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Picosecond Dynamics of Bacteriorhodopsin, Probed by Time-Resolved Infrared Spectroscopy†

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ABSTRACT: The photoinduced reaction cycle of bacteriorhodopsin (BR) has been studied by means of a recently developed picosecond infrared spectroscopic method at ambient temperature. BR – K difference spectra between 1560 and 1700 cm^{-1} have been recorded at delay times from 100 ps to 14 ns. The spectrum remains unchanged during this period. The negative difference OD band at 1660 cm^{-1} indicates the peptide backbone responds within 50 ps. A survey in the region of carboxylic side chain absorption around 1740 cm^{-1} reveals that perturbations of those groups, present in low-temperature FTIR spectra, are not observable within 10 ns, suggesting a slow conformational change.

Bacteriorhodopsin is the single protein in the purple membrane of *Halobacterium halobium* (Oesterhelt & Stoeckenius, 1973) and is widely studied as a model system for light to energy-transducing proteins and transmembrane ion pumps. It contains a retinal chromophore which is bound to the lysine-216 residue via a protonated Schiff base (SB). Absorption of a photon in the visible leads to a reaction cycle, during which the BR_{570}, J_{625}, K_{610}, L_{550}, M_{412}, N, and O_{640} states (subscript, approximate absorption maximum) of the protein are generated with time constants in the range from hundreds of femtoseconds to milliseconds (Lozier et al., 1975; Zinth et al., 1988; Mathies et al., 1988) and during which protons are pumped across the cell membrane. The generated chemical potential enables the bacterium to produce ATP under

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anaerobic conditions. An important step is the transient deprotonation of the Schiff base during the L to M transition and the successive reprotonation [for review, see Koyama et al. (1988) and Lanyi (1984)].

The initial, fast trans to cis isomerization around the C_{13}=C_{14} double bond of the chromophore triggers a series of relaxation processes of the retinal–protein complex which constitute the pump process along a pathway of proton acceptors and donors. Therefore, it is of great importance to study the dynamics of both the chromphore and the protein as well as their coupling on a microscopic level.

For this purpose, transient infrared difference spectroscopy is a powerful tool since the response to photoexcitation of each individual vibrational mode from the chromophore as well as from the protein contributes specifically to the observed signal.

During the past decades, several research groups have studied the BR photocycle on increasingly shorter time scales by means of time-resolved single-wavelength infrared methods [e.g., see Siebert et al. (1981)] and time-resolved Fourier transform infrared spectroscopic (FTIR) methods [e.g., see Gerwert and Hess (1987) and Braiman et al. (1991)]. However, low-temperature experiments in which the various intermediate states can be frozen in have frequently served to obtain IR difference spectra under steady-state conditions, as well as to study temperature effects on the reaction kinetics (Gerwert et al., 1989; Braiman et al., 1988; Ormos, 1991).

Especially FTIR spectra of the K intermediate, which is formed with a 5-ps time constant (Polland et al., 1986) and decays with a lifetime of 2 μs (Milder & Kliger, 1988) to the L state, could until recently (Noelker et al., 1992; Uhmann et al., 1991) only be obtained at low temperature. Although submicrosecond time-resolved FTIR studies are feasible now, the time resolution of a few nanoseconds, which is available by the improved step-scan FTIR method, represents its technical limit.

In previous reports (Moore et al., 1988; Anfinrud et al., 1988; Diller et al., 1991), a highly sensitive picosecond and subpicosecond time-resolved infrared spectroscopic apparatus and the first results on BR (Diller et al., 1991), namely, the BR – K difference spectrum, taken at room temperature in the range between 1560 and 1700 cm^{-1}, 100 ps after photoexcitation, were presented. This technique links the fast FTIR methods to those time regimes which are already covered by optical and resonance Raman (RR) spectroscopy. In the present work, we have extended the time regime from 100 ps previously reported (Diller et al., 1991) to 14 ns, addressing the K–KL transition repeatedly discussed in the literature (Shichida et al., 1983; Milder & Kliger, 1988; Doig et al., 1991). We discuss the contributions of protein bands in the spectra with respect to low-temperature data (Braiman et al., 1988; Ormos, 1991; Bagley et al., 1982; Gerwert & Siebert, 1986) and recently published FTIR studies, performed at room temperature (Braiman et al., 1991; Noelker et al., 1992). Furthermore, the spectral region from 1710 to 1760 cm^{-1}, in which protonated carboxyl groups of amino acid side chains absorb, has now been investigated. Strong absorption changes in that region during the photocycle have been described in the literature, indicating that these groups undergo deprotonation and reprotonation processes and are part of the proton wire (Gerwert et al., 1989; Braiman et al., 1988; Müller et al., 1991).

Materials and Methods

The sample is a humidified film of purple membrane between two 25-mm-diameter calcium fluoride windows [OD ~1.3 at 570 nm; purple membrane isolated from H. H. strain

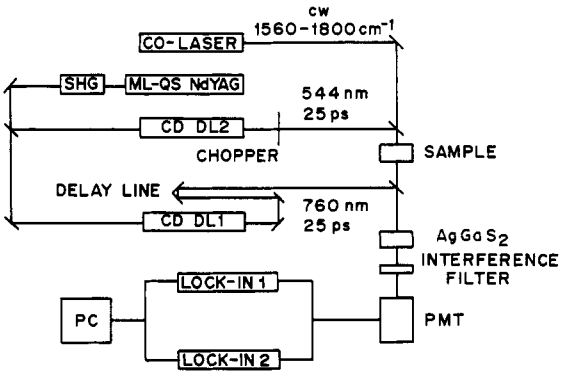

FIGURE 1: Picosecond visible pump infrared probe laser system. Abbreviations: continuous wave; CO, carbon monoxide; ML–QS, mode-locked; Q, switched; CD, cavity-dumped; DL1 and DL2, dye laser; PMT, photomultiplier; PC, computer.

ET 1001 as described by Oesterhelt and Stoeckenius (1974)].

The quality of the sample and the performance of the photocycle were checked by static infrared and optical spectroscopy and by transient absorption spectroscopy. Sufficient background illumination kept BR in the light-adapted state BR_{570}. The sample was rotated and moved up and down perpendicular to the focused laser beams in order to expose completely recovered portions with each laser shot. The experiments were performed at ca. 20 °C.

The laser system (Figure 1) consists of a frequency-doubled, mode-locked, Q-switched Nd:YAG laser (Quantronix 116, repetition rate 500 s^{-1}) which pumps two home-built, cavity-dumped dye lasers. The pulses of one of them (DL2, $W \sim 80$ nJ at 550 nm), chopped at 250 s^{-1}, are focused into the sample and initiate the photocycle. A slightly smaller area is probed by a continuous wave (cw) carbon monoxide laser beam (CO-3i-WTVD; Laser Photonics; line spacing ~4 cm^{-1}). The spectral output of this particular CO laser ranges from 1563 to 1790 cm^{-1} with a typical power of 20–40 mW.

The time resolution is introduced by gating the cw infrared beam after probing the sample with the pulses of the second dye laser (DL1, $W \sim 1$ μJ at 760 nm, repetition rate 500 s^{-1}) in a nonlinear crystal ($AgGaS_2$, Cleveland Crystals). The pulses generated at the sum frequency having intensities linear in the infrared power are detected with a photomultiplier. A narrow band-pass filter rejects all non-upconverted light. The electronic signal is then fed into two lock-in amplifiers, locked at 250 and 500 s^{-1}, respectively, allowing a direct measurement of the photoinduced infrared absorbance changes.

When the pulses the DL1 are optically or electronically (in increments of the 10-ns round trip time of DL1) delayed and the CO laser is set to a specific frequency, kinetics are obtained. When the delay of DL1 is set to a fixed value, the CO laser wavelength can be tuned, and a spectrum is recorded. The cross-correlation of DL1 and DL2 was about 60 ps.

Results

BR – K difference spectra were taken at delay times of 100 ps, 1 ns, and 14 ns between 1560 and 1700 cm^{-1}: No significant changes were observed. In Figure 2, the spectra are shown. They match well with the one previously reported at 100 ps (Diller et al., 1991). The kinetics depicted in Figure 3 show the time course of the absorbance change at 1658, 1650, 1637, and 1610 cm^{-1}. After the fast rise, introduced by the exciting laser pulse, the signals appear constant throughout the whole time range. Having also in mind that laser pulses of 60-ps FWHM were used, we conclude that the time constant for appearance of these populations is less than 50 ps.

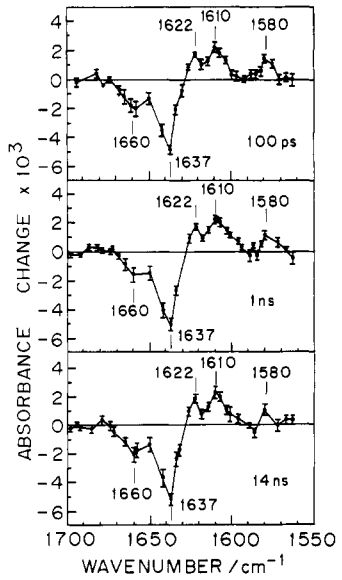

FIGURE 2: Infrared BR_{570} – K difference spectra. Excitation at 550 nm. Delay times as indicated at 100 ps (top), 1 ns (middle), and 14 ns (bottom).

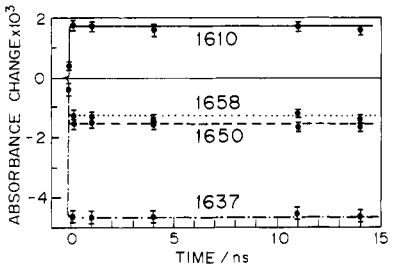

FIGURE 3: Infrared absorbance kinetics following the excitation of BR_{570} at 550 nm. Probe as indicated at 1658, 1650, 1637, and 1610 cm^{-1}. (Spectra normalized to signal at 1610 cm^{-1}.)

The negative bands (bleach) are due to the depletion of the unphotolyzed state BR_{570}. Both negative lines at 1660 and 1637 cm^{-1} lie in the region of amide I absorption. The strongest contribution to the line at 1637 cm^{-1} is the chromophoric C=NH stretch vibration of the Schiff base in BR_{570} (Noelker et al., 1992; Bagley et al., 1982; Gerwert & Siebert, 1886; Massig et al., 1982) which shifts to about 1610 cm^{-1} (Rothschild et al., 1984) or 1624 cm^{-1} (Lohrmann et al., 1991) in the K state, representing some of the absorbance at those positions.

The shoulder at 1660 cm^{-1} appears to be due to CO stretch vibrations of the amide I band. There is no resonance Raman evidence for chromophoric bands in this spectral position whereas absorbance changes in this region have been observed in transient (Braiman et al., 1991; Ormos, 1991) and low-temperture (Braiman et al., 1988; Bagley et al., 1982; Gerwert & Siebert, 1986) FTIR experiments. This band, like the others studied, appears within the experimental time resolution.

The positive bands at lower frequency than 1630 cm^{-1} represent various chromophore and protein contributions of the K intermediate and have not yet been assigned to particular vibrational modes. However, it is noteworthy that the absorbance below 1580 cm^{-1} diminishes toward 1560 cm^{-1} whereas low-temperature FTIR spectra [e.g., see Braiman et al. (1988), Bagley et al. (1982), and Gerwert and Siebert (1986)] show a band at 1556 cm^{-1}.

Figure 4 shows the photoinduced absorbance changes after 10 ns in the region where protonated carboxyl groups (aspartic acid, glutamic acid) of amino acid residues absorb. The noise of these data is considerably smaller than that in Figure 2.

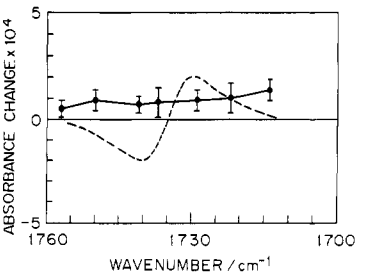

FIGURE 4: Infrared absorbance changes in the carboxylic region, 10 ns after excitation at 550 nm. Dashed line, approximate signal observed in FTIR BR – K spectra [e.g., see Braiman et al. (1988), Noelker et al. (1992), and Gerwert and Siebert (1986)].

This is due to the drop of the amide background absorption to less than 10% above 1710 cm^{-1} and the availability of more power in the respective CO laser lines. The spectrum is flat within an error of less than 1×10^{-4} OD units.

In addition to the experimental data, an approximate spectrum is drawn of the signals observed in low-temperature (Braiman et al., 1988; Gerwert & Siebert, 1986) BR – K spectra and, more recently, at room temperature after 0.5 μs (Noelker et al., 1992).

Discussion

K–KL Transition. The lifetimes of the first intermediates in the BR photocycle have been revealed by transient optical absorption spectroscopy. The earliest electronic ground-state intermediate, J, is formed on the subpicosecond time scale (Zinth et al., 1988; Mathies et al., 1988) and decays to the K state within 5 ps (Polland et al., 1986). A large blue shift in the absorption maximum occurs within 2 μs when the L_{550} state is formed (Milder & Kliger, 1988).

However, in several room temperature studies, small transient changes in the optical (Shichida et al., 1983; Milder & Kliger, 1988) as well as in the resonance Raman (Doig et al., 1991) spectra of K have been observed, and an additional intermediate state, KL, located between K and L, has been suggested. Shichida et al. (1983) observed two transitions within 50 ps and 150 ns and assigned them to the associated states K and KL, respectively. Milder and Kliger (1988) reported the K–KL transition within 10 ns.

In a recent resonance Raman study, Doig et al. (1991) concluded, by following the kinetics of the chromophoric hydrogen out-of-plane vibrations and the ethylenic stretch vibration, that the chromophore–protein complex undergoes a transition from K to KL with a time constant of about 70 ps. These authors suggested that this relaxation process involves the formation of stronger hydrogen bonds between a protein counterion and the Schiff base and a renewed twist of chromophoric C–C single bonds. To address such questions with the present data, the assignments of changes in the spectrum to conformational changes of either the protein or the chromophore is required.

The C=NH stretch vibration of the Schiff base in K has not yet been definitely assigned, but it is surely located between 1609 and 1626 cm^{-1} [1609 cm^{-1}, FT low temperature (LT) (Rothschild et al., 1984), RR LT (Stern & Mathies, 1985); 1613 cm^{-1}, FT LT (Gerwert & Siebert, 1986); 1623 cm^{-1}, RR LT (Braiman & Mathies, 1982); 1624 cm^{-1}, RR room temperature (RT) (Lohrmann et al., 1991); 1626 cm^{-1}, RR RT (Terner et al., 1979)].

Picosecond time-resolved resonance Raman K spectra, taken at room temperature, do not show bands above the noise level in the C=NH stretch region [e.g., see Doig et al. (1991) and Brack and Atkinson (1989)]. Studies using considerably

decreased time resolution or taking the spectra at low temperature have located the C=NH stretch vibration at 1609 cm^{-1} (Stern & Mathies, 1985) or at about 1625 cm^{-1} (Lohrmann et al., 1991; Braiman & Mathies, 1982; Terner et al., 1979). In low-temperature FTIR difference spectra of the BR–K transition, the spectral region between 1630 and 1590 cm^{-1} is fairly congested, but using isotopically substituted retinal chromophores, the C=NH stretch vibration of K has been associated with bands at 1609 cm^{-1} (Rothschild et al., 1984) or 1613 cm^{-1} (Gerwert & Siebert, 1986). For the present discussion, we therefore assume that the C=NH stretch of the K state contributes to the absorption strength of one of the positive signals of 1622 and 1610 cm^{-1}.

The ambiguity in the C=NH stretch assignment may indicate that different K states are being detected, dependent on the sample temperature, the solvent environment, and the time resolution. As has been shown in experimental studies (Baasov et al., 1987) and quantum chemical calculations (Gilson et al., 1988), the frequency of the C=NH stretch depends on both the C=N stretching force constant and the C=N—H bending force constant. This makes the C=NH stretch frequency sensitive to the electrostatic Schiff base counterion interaction *and* to the hydrogen-bonding environment of the Schiff base nitrogen.

Since our data show no significant change in the relevant C=NH spectral region (Figures 2 and 3), we conclude that between 100 ps and 14 ns neither the distance between the Schiff base nitrogen and the counterion nor the strength of the Schiff base hydrogen binding with protein groups is altered. Thus, even if a modification of the K state takes place during this time, the constituting changes are not likely to occur in the vicinity of the retinal lysine linkage, but rather along the chromophoric polyene chain closer to its β-ionone ring.

The observed changes in the optical absorption maximum (Milder & Kliger, 1988) on this time scale do not contradict this conclusion. Baasov et al. (1987) have shown that perturbations in the vicinity of the ring can indeed shift the absorption maximum and the frequency of the chromophoric C=C stretches, without altering the C=NH stretch frequency. However, since it is likely that the bands at 1622 and 1610 cm^{-1} originate mainly in C=C stretches (Curry et al., 1982), the lack of changes in our data suggests that those perturbations as well do not take place on the time scale 100 ps–14 ns.

Besides these chromophore-related vibrations, the indicated protein-based bands at 1660 and 1580 cm^{-1} also remain constant. This suggests that the portion of the protein signalled by these bands is not changing structure. The K–KL transition, as suggested in the literature so far, should be accompanied by a protein structure change. Hence, we conclude that, if it occurs at all, an alteration of the K state (K–KL) takes place either within the first 50 ps, before the present observations, or on the much longer time scale of tens or hundreds of nanoseconds.

The RR study by Doig et al. (1991) suggests a time constant of 70 ps for the formation of KL, and this state should be seen evolving through the IR spectrum. However, the bands (C=C double bond stretch, ca, 1520 cm^{-1}; hydrogen out-of-plane, 955–1000 cm^{-1}) on which this time constant is based are exclusively of chromophoric origin and do not lie in the spectral region of IR sources used in this work. In a future experiment, the first hundred picoseconds will be explored in a broader spectral range to see whether the processes which induce the structural changes of the chromophore seen in the RR spectrum are also being indicated in the C=NH stretch, the amide I and amide II region. In a previous study (Diller et al., 1991), the appearance of the positive band at 1610 cm^{-1} was monitored using laser pulses of ca. 25-ps FWHM, and it showed a rise time of less than 10 ps; hence, at least this band does not change significantly between 10 ps and 14 ns.

Amide Contributions. The negative band at 1660 cm^{-1} and the positive band at 1580 cm^{-1} (Figure 2) are most likely of protein origin. The 1660-cm^{-1} band lies almost in the center of the strong amide I absorption line. A band at that position has not been detected in any of the BR photocycle intermediate states by resonance Raman, whereas IR spectra show bands in this region under various experimental conditions.

In low-temperature FTIR spectra of the BR–K transition [e.g., see Braiman et al. (1988), Bagley et al. (1982), and Gerwert and Siebert (1986)], a more or less structured shoulder between 1650 and 1670 cm^{-1} is always present. The 0.5-μs room temperature BR – K spectrum reported by Noelker et al. (1992) shows a band at the same position. Time-resolved FTIR BR – M spectra taken at 240 and 260 K (Ormos, 1991) as well as at 16.5 °C (Braiman et al., 1991), addressing the L, M, and N states, show strong absorbance changes in this region, which have been interpreted as directly reflecting protein structural changes. We therefore assume that the band at 1660 cm^{-1} arises from amide I CO stretch vibrations. Assignments to particular amino acids were not yet possible.

Since the protein band is certainly present by 50 ps at room temperature as shown in this study and since it is seen in 77 K FTIR BR – K spectra, we conclude that it originates in one or more peptide carbonyl groups which are essentially in van der Waals contact with the chromophore. The immediate proximity of the chromophore would permit the peptide vibration to be directly perturbed by the initial photoisomerization. The perturbation could consist of a change in the electrostatic environment due to the Schiff base nitrogen charge displacement or a sterically induced alteration of the respective peptide group force constants. Since peptide N–H in-plane bending vibrations are involved in the amide I (and amide II) mode, the amide I frequency of a specific group should be sensitive to changes in peptide hydrogen binding. In any case, the affected amino acid is suggested by the results to be in the close vicinity of the Schiff base group. Since the band at 1660 cm^{-1} maintains its metastable character—it remains constant throughout 14 ns, possibily as long as 500 ns (Noelker et al., 1992), the corresponding peptide group appears to be strongly coupled to the K (or KL)-specific structure of the chromophore.

For the band at 1580 cm^{-1}, similar arguments apply, though it is located in the wing of the amide II absorption which concerns the peptide C–N stretch. Chromophoric bands could contribute to the absorption in this region though none are assigned.

This work shows that the absorption near 1580 cm^{-1}, where evidence of the band at 1556 cm^{-1} seen in low-temperature FTIR BR – K spectra is expected, is not present between 50 ps and 14 ns. It is also not seen in the room temperature 0.5-μs BR – K spectrum reported by Noelker et al. (1992). The spectral position and different behavior with respect to the state of the protein (frozen or at ambient temperature) suggest an amide II origin for this absorption. In that case, a qualitatively different protein response compared with that indicated by the band at 1660 cm^{-1}, which is present in low-temperature FTIR spectra, is suggested. At low temperature, the isomerization apparently perturbs irreversibly the corresponding peptide group (or groups): The perturbed conformation is fixed. At

ambient temperature, however, the peptide group and its local protein environment may have enough flexibility and degrees of freedom to adjust rapidly to the perturbation and reestablish a "relaxed" conformation. This suggests that a transient signal at 1556 cm^{-1} may occur within the first 50 ps.

It is known [e.g., see Ormos (1991) and Kalisky and Ottolenghi (1982)] that the BR photocycle shows non-Arrhenius behavior so that at different temperatures the photocycle reaction scheme changes qualitatively. Therefore, the low-temperature spectra may indeed depict a BR conformation that is never realized at ambient temperatures. In any case, it would be interesting to see whether transient absorbance changes occur in this region prior to 50 ps.

Carboxylic Groups. Studies using isotopically labeled BR as well as mutants have given evidence that the carboxyl groups of the aspartic acids Asp85, -96, -115, and -212 are directly involved in the proton pump mechanism (Gerwert et al., 1989; Braiman et al., 1988; Müller et al., 1991). Protonated carboxylic carbonyl groups absorb between 1760 and 1730 cm^{-1}, whereas the ionized group absorbs around 1550 and 1350 cm^{-1} [e.g., see Bellamy (1957)]. Deprotonation and reprotonation processes of these groups are indicated by IR absorbance changes seen on various time scales, beginning with the L–M transition.

However, low-temperature FTIR BR – K spectra only show small signals [e.g., see Braiman et al. (1988) and Gerwert and Siebert (1986)], in particular a small positive signal at about 1733 cm^{-1} and a negative one at about 1740 cm^{-1}. The approximate signal observed in FTIR is schematically depicted in Figure 4 (dashed line), which also shows the results of our room temperature experiment. These bands have been associated with a perturbation—not with a change in the protonation state—of the protonated Asp115 (Braiman et al., 1988; Eisenstein et al., 1987) during the BR–K transition. A comparable signal is present in the 0.5-μs FTIR BR – K spectrum (Noelker et al., 1992), recorded at room temperature. However, at 10 ns after photoisomerization, a signal of this magnitude is absent (see Figure 4). These results may be explained by a conformational change of the protein occuring between 10 and 500 ns. Asp115 is supposedly located in the retinal pocket (Henderson et al., 1990), in the close vicinity of the chromophore. Hence, one would expect a fast response of the carboxylic group to the isomerization. However, the slow evolution of the signal rather implies a more indirect coupling to the isomerization most likely through the protein backbone.

Concluding Remarks

This study presents the first examples of a protein response to a photoinduced process on a picosecond time scale. Three different types of responses should be considered: (a) an immediate response of a peptide carbonyl group to a change in the nearby chromophore (appearance of the amide I band at 1660 cm^{-1}); (b) a response involving both the chromophore and protein relaxation (the band at 1556 cm^{-1} which relaxes rapidly at ambient temperatures); (c) a slow nanosecond response, most likely involving a protein relaxation (slow rise of the signal in the carboxylic region). Type a direct responses to nearby chromophore changes may be the dominant ones. Another example of this can be found in studies of comparisons of light-induced signals from visible or infrared absorption and photocurrent (Müller et al., 1992; Gerwert et al., 1990) in which these parameters are found to show concerted kinetics. Type b responses illustrate the significance of time-resolved studies at ambient temperatures with respect to low-temperature studies. The SB protonation pathway and consequently the function of BR are critically dependent on the temperature and on very specific dynamic properties of the protein (Ormos, 1991). Both type b and type c responses may permit detailed structural studies of the origins of the non-Arrhenius-like characteristics of the BR dynamics.

At present, detailed structural conclusions from either time-resolved or stationary IR studies are still limited by the lack of assignments, especially for amide I and amide II bands which concern the protein backbone. Using the structural information from the work by Henderson et al. (1990), and isotopically labeling and genetic techniques, it should be possible to gain more insight into the proton pump path as the current research demonstrates, and also on the concomitant dynamics of other residues and the backbone responding to the primary process.

Especially for the first reaction steps of the BR photocycle, molecular dynamics simulations are helpful to describe the fast protein response (Nonella et al., 1991). Currently, studies are underway (e.g., F. Zhou, A. Windemuth, and K. Schulten, personal communication) which investigate the dynamics of the primary photoisomerization as well as the thereby-induced protein dynamics, assuming isomerization models still under discussion. From studies like that, it should be possible to deduce which parts of the protein do or do not undergo conformational changes and are or are not likely to be detected by differences in the IR spectrum. Molecular groups of interest are, for example, the covalently SB-bound Lys216 residue and its peptide group, the residues belonging to the SB counterion(s), further residues of the chromophore pocket, and nearby protein-bound water molecules.

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Identification of an Isoprenylated Cysteine Methyl Ester Hydrolase Activity in Bovine Rod Outer Segment Membranes†

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ABSTRACT: Proteins from eucaryotic cells which have a carboxyl-terminal CAAX motif are posttranslationally modified by isoprenylation. The pathway involves the linkage of an *all-trans*-farnesyl (C15) or an *all-trans*-geranylgeranyl (C20) moiety to the cysteine residue followed by proteolysis which generates the modified cysteine as the carboxyl-terminal residue. Carboxylmethylation of the modified cysteine residue completes the pathway. This latter methylation reaction is the only potentially reversible reaction in the pathway and thus of possible regulatory significance. A specific esterase is required to reverse the methylation. It is demonstrated here that simple isoprenylated cysteine derivatives, such as *N*-acetyl-*S*-farnesyl-L-cysteine methyl ester (L-AFCM) and *N*-acetyl-*S*-geranylgeranyl-L-cysteine methyl ester (L-AGGCM), are substrates for a rod outer segment (ROS) membrane esterase activity. The K_M and V_{max} values for L-AFCM and L-AGGCM are 186 μM and 2.2 nmol mg^{-1} min^{-1} and 435 μM and 4.8 nmol mg^{-1} min^{-1}, respectively. The enzyme(s) is stereoselective rather than stereospecific because D-AFCM is enzymatically hydrolyzed with K_M and V_{max} values of 157 μM and 0.46 nmol mg^{-1} min^{-1}, respectively. The enzyme(s) does not process *N*-acetyl-L-cysteine methyl ester, demonstrating that the isoprenyl moiety is required for substrate activity. Ebelactone B is a potent mechanism-based inactivator of the enzyme with a K_I = 42 μM and a k_{inh} = 3.7 × 10^{-3} s^{-1}. Importantly, L-AFCM, L-AGGCM, and ebelactone B all inhibit the demethylation of the endogenous ROS substrates, showing that the same enzymatic activity is involved in the processing of the synthetic and physiological substrates.

The γ subunits of heterotrimeric G proteins and the "small" G proteins, including ras, are posttranslationally modified by isoprenylation (Casey et al., 1989; Farnsworth et al., 1990; Hancock et al., 1989; Lai et al., 1990; Maltese, 1990; Mumby et al., 1990; Schafer et al., 1989). Isoprenylation involves three modifications at proteins containing a carboxyl terminus of CAAX, where C = cysteine, A = an aliphatic amino acid, and X is undefined (Casey et al., 1989; Hancock et al., 1989; Lowy

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