentration of L_{550} gradually decreases and becomes undetectable in dry films.

Discussion

Our experimental results and literature data [16] testify to similarity between the photochemical processes in humid purple membrane films (at about 100% relative humidity) and in water (or water/glycerol, 1 : 2, v/v) suspensions. In both cases the intermediates characterized by the same spectral parameters and the same life time are found to be involved in the photoreaction cycle. Dehydration of purple membranes causes changes in the chromophore state and the photoreaction cycle. In the absorption spectra an additional component at 506 nm appears. The dependence of the 506 nm band intensity upon humidity has a well-defined extremum in a range 50—55% (Fig. 5). The effect is observed only in the film. In liquid suspensions of purple membranes the intensity of the 506 nm band rises gradually, with increasing concentration of the organic solvents (e.g. dioxan) and decreasing activity of water molecules. No extrema are observed. Little can still be said about the chromophore state characterized by this absorption band. An analogous high-frequency band shift with the maximum at 460—480 nm in differential spectra is found to occur after addition of organic solvents to water suspensions [8,9,34], after heating purple membranes [10,26], and after titration of aqueous suspensions at high pH values (pH > 11) [10,31]. In accordance with the available literature data [10], the chromophore center in this complex is accessible to external chemical agents such as hydroxylamine. Our experiment shows that the polar properties of the atmosphere surrounding the film have a pronounced effect on the 506 nm complex. Polar gases HCl, H_2S, NH_3, like vapours of H_2O and ^2H_2O, are able to reversibly convert the chromophore from the 506 nm complex to the initial state (unpublished results). When exposing the film to non-polar gases H_2, O_2, N_2, CO_2, the 506 nm complex is retained and the above-mentioned photoinduced 'red' shift is not observed. Dehydration of purple membranes was supposed to inhibit the photoisomerization reaction [16]. Our low-temperature studies of purple membranes support this assumption. The rate of M_{412} formation, $\nu_M = (d[M_{412}]/dt)$ (after fast cooling to —90°C) determined at the initial moment of illumination, shows the hydration dependence to be close to that obtained at room temperature in flash experiments [16]. For dark-adapted (DA) bacteriorhodopsin ν_M^{DA} remains approximately constant on water removing. For light-adapted (LA) bacteriorhodopsin ν_M^{LA} is found to be higher than ν_M^{DA} by a factor of two. The difference between ν_M^{DA} and ν_M^{LA} correlates with the increasing of the *all-trans* retinal from during the dark-light adaptation. On dehydration ν_M^{LA} decreases up to the value

observed in the dark-adapted state (ν_M^{DA}) probably due to the inhibition of the isomerization reaction. At the same time on constant illumination of the dehydrated films (by vacuum pumping at 20°C for 3—4 h) the major part of the chromophores can be easily converted at low temperatures to the M_{412} intermediate associated only with the photocycle of the *trans* form. Evidently the isomerization reaction decreasing sharply in the rate does not stop completely on water removing. The photoreaction cycle of dry purple membranes is devoid of the 550 nm transient and incorporates the long-lived 412 nm complex, which can be observed at room temperature. Dehydration of purple membranes inhibits strongly the backward 412 → 506 nm reaction. In water suspensions of bacteriorhodopsin the reaction 412 → 560 nm is associated with the Schiff-base protonation in the chromophore center [8,33]. In accordance with X-ray data [11,12], dehydration alters insignificantly the structure of purple membranes. The main effect is some disorder of the hexagonal array. Our spectroscopic studies of dehydrated purple membranes do not reveal any remarkable changes in the α-helical conformation of the protein polypeptide chain. The main parameters of the amide bands (position, intensity, shape, half-width) were found to be similar for both the humid and dry states. Presumably, the 506 nm band may be attributed to local rearrangements in the bacteriorhodopsin structure or to displacement of the whole α-helical segments.

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