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Purple Membrane of Halobacteria: A New Light-Energy Converter

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Since the discovery of photosynthesis all organisms investigated have been found to use chlorophylls as the primary light energy absorbing pigments. According to the now generally accepted chemiosmotic theory, a series of oxidation–reduction reactions converts the absorbed energy to an electrochemical proton gradient across the membrane, which contains the pigments and components of the redox chain.[1] Recently, however, a new pigment has been discovered which, when illuminated, can directly generate an electrochemical proton gradient across a membrane. This pigment, bacteriorhodopsin, occurs in the cell membrane of halobacteria. These prokaryotic organisms can only live in environments with high NaCl concentrations and are naturally found in salt lakes, where the salt concentration is near saturation. Both in bacteriorhodopsin- and in chlorophyll-based photosynthesis the energy stored in the electrochemical proton gradient is used by cells to synthesize ATP and to drive other transport processes, such as the uptake of nutrients or the ejection of Na^+. In prokaryotes locomotion is also directly driven by the proton gradient.[2,3]

This Account will briefly describe our present knowledge about the structure and function of bacteriorhodopsin and will be based mainly on work from my own laboratory. Several more comprehensive reviews[4-8] and a recent symposium[9] give a more detailed picture and may be used as a source for references to the rapidly expanding literature on this subject.

Bacteriorhodopsin (bR) is a protein with a molecular weight of 26000 which is amazingly similar to the visual pigments of animals. Its chromophore, retinal (vitamin A aldehyde), is bound as a protonated Schiff base to the ϵ-amino group of a lysine residue which is 41 residues distant from the N-terminal amino acid of the molecule. The complete amino acid sequence has recently been determined by Ovchinnikov's group.[10] The NH_2-terminal residue, a pyroglutamic acid, is located on the exterior surface of the membrane and the COOH-terminal on the cytoplasmic side. Hydrophobic amino acids constitute 62% of the polypeptide chain, which is largely buried in the membrane traversing it seven times. However a hydrophilic COOH-terminal "tail" of 17 amino acids containing 3 glutamate residues and 1 aspartate residue is accessible to proteolytic digestion on the cytoplasmic surface of the intact membrane. Extensive treatment with papain also cleaves the polypeptide between residues 72 and 75 and removes the three NH_2-terminal amino acids (Figure 1).

A lysine–retinal Schiff base would be expected to show an absorbance maximum at approximately 370 nm. However, bR has a strong absorbance band around 568 nm and only three minor bands around 400 nm (Figure 2, curve 2). This large red shift of λ_{max} is due to protonation of the imine and additional noncovalent interaction with the protein. It has been shown, for instance, that twisting around double bonds[11] or placing a positive charge near the β-ionone ring of the protonated retinylidenimine would result in a red shift of the observed magnitude in a retinylidene protein. A number of spectroscopic observations also indicate noncovalent interactions with aromatic amino acid residues, presumably tryptophan.[12,13] The actual mechanism of

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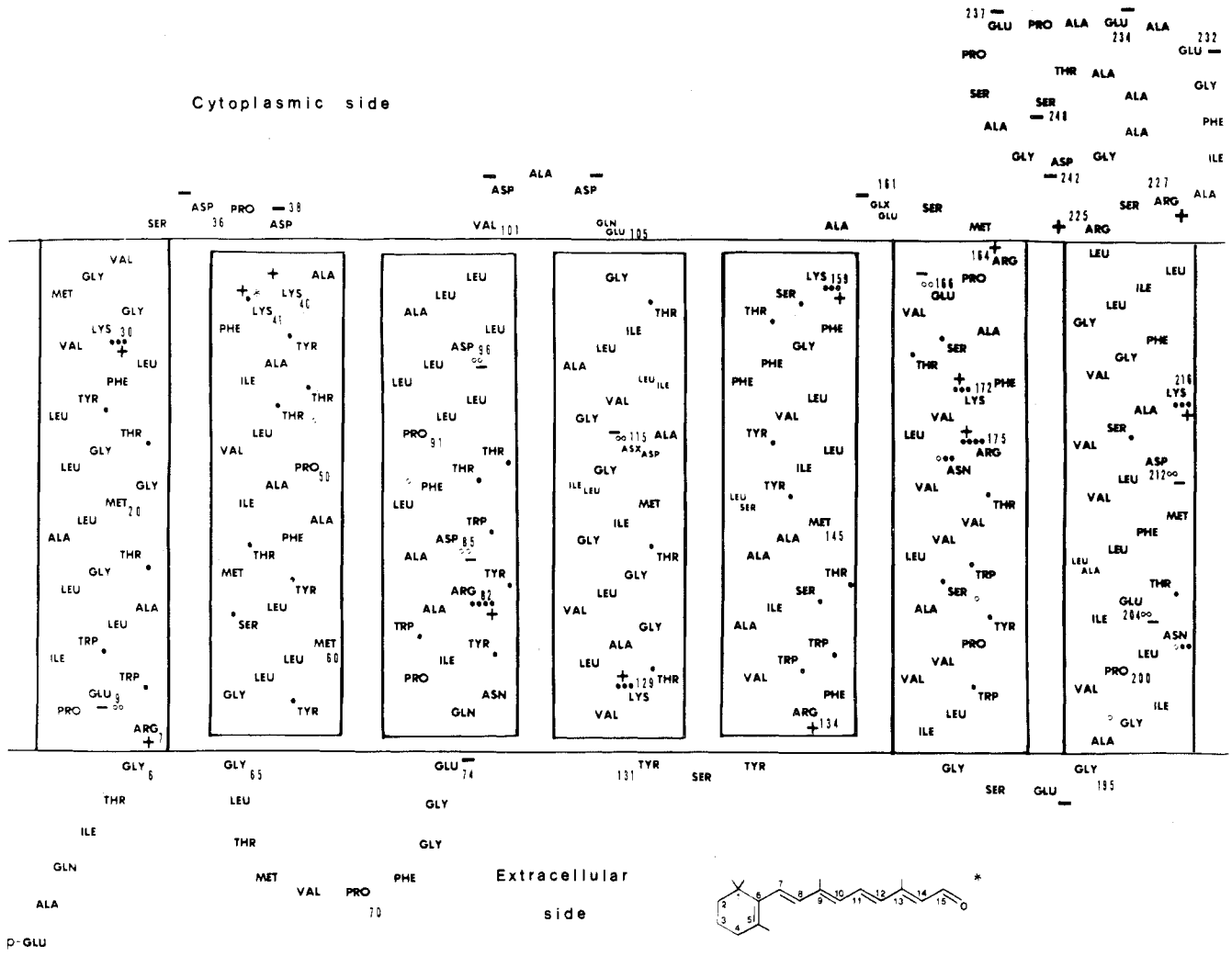

Figure 1. Amino acid sequence of bacteriorhodopsin arranged in seven α-helical segments of suitable length and character to span the bilayer of the purple membrane. The horizontal lines delineate the peak electron density associated with lipid head groups on either side of the membrane. Residue positions are projected onto the surface of a cylinder around each helix axis with 3.5 residues per helical turn of pitch 5.1 Å. The positions of potential proton donors (●) and acceptors (○) relative to the α-carbon locations and groups normally charged in the aqueous phase are indicated. The sequence is that of H. G. Khorana et al. (*Proc. Natl. Acad. Sci. U.S.A.*, **76**, 5046–5050 (1979)) with differences in the sequence of Ovchinnikov et al.[10] indicated below. Tryptophan-138 is not present in the sequence of Ovchinnikov. The retinal chromophore attached to lysine-41 as a protonated Schiff base is shown in its all-trans configuration in the lower right (R. M. Stroud, N. V. Katre, and D. A. Agard unpublished).

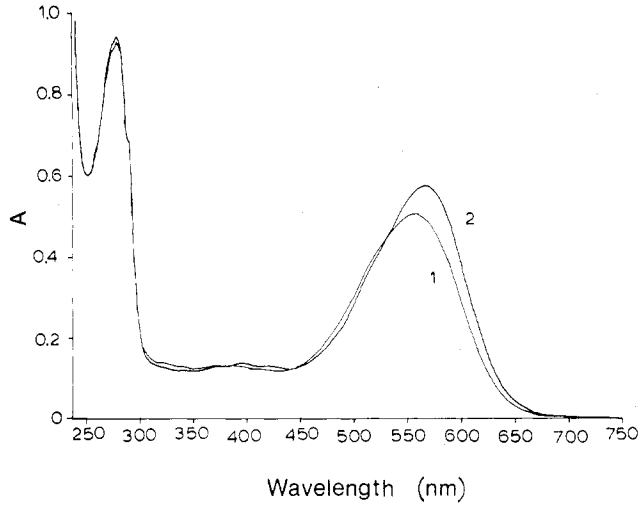

Figure 2. Absorbance spectra of isolated purple membrane in the dark-adapted (curve 1) and the light-adapted state (curve 2).

the red shift, however, still remains to be established and will probably require solution of the protein tertiary structure at high resolution. Ottolenghi has recently reviewed the spectroscopy of bR in detail.[14] In the intact membrane the chromophore is apparently not readily accessible from the aqueous phase. Evidently, the Schiff base is quite stable, reacting with hydroxylamine or borohydride only under intense illumination and even then only slowly. In the case of hydroxylamine the reaction leads to formation of retinal oxime and the apoprotein, bacterioopsin (bOp), which can be reconstituted by the addition of retinal or retinal analogues.[15,16]

If bR is kept in the dark, its absorbance maximum shifts to 558 nm and the absorbance decreases by approximately 15% (Figure 2, curve 1). This form is known as dark-adapted bR (bR_{558}^{DA}). The rate of this reaction is temperature and pH dependent, but at neutral pH and room temperature takes several hours to complete. Illumination with moderate light inten-

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sities restores the light-adapted form (bR_{568}^{LA}) within seconds. Extraction of retinal from bR_{568}^{LA} yields nearly exclusively *all-trans*-retinal while bR_{558}^{DA} yields equal amounts of the 13-cis and all-trans isomers.[17,18] It would seem that the conformation of the protein in bR_{558}^{DA} stabilizes the 13-cis configuration of the retinal and that in bR_{568}^{LA} the all-trans configuration. Later we have to qualify this statement somewhat.

Purple Membrane

In the bacterial membrane bR is concentrated in patches, where it forms a two-dimensional hexagonal lattice (plane group p3) excluding all other membrane proteins.[19] The patches can easily be isolated in relatively large quantities. They can constitute more than 50% of the total cell membrane and remain intact at low ionic strength, which dissociates the rest of the surface membrane. Because of their deep purple color, they are known as the purple membrane (pm). Lipids, which constitute 25% of the mass of pm, consist mainly of the diether analogue of phosphatidyl glycerophosphate, which is also the main lipid found in the rest of the surface membrane. Of the pm lipids 30% are glycolipids, triglycosyl diether and glycolipid sulfate. Phosphatidyl glycerosulfate, phosphatidylglycerol, and small amounts of neutral lipids are also present. The sulfolipids are said to be present only in the pm, and the carotenoids present in the rest of the surface membrane are excluded from it.[20] All polar lipids contain only ether-bonded phytanol as their hydrophobic moiety.

The well-ordered crystalline lattice of pm has made it possible to determine the structure of bR with a higher resolution than that of any other membrane protein. Since only the small two-dimensional crystals of the native membrane are available so far, Henderson and Unwin[21] resorted to low-dose electron microscopy of isolated pm patches to obtain the structure at 7-Å resolution. Figure 3 shows a projection of the electron scattering density distribution onto the plane of the membrane. Tilting the specimen in the electron microscope yielded a three-dimensional map but with a somewhat poorer resolution in the third dimension. In the two-dimensional map a region of high density must correspond to three bR molecules clustered around a threefold axis of the lattice. It is resolved into an inner ring of 9 α helices with their long axis normal to the membrane plane and an outer ring of 12 α helices which are slightly inclined so that their projections overlap. One bR molecule thus contains seven α-helical segments. In the most likely assignment one molecule consists of a row of three closely spaced α helices near the threefold axis and a parallel outer row of four which are slightly spread apart on one surface and closer together on the other (Figure 3). It has been determined by electron microscopy and electron diffraction that the surface toward which the segments fan out is the cytoplasmic surface of the membrane.[22,23] The high α-helix content of the protein and the orientation of the α helices are also confirmed by X-ray diffraction data, circular dichroism (CD), and infrared spectra.

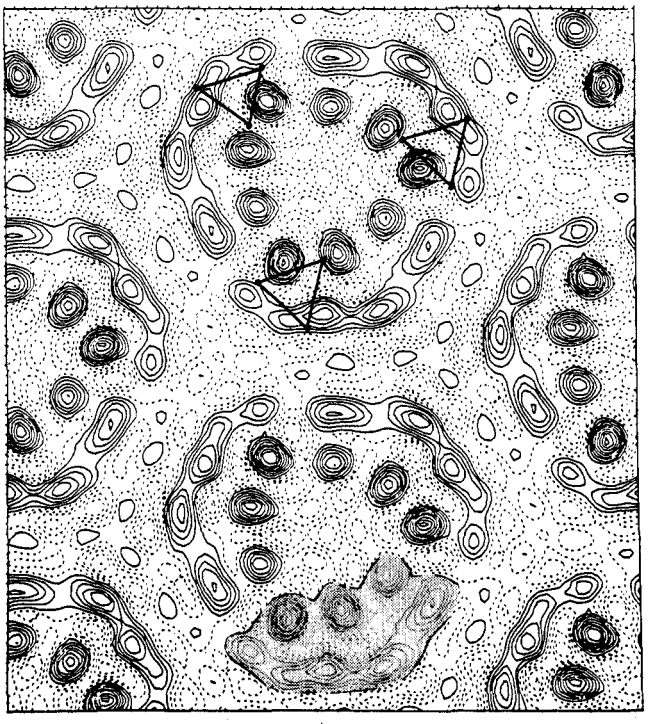

Figure 3. A projection of the electron scattering density distribution onto the plane of the membrane. Regions of higher than average density are indicated by solid lines and of lower than average density by broken lines. The shaded area at the bottom of the figure outlines one bR molecule. The triangles indicate the most likely localization of the retinal. The highest density regions with a diameter of ~10 Å are due to α-helical segments of the protein, which cross the membrane at right angles.

The spaces between the protein molecules are occupied by lipid molecules in the typical bilayer orientation. Unfortunately at present resolution, neither the connections between the α helices nor the COOH and NH_2 terminals of the polypeptide chain are resolved. Ovchinnikov et al.[10] have proposed an arrangement based on their sequence data which yields seven α-helical segments of approximately 30 amino acids each. The necessary short connections are located on the membrane surfaces, and the 18 amino acid long hydrophilic C-terminal tail is on the inner membrane surface. So far, the postulated helical segments of the sequence have not been identified with helical segments in the map, and the proposed arrangement requires that a significant number of hydrophilic residues are buried in the membrane. Moreover, one would not expect that some sequences, which according to this proposal are located in the membrane, would readily form an α helix.[24] A slightly different arrangement of helical sequences is shown in Figure 1. Considerable further work is clearly needed before the structure of bR in pm is resolved at a level where it can be linked to its function.

At the present resolution it is not possible to locate the retinal in the map. However, we have replaced the native retinal in pm with fully deuterated retinal and

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used neutron diffraction to localize it. In a difference map of the membrane profiles from pm reconstituted with retinal and fully deuterated retinal, respectively, we find a pronounced peak in the center of the membrane.[25] Because so far we have not resolved the shape of the retinal molecule, we assume that we are seeing the position of the β-ionone ring around which most of the hydrogen atoms are concentrated. However, we know the angle between the transition moment of the chromophore and the plane of the membrane, which should roughly correspond to the orientation of the side chain and which has been determined in several laboratories. Most values fall between 17 and 27°. Using our own value of 24 ± 1°[26] would place the Schiff base ~17 Å below the nearer membrane surface. According to the sequence data this should be the cytoplasmic surface, because the N-terminal of the polypeptide chain is on the exterior surface and it would require a large stretch of nonhelical configuration to place lysine-41, to which retinal is bound, nearer to the exterior surface. However, the proposed localization needs to be confirmed by higher resolution data and/or energy-transfer experiments.

Neutron diffraction from substituted, deuterated pm can also be used to localize retinal in the membrane lattice. Unfortunately, the result is not as unequivocal as in the case of profile diffraction. The difference map shows numerous peaks, many, but not all, of them obviously spurious. The three most prominent peaks are grouped closely together and are localized in the protein region as indicated in Figure 3.[27] The appearance of three peaks related by angles of 60° might be an artifact. On the other hand, they could correspond to the positions of the Schiff base and β-ionone ring for the 13-*cis*- and *all-trans*-retinal isomers in the protein, at least if we assume that the position of the Schiff base is fixed. The high absorbance of the sample and the long exposure time required for neutron diffraction have not permitted us to test this on light-adapted bR, which contains only the all-trans isomer.

There are additional complications in the calculation and interpretation of the neutron map. For instance, the phases necessary for the calculation of scattering density distribution were taken from Henderson's and Unwins's electron microscopy work and the state of the membranes prepared for microscopy is uncertain, because these are necessarily dried preparations which have a blue-shifted absorbance maximum and the light–dark adaptation phenomenon disappears during drying. However, using the neutron diffraction data and electron microscopy phases yields a map very similar to the electron microscopy map, and recent X-ray diffraction data indicate that the effect of light adaptation on the lattice structure is small (J. Stamatoff, personal communication) and so is the effect of drying. On the other hand, the expected change in the orientation of the chromophore during light adaptation has so far not been detected, and resonance Raman spectra indicate conformations of the chromophore in the protein which are not the same as those of either 13-*cis*- or *all-trans*-retinal in solution (see below). We conclude that the chromophore is probably localized in the region delineated by the triangle shown in Figure 3, but that a more precise localization and interpretation of the peaks will have to await higher resolution data.

The position of the chromophore shown in Figure 3 results in a rather uniform distribution throughout the lattice, which has some implications for the interpretation of the CD spectra. Heyn et al.[28] first showed that the observed CD bands in the visible region of the spectrum can be attributed to an intrinsic positive band and a superimposed positive–negative band which is due to exciton interaction between chromophores. Dissociation of the lattice by the action of detergents or organic solvents eliminates the exciton bands. Ebrey et al.[29] assumed that exciton interaction took place between chromophores of the three protein molecules clustered around the threefold axis—usually referred to as the trimer—and calculated chromophore distances and orientation. The results disagree with the neutron diffraction data and the chromophore angle determined by others. The theoretical treatment must be reevaluated if the chromophores are nearly equidistant throughout the lattice.

In the meanwhile the negative CD band near 600 nm is still useful as an indicator for the existence of the lattice. While in the native membrane the lattice is always found, bleaching with hydroxylamine disorders it and detergents, e.g., Triton X-100, can completely dissociate pm into bR monomers.[30] Even more interesting, lipid vesicles containing bR can be formed with synthetic or natural membrane lipids. If the lipid to protein ratio is high, bR exists in the monomer form above and in the aggregated state below the liquid–gel phase transition of the lipids.[31] These preparations can obviously be used to inquire about possible effects of the lattice on the function of bR.

Studies have begun only recently, and it has already been established that monomeric bR can pump protons.[32] However, whether or not it does so with the same efficiency as in the lattice remains to be seen. There are reasons to suspect that the monomer may be less efficient. Also, the lattice favors the light-adapted state of bR and this appears to depend on protein–protein interaction in the lattice and not on the state of the lipid.[33] Lipid vesicles of this kind have been used early to unequivocally demonstrate the proton-pumping function of bR[34] and, incidentally, have lent strong support to the chemiosmotic theory of energy coupling.[35] These model preparations as well as planar lipid films containing pm or combinations of both are now widely used to investigate the function of bR. They show that the proton translocation is electrogenic and that the protons can be pumped against potentials of up to 300 mV.[36] However, little or no attention has

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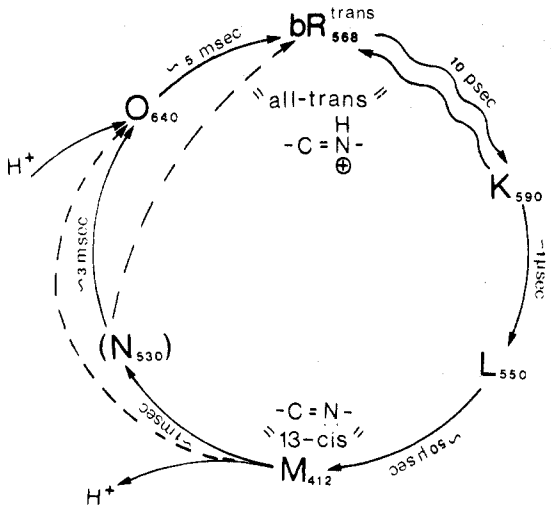

Figure 4. Tentative scheme of the bR photoreaction cycle. The subscripts indicate the calculated absorbance maxima for the intermediates. The protonation state of the Schiff base and the retinal isomers present are indicated for bR_{568} and M_{412}. The indicated release and uptake of protons occur on opposite sides of the membrane.

been paid in most of these studies to the state of bR in the model systems and the usually less than perfect orientation of bR across the lipid bilayers. This must be remedied before accurate quantitative data can be obtained.

Photoreaction Cycle

The function of pm as a light-driven proton pump requires that bR undergo a cyclic photoreaction resulting in the release and uptake of protons on opposite sides of the membrane. The low rate of dark adaptation precludes a direct role of this reaction in proton pumping and focused attention on the photoreactions of bR_{568}^{LA} which can be considered as a homogeneous species containing *all-trans*-retinal only. One might expect difficulties in the investigation of its photoreaction cycle because an efficient pump should have a short cycling time and reaction cycle intermediates should not accumulate in significant amounts under physiologic conditions. Fortunately, the chromophore shows promounced absorbance changes during the reaction cycle, so that the high time resolution and sensitivity of flash spectroscopy can be used to characterize the cycle. Complementary data have been obtained from absorption spectroscopy of pm exposed to low temperature, high pH, organic solvents, or other unphysiologic conditions which can drastically change the kinetics of the photoreaction cycle. At present, isomerization of the retinal and protonation changes of its Schiff base appear as the functionally most important observable events in the photoreaction cycle. Since the free-energy changes for these reactions in solution are small, they must be strongly modified by interactions with the protein; but no experimental approach to this important aspect of the problem has so far yielded interpretable data. (For a recent review of the photoreaction cycle, see also ref 14.)

Figure 4 shows a presumably oversimplified scheme of the reaction cycle based on flash spectroscopic data from isolated pm preparations at near physiologic temperature and pH but low ionic strength.[37] Because the intermediates show broad, overlapping absorbance bands and preparations containing only one intermediate in pure form have not been obtained, neither λ_{max} nor the absorption coefficients can be rigorously determined. Therefore some a priori assumptions had to be made in the construction of the scheme. We originally assumed a unidirectional unbranched cycle of first-order, or pseudo-first-order, reactions and we estimated amount of pigment cycling by the depletion of bR_{568}^{LA} absorbance ~1 ms after the flash when most of the cycling pigment was present as M_{412}, the intermediate which shows the largest blue shift. Except for small differences in the calculated λ_{max}, the scheme is consistent with low-temperature data.[38] Our assumptions are certainly not entirely correct, and data have been obtained by us and others which cannot be explained by the scheme presented. Significant back-reactions and/or branching reactions will probably have to be introduced. Nevertheless, the scheme is apparently valid in its main features.

Some of the possible additional or alternative reactions for which evidence has been obtained under approximately physiologic conditions are indicated by dashed lines. Recently we attempted a more rigorous analysis analyzing the flash-induced absorbance changes at different wavelengths and temperatures for the minimum number of first-order components that give a satisfactory fit at all temperatures and wavelengths. The result should unequivocally determine the number of spectroscopically detectable intermediates, but not their interconnecting pathways.[39] Resolution of the latter will require additional assumptions. The main difference to the cycle presented in Figure 4 is that M may be split into two components with similar spectra and lifetimes, a result which agrees with earlier conclusions drawn by others from different experimental observations.

The first well-established intermediate, K_{590}, rises in 11 ps and decays with a half-time of 1 μs.[40] It is not an excited state of bR because it is indefinitely stable at 77 K. It may be preceded by a farther red-shifted intermediate, which rises in ~1 ps.[14] The uncertainty of the calculated λ_{max} for K is rather large; low-temperature data gives consistently longer wavelengths than flash data with values up to 630 nm. K_{590} is converted back to bR by illumination, as are apparently all other intermediates of the cycle. However, this is especially significant in the case of K because of its short rise time and because its absorbance band completely overlaps that of bR on the short-wavelength side so that at best only 50% of bR can be converted to K_{590}. The conversion of bR_{568} to K_{590} (or its percursor) is the only reaction of the photocycle which requires light. This implies that the energy necessary for the completion of the cycle and for the translocation of protons against a considerable electrochemical gradient must be stored in the conformational state of K_{590}, which has

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a lifetime five orders of magnitude larger than its rise time.

The reaction $bR_{568} \rightarrow K_{590}$ very closely resembles the first resolvable step in the photoreaction of visual pigments; for this reason and because few solid data are available, the conformational state of K_{590} has been the subject of a considerable amount of speculation. Its short rise time and the fact that K_{590} is formed even at 2 K preclude a large conformational change. The low-temperature data and an isotope effect in D_2O strongly suggest that a proton transfer is involved. However, it is probably not the Schiff base proton, as originally suggested, because the Schiff base of K_{590} is still protonated according to the resonance Raman data from several laboratories.[41,42] The evidence for an isomerization of a retinal double bond during the photoreaction is much weaker for bR than it is for the visual pigments. Nevertheless, it still seems likely that an all-trans to 13-cis isomerization does occur and that this conformational change may be initiated during the formation of K_{590}. The energy barrier for this isomerization should be relatively low in the excited state. Consequently K_{590} could be regarded as a chromophore configuration similar to that of the excited state, which has been stabilized by an induced conformational change in the protein. A likely mechanism for the energy storage would be a charge separation in the protein. Rather detailed models for the formation of K_{590} and its role in proton pumping have been published but remain highly speculative.[43-45]

The next photocycle intermediate, L_{550}, decays in $\sim$40 μs. The resonance Raman spectra indicate that its Schiff base is still protonated and in the 13-cis configuration. The next intermediate, M_{412}, has the farthest blue-shifted absorption maximum and has been the most important intermediate for our efforts to understand the photoreaction cycle. Its Schiff base is unprotonated according to the resonance Raman spectra,[42,46] and this may mainly account for the large blue shift of the absorbance maximum. Its decay time is more temperature and pH dependent than that of the earlier intermediates. At unphysiologically high pH, low temperature, and high light intensity it can be accumulated in relatively high concentrations in the photosteady state, which may approach 100%.

M_{412} appears to be stable below −60 °C. Consequently its absorbance spectrum is known better than that of the other intermediates. The spectrum shows subsidiary maxima near 425 and 395 nm and has a molar extinction coefficient for the main peak of ϵ = 46 000 M^{-1} cm^{-1} based on ϵ = 58 000 M^{-1} cm^{-1} for bR_{568}. Its absorbance is often used to estimate the amount of pigment cycling; however, in most cases and certainly under physiologic conditions other intermediates are present at the same time and, if they are neglected, will cause significant errors in the estimate. (Unfortunately this is also true for determination from the depletion of the bR_{568} absorbance.) The main evidence for an isomerization of the C_{13}=C_{14} bond during the photoreaction cycle is derived from the extraction data of M intermediates stabilized by the presence of high salt concentrations and organic solvents or guanidinium hydrochloride and high pH.[18] Extractions under these conditions yield mainly 13-*cis*-retinal.

The late stages in the photoreaction cycle show intermediates with rise and decay times in the range of a few milliseconds and a rather strong dependence on pH and temperature. The decay of M_{412} back to bR_{568} under most conditions is clearly biphasic and seems to involve two more intermediates. N_{520} appears only as a shoulder on the calculated spectrum of M_{412} and O_{640} becomes prominent only at elevated—but still physiologic—temperatures and at acidic pH. We originally assumed a linear pathway from M_{412} through intermediates N_{520} and O_{640} to bR_{568}. Others have proposed that O_{640} lies on a minor side path and that M_{412} reverts directly to bR_{568}. We have recently reevaluated the situation and concluded that even more complex schemes may be required to satisfactorily explain all data. However, some simpler models have not yet been tested and may well give satisfactory solutions. There thus exists considerably more uncertainty about the sequence and number of intermediates in the second half of the photoreaction cycle than in the first.

Evidence for the protonation changes of the Schiff base during the photoreaction cycle is based on resonance Raman data whereas evidence for the retinal isomerization comes mainly from extraction experiments followed by chromatography.[18] The Raman spectra, of course, also contain information on the isomeric state of the retinal, and a considerable effort has been made to extract this information. Spectra of retinylidene Schiff bases in solution show distinctive features for the different isomers especially between 1100 and 1400 cm^{-1}, the so-called fingerprint region.[47] Unfortunately, none of these model spectra resemble closely enough the spectra of dark- and light-adapted bR and its intermediates to allow an identification. This is undoubtedly due to the interaction with the protein. The questions then arise whether the spectra resemble more closely those of 13-*cis*- or *all-trans*-retinal and whether the criteria for resemblance should be the intensity, the wavenumber, or the relative position of bands. These questions have not been resolved. Most investigators in their more recent papers, however, agree that the data strongly suggest that isomerization does occur during the photoreaction cycle and that the spectra are at least compatible with an all-trans configuration for bR_{570} and a 13-cis configuration for M_{412}.[48]

The determination of isomer configuration by retinal extraction with protein-denaturing solvents also has its problems. In order to minimize thermal isomerization in the extract, rapid procedures with low yield have been used and if different isomers should be present preferential extraction of one over the other cannot be excluded. Moreover, one determines only the isomeric configuration to which the pigment relaxes when its interaction with the protein is destroyed, not the configuration it assumes in the protein. Nevertheless, no

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evidence for preferential extraction has so far been found, artifactual isomerization around double bonds also appears to be unlikely, and one may talk about 13-*cis*- and *all-trans*-retinal-containing bR but should keep in mind that the configuration in the protein may not be exactly the same as that of the isomers in solution.

Protons and the Photoreaction Cycle

As expected, the photoreaction cycle is accompanied by the release and uptake of protons. Flash spectroscopy of the isolated pm in the presence of pH indicator dyes shows the release of protons with halftimes slightly longer than the rise but shorter than the decay time of M_{412}. Uptake occurs during the re-formation of bR, but, at least so far, cannot be related to a specific intermediate because of the uncertainties about the reaction pathway.[37] The rather large red shift of the absorption maximum in N_{520} and O_{640} suggests that the Schiff base has already been reprotonated in these intermediates but probably not directly by a proton from the medium (see below). If the protonation changes in isolated pm sheets are an expression of the proton-pumping activity of bR, release must occur on the external and uptake on the cytoplasmic side of the membrane. Comparison of the pH change kinetics in suspensions of cell envelopes, which are "right side out", and bR-containing lipid vesicles, which are "inside out" with respect to the bR orientation in cells, showed that the proton release and uptake processes observed in isolated pm do, indeed, occur on the expected opposite surfaces of pm.[49]

The stoichiometry of proton release and uptake cannot be unequivocally determined until the kinetics of the photoreaction cycle have been definitively resolved. The problem is further complicated by the possibility that groups not directly involved in the transmembrane movement of protons also exchange protons with the suspension medium during the photoreaction cycle. Such protons—often called "Bohr protons" in analogy to the Bohr effect observed in hemoglobin—may be responsible for apparent variations in the pump stoichiometry when the ionic strength of the suspending medium is changed.[50,51] Our own recent data are not entirely consistent with our original finding that in isolated pm at low ionic strength and in cell envelopes only one proton is translocated in one cycle. In intact cells we have measured initial rates of proton ejection which suggest that two protons may be translocated in one cycle;[52] these calculations, however, are based on a quantum yield for cycling of ~0.3 determined by others,[53,54] and an unambiguous determination of this value is also impossible until the kinetics of the photoreaction cycle are better known. One early report, which we, however, are unable to confirm, gives the quantum yield as 0.79.[55]

It should be noted that, mainly for technical reasons, so far all detailed spectroscopic data on the photoreaction cycle have been obtained under unphysiologic conditions. Even if the experiments have been carried out in high salt concentrations the electrochemical gradients always present in living cells have not been duplicated. One would expect that these can influence the kinetics of the cycle significantly.

Proton Pumping Mechanism

If bR functions as a light-driven proton pump it must provide a pathway for protons across the membrane. A large conformational change or rotation of the protein, which could carry a protonated group from the inner surface to the outer surface and return it unprotonated, is unlikely. Spectroscopic techniques designed to detect movements with the necessary time resolution have failed to show any large change in bR orientation during the photoreaction cycle. Also, intra- and intermolecular cross-linking does not seen to abolish the photoreaction cycle. An aqueous pore large enough to allow a diffusion of hydrated protons can be ruled out, because it should be detectable in neutron diffraction maps of pm in D_2O and these show no indication of such a channel.[56] A narrow channel containing bound hydrogen-bonded water molecules, however, cannot be ruled out. Protons could move through such a channel by the same "hopping" mechanism responsible for the high proton conductivity of ice and of hydrogen-bonded organic crystals. Alternatively, a transmembrane chain of hydrogen bonds might be provided by the hydrophilic groups of amino acid side chains, and detailed models for such proton-conduction chains have been proposed.[57] Finding plausible models for a proton conduction path across the membrane thus does not present insurmountable difficulties. It should, however, be emphasized that the details of structural requirements and the conduction mechanism are far from trivial. The latter recently have been discussed in some depth.[58] The nature of the proton channel will presumably be revealed when a higher resolution map of the pm structure becomes available.

The channel must, of course, allow the flow of protons in only one direction, otherwise the membrane could not sustain a proton gradient. In addition a driving force must be provided. The mechanism should, therefore, include what may be termed a gate and an impeller. We have proposed that the Schiff base serves both functions.[59] Assume that a gap exists in the channel and that the Schiff base is hydrogen bonded to the segment of the channel leading to the cytoplasmic surface of the membrane. Retinal isomerization during the photoreaction could move the Schiff base across the gap, so that it would transiently connect to the segment of the channel leading to the exterior membrane surface. If at the same time a transient decrease in the Schiff base pK occurred, its proton would be injected into the exterior segment of the channel and conducted to the outside medium. A proton would be restored to the Schiff base from the cytoplasmic side, when it returns to its original high pK and position connected to

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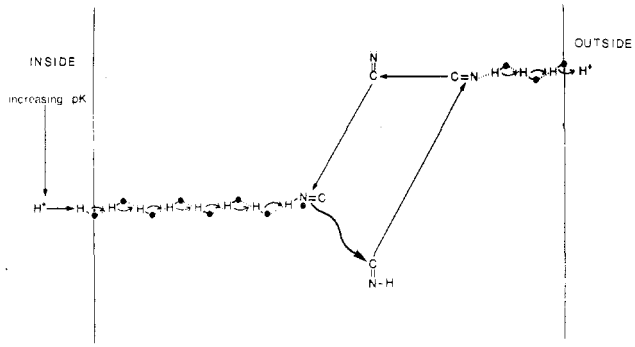

Figure 5. The proposed mechanism for proton translocation. For explanation, see text.

the interior segment of the channel (Figure 5). Since the maximum gradient the pump can establish appears to be 280–300 mV, the change in pK should be ~5 pH units for efficient pumping. It is not absolutely necessary to postulate any change in pK if instead we assume that the isomerization which carries the Schiff base back to its original position can take place only in the unprotonated state; it may, however, be difficult to achieve a reasonable efficiency if only this principle is invoked.[60]

While the model is consistent with most of the observations on pm, it will have to be extensively modified or discarded if it is conclusively shown that two protons are translocated during each cycle. Nevertheless, the two main postulates, that retinal isomerization and pK changes drive the photoreaction cycle and that proton conduction along hydrogen bonds provides a specific low resistance pathway across the membrane, could probably be retained. They are open to experimental verification. Similar much more detailed models have been proposed by others, but they are necessarily even more speculative. At present it seems best to await the collection of more experimental data before proceeding with modifications or elaborations of the model.

Concluding Remarks

The discovery of bacteriorhodopsin was a lucky accident. Not only is it a second photosynthetic pigment, it also proved to be so simple a light-energy converter, compared to the chlorophyll-based systems, that in a few years we learned more about its structure and function than we know about any other biologic energy converter or intrinsic membrane protein. Its similarity to the visual pigments of animal makes it an interesting model system for the early events in visual excitation and has profound implications for our thinking about the evolution of life and biologic energy-converting and sensory systems. We can expect that our progress in understanding bR will continue to be fast. The photoreaction cycle kinetics can probably be definitively resolved, and the structural analysis has not even made full use of all available data. Increased resolution diffraction data from purple membrane can be obtained and should allow us to locate the retinal Schiff base and β-ionone ring. This would be a starting point to coordinate the amino acid sequence data with the structural model. Heavy atom labeling of specific amino acid residues in conjunction with image reconstruction from low-dose electron micrographs as well as neutron diffraction from purple membrane with selectively deuterated amino acids are pursued in several laboratories. The results should allow complete assignment of the segments of the amino acid sequence to the transmembrane α helices and their connections in the map. If still higher structural resolution is necessary, there is no obvious reason why the efforts to grow three-dimensional bR crystals suitable for X-ray diffraction should not eventually succeed. The resulting structural data should yield a much improved basis for modeling proton pumping. The experience with enzyme research, however, suggests that even atomic resolution of the structure may not be sufficient to deduce the mechanism of action. In that case the structure of photocycle intermediates at low temperature or time-resolved X-ray studies with synchrotron radiation or equivalent sources may supply crucial data. Other promising techniques are modification of specific groups in the proteins, reconstitution of bR with retinal analogues, resonance Raman spectroscopy of these modified compounds, and the measurement of photoelectric effects and transport in lipid vesicles and planar lipid films containing bR. Finally, the powerful genetic techniques for studying structure and function of energy and sensory transduction in prokaryotes, while so far little developed in halobacteria, can be used, and the genetics of halobacteria are of great interest in their own right.

All these approaches to pm structure and function are presently used or developed, mainly by research groups in Russia, Germany, USA, and Japan. Some short cuts may be discovered and some unexpected difficulties may arise. However, bR will probably be the first fully understood biologic energy transducer, and the knowledge gained will be of considerable help in many related fields of research.

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