Photoactivation of Rhodopsin Causes an Increased Hydrogen-Deuterium Exchange of Buried Peptide Groups

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ABSTRACT A key step in visual transduction is the light-induced conformational changes of rhodopsin that lead to binding and activation of the G-protein transducin. In order to explore the nature of these conformational changes, time-resolved Fourier transform infrared spectroscopy was used to measure the kinetics of hydrogen/deuterium exchange in rhodopsin upon photoexcitation. The extent of hydrogen/deuterium exchange of backbone peptide groups can be monitored by measuring the integrated intensity of the amide II and amide II′ bands. When rhodopsin films are exposed to D_2O in the dark for long periods, the amide II band retains at least 60% of its integrated intensity, reflecting a core of backbone peptide groups that are resistant to H/D exchange. Upon photoactivation, rhodopsin in the presence of D_2O exhibits a new phase of H/D exchange which at 10°C consists of fast (time constant ~30 min) and slow (~11 h) components. These results indicate that photoactivation causes buried portions of the rhodopsin backbone structure to become more accessible.

INTRODUCTION

Rhodopsin is a 7-helix integral membrane protein found in photoreceptor membranes, which belongs to the family of G-protein coupled receptors (for reviews see Hargrave and McDowell, 1992; Khorana, 1992). It contains an 11-*cis* retinylidene chromophore covalently linked by a protonated Schiff base to Lys-296 of the apoprotein opsin. Light triggers a femtosecond isomerization of this chromophore to an all-*trans* configuration (Green et al., 1977; Schoenlein et al., 1991) followed by a series of rapid thermal dark reactions (Batho→Lumi→Meta I→Meta II) (Wald, 1968; Yoshizawa and Wald, 1963) culminating in the binding and activation of the G-protein, transducin (Vuong et al., 1984). Meta II decays via a much slower set of reactions to Meta III or to opsin plus all-*trans* retinal (Ostroy, 1977). Of all the intermediates, only Meta II is known to bind and activate transducin (Kibelbek et al., 1991; Vuong et al., 1984). A central goal for understanding receptor activation is the elucidation of the conformational changes at the Meta II stage which lead to the binding and activation of transducin. Such information is also important for understanding the mechanism of other 7-helix ligand-activated G-protein coupled receptors, a family that underlies a large array of cellular signal transduction mechanisms (van Rhee and Jacobson, 1996).

One approach to monitoring conformational changes in receptors and other membrane proteins is the method of FTIR difference spectroscopy (Baenziger et al., 1992; Braiman and Rothschild, 1988; Rothschild et al., 1981). In the case of rhodopsin, conformational changes of the retinylidene chromophore, protonation, and/or hydrogen bonding changes of Asp, Glu, and Cys residues, structural changes of the peptide backbone and structural changes of the membrane lipid matrix have been detected at different stages of the photoactivation cascade (Bagley et al., 1985; DeCaluwé et al., 1995; DeGrip et al., 1988; Fahmy et al., 1994; Klinger and Braiman, 1992; Nishimura et al., 1996; Rath et al., 1993, 1994; Rothschild et al., 1983; Rothschild and DeGrip, 1986; Siebert et al., 1983).

Infrared spectroscopy can also be used to probe rhodopsin structure by monitoring the extent and kinetics of H/D exchange (Downer et al., 1986; Englander et al., 1982; Osborne and Nabedryk-Viala, 1977). For example, the amide II mode (peptide NH bending) shifts from ~1545 to 1445 cm^{-1} (amide II′) upon NH→ND exchange of backbone peptide groups (Blout et al., 1961) (note the region around 1445 cm^{-1} also contains contributions from the CH bending mode of CH_3 groups that should not undergo H/D exchange). Compared to tritium-labeling studies, which detect all exchangeable hydrogens in a protein including those from side-chain groups, infrared thus provides a means to selectively monitor H/D exchange in the protein backbone. The inherent sensitivity and fast time-resolution of FTIR as well as the ability to measure samples in an aqueous medium using ATR (Braiman and Rothschild, 1988; Marrero and Rothschild, 1987) also makes this an attractive method for monitoring H/D exchange under near physiological conditions.

In the current study, both transmission and ATR-FTIR spectroscopy have been used to monitor the H/D exchange

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Abbreviations used: Meta I: metarhodopsin I; Meta II: metarhodopsin II; ATR: attenuated total reflection; D_2O: ^2H_2O; FTIR: Fourier transform infrared; H/D: hydrogen/deuterium; Rho: rhodopsin; ROS: rod outer segments.

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rate of rhodopsin upon photobleaching. Unbleached rhodopsin has a core of peptide groups that are resistant to H/D exchange (Downer et al., 1986; Haris et al., 1989; Osborne and Nabedryk-Viala, 1977; Rothschild et al., 1980a). Upon photoactivation, rhodopsin exhibits a new phase of H/D exchange. At 10°C in D_2O this consists of a fast phase with time constant (τ) $\sim$ 30 min and a much slower phase with $\tau \sim$ 11 h. These results indicate that a part of the rhodopsin structure becomes exposed to the aqueous medium upon photoactivation.

MATERIALS AND METHODS

Sample preparation

Rhodopsin membranes were prepared from bovine ROS according to methods previously described (DeGrip et al., 1980). The A_{280}/A_{500} ratio of the resulting washed photoreceptor membrane was typically 2.0 $\pm$ 0.1. Membrane suspensions at a concentration of 55 nmol/ml rhodopsin were stored at −20°C until further use. Sample films for transmission spectroscopy were prepared by isopotential spin drying (Clark et al., 1980; Rothschild et al., 1980c) of an aqueous suspension of photoreceptor membranes in H_2O, containing ~1–3 nmol rhodopsin, onto an AgCl window. The film was then rehydrated before insertion into a sealed transmittance cell as previously described (Rath et al., 1993). For H/D exchange measurements, the rhodopsin film was first dried for more than 12 h in a dry-air box in order to remove residual H_2O. The dried film was then exposed to bulk D_2O for more than 24 h by putting ~10 μl D_2O directly on the film and sealing it with a second AgCl window. It was then redried in a dry-air box and assembled into a sealed IR cell using a second AgCl window containing small drops (~3 μl) of D_2O placed outside of the IR beam path.

Transmission FTIR difference spectroscopy

Transmission Fourier transform infrared difference spectra of hydrated rhodopsin films were recorded using methods similar to those previously reported (DeGrip et al., 1988; Rothschild et al., 1987). The H_2O or D_2O content of the sample was monitored by measuring the intensity ratio of the 3400 cm^{-1} band (O—H stretch mode) or 2600 cm^{-1} band (O—D stretch mode) to the methyl and methylene C—H stretch bands of the protein and lipids in the 2800–3000 cm^{-1} region. The Rho→Meta II difference spectra were recorded at 10°C. The sample was photobleached for 3 min using light from a 150-W tungsten illuminator (Model 180, Dolan-Jenner industries, Lawrence, MA) filtered by a 500 nm long-pass filter (Corion Corp., Holliston, MA) and several heat filters and transmitted to the sample with an annular optical fiber. Spectra were recorded at 8 cm^{-1} resolution and 11 min intervals for several hours before and after illumination (3000 scans for each spectrum) on a BioRad FTS-60A spectrometer (BioRad, Digilab Division, Cambridge, MA) equipped with a Mercury-Cadmium-Teluride (MCT) detector.

ATR-FTIR difference spectroscopy

ATR-FTIR difference spectra were recorded using an apparatus previously described (Rath et al., 1994). All sample manipulations were performed under dim red light. Approximately 4 nm of ROS membranes in an 80-μl volume were added to 20 μl phosphate buffer (5 mM sodium phosphate, 5 mM KCl, 2 mM $MgCl_2$, 3 mM $CaCl_2$, and 250 mM NaCl at pH 6.8). The resulting solution was then quickly transferred onto a 50×20×2-mm germanium internal reflection element (IRE) and dried under a slow stream of argon gas to form a multilamellar film. The IRE was then mounted in a modified temperature-controlled ATR cell (MEC-1TC, Harrick Scientific Corp., Ossining, NY) equipped with a quartz window for sample illumination. The rhodopsin film was then cooled to 10°C by flowing coolant in the cell jacket. In order to further dry the film, a slow stream of N_2 gas was flowed through the cell for 1–2 h. A BioRad-Digilab FTS-60A FTIR spectrometer (BioRad, Digilab Division, Cambridge, MA) equipped with an MCT detector was used to collect infrared spectra consisting of an average of 5 min of data collection (1350 scans) at 8 cm^{-1} resolution. In order to monitor the H/D exchange in dark, D_2O was injected into the cell containing the dry film while spectra were being recorded. This causes a drop in infrared absorbance of the sample due to swelling of the film, which stabilizes in ~15 min. The film was then allowed to exchange for more than 24 h before photobleaching. A 150-W tungsten-halogen lamp (Model 180, Dolan-Jenner Industries, Lawrence, MA) with several heat filters and a 500-nm long-pass filter (Corion Corp., Holliston, MA) were used for sample illumination.

Data analysis

Spectral data analysis was performed with GRAMS/32 (Galactic Industries, Nashua, NH) using the macro programs baseline.ab, integrat.ab, and curvefit.ab. In the case of the amide II bands, the integrated intensity was calculated after baseline correcting the band over the limits 1525 to 1575 cm^{-1} and then calculating the integrated intensity. Alternatively, the 1500–1800 cm^{-1} region of the spectrum was curve-fit following Downer et al. (1986) and the integrated intensity of the amide I and II bands determined. A total of eight bands were fit including bands near 1740, 1680, 1655, 1636, 1621, 1580, 1543, and 1516 cm^{-1}, which correspond to bands observed in the spectrum after spectral deconvolution. H/D exchange of the peptide groups in rhodopsin was calculated by measuring the fractional decrease (increase) in the amide II (amide II′) intensity or by calculating the change in the ratio w ($A_{\text{amide II}}/A_{\text{amide I}}$), where $A_{\text{amide II}}$ and $A_{\text{amide I}}$ are the integrated intensity of the amide II and amide I bands, respectively. The total fraction of unexchanged peptide groups in rhodopsin (f) was estimated from the relation $f = w'/w$, where w' is the amide I and II intensity ratio measured for samples exposed to D_2O and w the intensity ratio before exposure (Downer et al., 1986). Kinetics of exchange were determined by curve-fitting integrated intensity of the amide II band to a sum of exponentials using a nonlinear curve-fitting package (Peakft Version 4, Jandel Scientific, San Rafael, CA).

RESULTS

Hydrogen/deuterium exchange in unbleached rhodopsin

Fig. 1 compares the FTIR absorption spectra of a dehydrated rhodopsin film and the same film after it has been kept in the dark, exposed to bulk D_2O for at least 48 h, and then dehydrated again (see Materials and Methods). For both the transmission (Fig. 1 *A*) and ATR-FTIR (Fig. 1 *B*) spectra, the major spectral change is a downshift of the amide II mode from 1543 cm^{-1} to near 1450 cm^{-1}. Since the amide II band reflects primarily the N—H bending mode of peptide amide groups (Miyazawa et al., 1958), this downshift reflects the H/D exchange of peptide backbone groups. In contrast, the amide I band near 1655 cm^{-1}, due mainly to the C═O stretch of peptide groups, is much less affected by H/D exchange. Only a small 2 cm^{-1} downshift is observed, characteristic of predominantly α-helical proteins (Susi et al., 1967). In general, these results are consistent with earlier infrared H/D exchange measurements and resonance Raman measurements on photoreceptor membrane (Downer et al., 1986; Haris et al., 1989; Osborne et al., 1978; Rothschild et al., 1976, 1980a). The increased intensity at lower frequency of the amide I band most likely

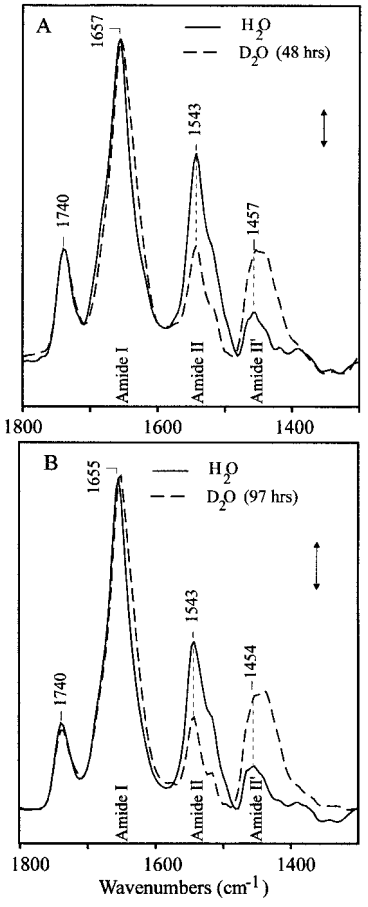

FIGURE 1 FTIR spectra of a dehydrated rhodopsin film. Spectra shown in the amide I and amide II region were recorded before (*solid lines*) and after (*dashed lines*) immersion in D_2O for 48 h (*A*) and 97 h (*B*). Spectra shown were recorded at 8 cm^{-1} resolution using transmission (*A*) and ATR-FTIR (*B*) techniques. The scale bars shown are 0.05 optical density (O.D.) units.

reflects the non-α-helical structure which undergoes rapid H/D exchange (Downer et al., 1986; Pistorius and DeGrip, 1994; Rothschild et al., 1980a).

The fraction of peptide groups undergoing H/D exchange can be estimated from the integrated amide II intensity. In the case of transmittance measurements (Fig. 1 *A*) this band decreases to 67% of its original area after 48 h of D_2O exposure, indicating that only 33% of the rhodopsin peptide groups have exchanged. Calculation of the *f* value (see Materials and Methods) results in a similar estimate of 27% H/D exchange. While this value is lower than the earlier estimates of 40–50% H/D exchange based on FTIR measurements of rhodopsin suspensions (Downer et al., 1986; Haris et al., 1989), the use of a multilamellar film which is oriented perpendicular to the incident light favors the absorption of the amide II mode of α-helical structure, which is oriented perpendicular to the membrane plane. In particular, polarized FTIR spectroscopy of oriented multilamellar films of photoreceptor membrane have shown that the amide II absorption is increased relative to what is expected for suspensions of photoreceptor membrane (Rothschild et al., 1980c). This enhancement originates from the predominantly perpendicular net orientation of the core α-helices of rhodopsin relative to the membrane plane, the predominantly perpendicular orientation of the amide II transition moment relative to the α-helix axis, and the predominantly perpendicular orientation of photoreceptor membranes relative to the incident light as discussed in more detail previously (Rothschild and Clark, 1979; Rothschild et al., 1980c). Thus, our estimates of H/D exchange, which are based on measurements of the amide II absorption, reflect primarily the H/D exchange of the core 7-helix bundle of rhodopsin as compared to the more peripheral surface regions, which are less oriented and expected to undergo more rapid H/D exchange. A similar effect was also found when polarized infrared light was used to probe H/D exchange in bacteriorhodopsin (Earnest et al., 1986).

Absorption measurements were also performed using ATR-FTIR spectroscopy, which allows the film to be completely immersed in solution (Harrick, 1967; Marrero and Rothschild, 1987; Rath et al., 1996). In this case, FTIR spectra of films could be continuously measured after immersion in D_2O (see below). Based on analysis of spectra obtained from a dehydrated film before adding D_2O and the same film dried after 97 h of immersion (Fig. 1 *B*), 32% of the peptide groups (based on *f* value) had undergone H/D exchange. Analysis of spectra recorded during the first 10 min after the film has been exposed to D_2O indicates that 25% of the peptides groups undergo H/D exchange during the initial 10-min period.

H/D exchange after photobleaching of rhodopsin

In order to investigate the effect of photobleaching on the H/D exchange rate in rhodopsin both time-resolved transmission and ATR-FTIR measurements of rhodopsin films were made after the samples had been exposed to D_2O for more than 24 h, during which time the H/D exchange rate has leveled off. FTIR spectra were recorded continuously with 5 min (ATR) and 11 min (transmission) intervals for several hours before and after photobleaching. As seen in Fig. 2, *A* and *B*, H/D exchange after photobleaching causes a reduction in the intensity of the amide II band near 1545 cm^{-1} that is much larger compared to the same time period before photobleaching. A concomitant rise in the intensity of the amide II′ band near 1454 cm^{-1} is also found, confirming that this effect is due to H/D exchange and not to instrumental baseline drift.

FTIR difference spectra consisting of a subtraction of the first spectrum recorded after photobleaching from subsequent spectra (Fig. 3, *A* and *B*) also show that the dominant spectral change is an increase in the intensity of the negative/positive bands near 1540/1450 cm^{-1} corresponding to the downshift of the amide II band induced by H/D exchange seen in Figs. 1 and 2. An additional negative/positive band is observed near 1660/1632 cm^{-1} which may correspond to the downshift of the amide I contributions

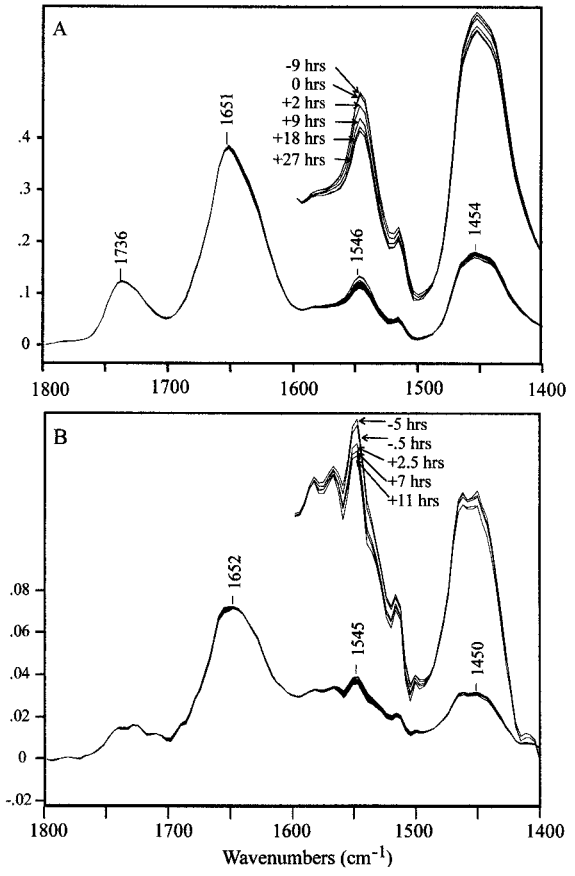

FIGURE 2 FTIR spectra of a rhodopsin film undergoing H/D exchange. Spectra were recorded using transmittance (*A*) and ATR-FTIR (*B*) (see Materials and Methods). Times before and after illumination of the film are shown in inset. *y*-axis labels are in optical density units (O.D.).

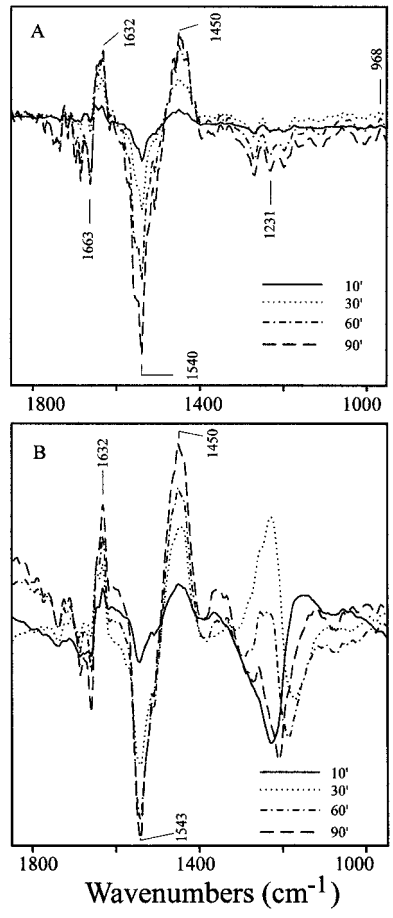

FIGURE 3 FTIR-difference spectra of a rhodopsin film at 10°C undergoing H/D exchange. Spectra were recorded using transmission (*A*) and ATR-FTIR (*B*) methods. Rhodopsin films were exposed in the dark to D_2O for more than 24 h prior to measurements (see Materials and Methods). The differences represent the changes occurring after photobleaching and were obtained by subtracting the first spectrum of the photoreceptor membrane film recorded immediately after light irradiation from the successive spectra recorded thereafter at the times indicated.

from non-α-helical structure (Pistorius and DeGrip, 1994; Rothschild et al., 1980a). These spectra also contain small contributions from the decay of the Meta II intermediate to Meta III and opsin and the associated refolding of rhodopsin (Klinger and Braiman, 1992; Rothschild et al., 1987). For example, small negative/positive bands observed in the earliest spectra near 1740/1724 cm^{-1} are the reverse of those seen during the Meta I to Meta II transition, and one of them (1740 cm^{-1}) has been assigned to Asp-83 residue in Meta II (Rath et al., 1993). None of these bands, however, contributes significantly to the amide II/amide II′ region and cannot account for the observed H/D exchange-induced spectral changes that are not seen in corresponding difference spectra of rhodopsin samples in H_2O.

The rate of H/D exchange in rhodopsin films can be determined by plotting the change in integrated intensity of the amide II band or amide II′ band as a function of time. In the case of transmittance FTIR measurements (Fig. 4 *A*), the fraction of peptide hydrogens undergoing exchange decreases slowly before illumination, reflecting the residual exchange that occurs after 48 h of exposure to D_2O. For example, after a 5-h period before photoactivation, only an ~0.2% decrease in the amide II band intensity was observed. In contrast, 5 h after photoexcitation, a 10% decrease in the amide II intensity was observed, which continued to decrease over the course of the measurements. A curve fit of the data recorded with 11 min time resolution revealed that the decay could be resolved into two components with $\tau \sim 1.5$ h and 18.5 h (Fig. 4 *A*). A curve fit of the amide II′ intensity gave a similar result (1.8 h and 18 h). In contrast, the integrated intensity of the ester carbonyl C═O stretch mode (1740 cm^{-1}) arising mainly from lipids in the photoreceptor membrane remained relatively constant after illumination with only a 1.5% drop, which occurs within a few minutes after bleaching and may reflect a change in absorption of Asp and Glu groups seen in FTIR difference spectra of the Rho→Meta II transition.

The results obtained from ATR-FTIR measurements (Fig. 4 *B*) show a more rapid H/D exchange for both the fast and slow components of the amide II and II′ bands ($\tau \sim 30$ min and 11 h). [The slow component of the amide II′ band could not be fit accurately and is not included in this

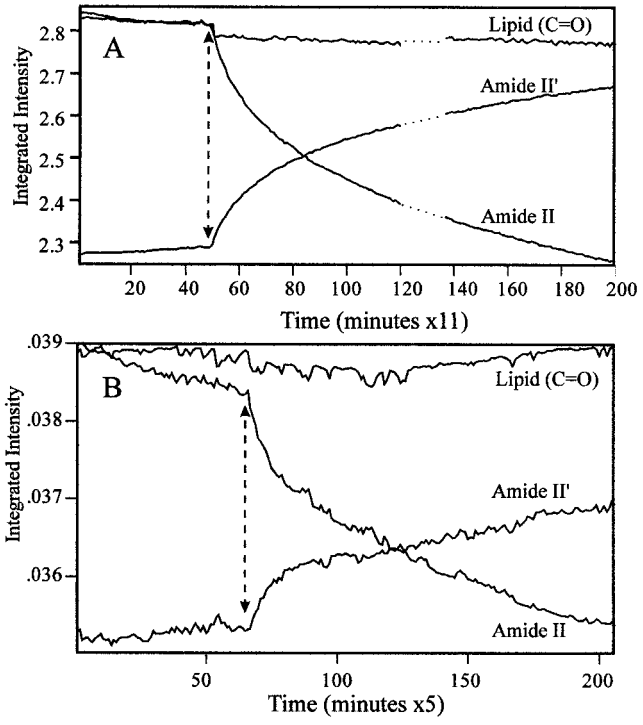

FIGURE 4 Kinetics of H/D exchange before and after photobleaching of rhodopsin. Changes in the integrated intensity of the amide II, II′, and lipid ester carbonyl bands for samples measured in D_2O before and after illumination (*dotted double arrow*) at 10°C using transmission (*A*) and ATR-FTIR (*B*) methods (see Materials and Methods). The dotted portion of the curves in (*A*) denote the times for which data were not obtained.

estimate.] This may be due to the exposure of the photoreceptor membrane film deposited on the ATR crystal to bulk D_2O compared with the transmittance measurements where the film was in equilibrium in a sealed cell with vapor from drops of D_2O. In the latter case, it is possible that only incomplete rhodopsin hydration occurs. However, FTIR difference spectra obtained under similar conditions (DeGrip et al., 1985) show little evidence of Meta I accumulation upon photoactivation, indicating that the membranes are fully hydrated upon exposure to vapor. The difference in H/D exchange rates is more likely to reflect a difference in the effective pH for the respective films, which was undetermined for these experiments. It is well known that H/D exchange rates of peptide groups are pH-dependent (Downer and Englander, 1982; Englander and Englander, 1977; Englander and Mayne, 1992). This has also been noted as a possible reason for differences in exchange rates previously measured for bovine and frog rhodopsin made in suspension (Downer and Englander, 1982).

DISCUSSION

A key step in the activation of G-protein coupled receptors is a conformational change triggered by ligand binding. In the case of rhodopsin, the active ligand is formed upon photoisomerization of the 11-*cis* retinylidene chromophore to an all-*trans* configuration. This leads to a series of conformational changes that culminates in formation of Meta II, the only intermediate that activates the G-protein (Kibelbek et al., 1991; Vuong et al., 1984).

One method of probing these structural changes is by measuring the accessibility of rhodopsin and its bleaching intermediates to the bulk medium via hydrogen exchange of its peptide groups. Earlier studies using infrared spectroscopy revealed that there exists a core region of rhodopsin where >50% of the total peptide groups are resistant to H/D exchange (Downer et al., 1986; Haris et al., 1989; Osborne and Nabedryk-Viala, 1977; Osborne et al., 1978; Rothschild et al., 1980a). As indicated by the current results, this region most likely reflects the 7-helix portion of rhodopsin that is buried within the lipid bilayer and consists primarily of α-helical structure oriented perpendicular to the membrane plane (Rothschild et al., 1980c).

There is little agreement, however, about how photoactivation affects the H/D exchange of rhodopsin. An early infrared study on H/D exchange in bovine rhodopsin reported that illumination does not increase the number of peptide hydrogens that exchange (Osborne and Nabedryk-Viala, 1977) except when rhodopsin is solubilized in detergent where it loses its native conformation upon bleaching (Osborne and Nabedryk-Viala, 1978). A more recent study, which analyzed the infrared spectra of unbleached and bleached rhodopsin in H_2O and D_2O, found no significant changes in structure or the level of H/D exchange (Haris et al., 1989). In contrast, hydrogen-tritium exchange measurements that do not distinguish between peptide and side-chain hydrogens revealed a fast-exchanging intermediate in the bleaching sequence of frog disk membrane that was attributed to Meta II (Downer and Englander, 1977). Similar results, however, were not obtained for bovine disk membranes, possibly due to the low pH used for this experiment (Downer and Englander, 1977).

Our current results based on time-resolved FTIR measurements of H/D exchange in rhodopsin clearly establish that photoactivation produces a new phase of H/D exchange of peptide groups. In rhodopsin films exposed to bulk D_2O (ATR measurements), two distinct components of H/D exchange with time constants of 30 min and 11 h were found. The faster rate is in the range expected for the decay of Meta II to Meta III at 10°C, which is ~5 min in rhodopsin films and aqueous suspensions of photoreceptor membranes at room temperature (Rothschild et al., 1980b; Van Breugel et al., 1979) and 35 ± 5 min at 10°C (Bovee, P. H., and W. J. DeGrip, unpublished). The decay of Meta II also leads to the formation of opsin plus retinal either directly or through the decay of Meta III, which is much slower at 20°C (Klinger and Braiman, 1992).

It is not yet possible on the basis of our measurements to correlate the fast and slow phases of the H/D exchange with the formation and/or decay of the Meta II, Meta III, and opsin states. In particular, photoactivated H/D exchange could reflect an increased accessibility of rhodopsin's core structure during the lifetime of the Meta II intermediate

which is at least partially shut off by Meta II decay. This would fit with the finding that rhodopsin undergoes a structural change upon formation of the Meta II intermediate, which is reversed upon its decay to Meta III (Farahbakhsh et al., 1993; Klinger and Braiman, 1992; Rothschild et al., 1987). In this case, the initial phase of H/D exchange kinetics we observe would depend both on the absolute rate of H/D exchange of newly exposed peptide groups and the rate of loss of these exchangable groups during Meta III formation. Alternatively, a very fast H/D exchange might be initiated upon formation of the Meta III intermediate. In this case, the H/D exchange kinetics would be expected to closely match the Meta II decay. Such an effect could occur, for example, if new peptide groups became exposed due to removal of the retinal chromophore from the binding pocket (Van Breugel et al., 1979). Future studies will be directed at distinguishing between these two possible cases.

The region(s) of the rhodopsin structure exhibiting increased H/D exchange upon photoactivation also remain to be determined. Since these regions were resistant to H/D exchange for almost 48 h prior to photoactivation, they are likely to be buried in the interior of the protein and may be located inside the 7-helix bundle. Part of the exchange may occur in peptide bonds lining the retinal binding site that lose the chromophore during Meta II decay as noted above (Van Breugel et al., 1979). Increased H/D exchange could also occur, for example, if a small rearrangement of α-helical orientation allowed increased accessibility of these core regions to the external medium. For example, such a rearrangement of α-helices is found to occur during the M$\rightarrow$N transition of bacteriorhodopsin (Ludlam et al., 1995). Alternatively, a portion of the rhodopsin structure that is buried in the membrane interior might move to a more surface-accessible region, for example, near the site for G-protein interaction. An increase in accessibility and reactivity of sulfhydryl groups (DeGrip and Daemen, 1982; Regan et al., 1978) and the appearance of new regions accessible for proteolysis upon bleaching support this picture (Kuehn and Hargrave, 1981).

One possible method for identifying regions of increased H/D exchange would be to utilize SDIL of peptide groups in conjunction with FTIR, as recently demonstrated for the case of bacteriorhodopsin and phospholamban (Ludlam et al., 1996). Alternatively, residues with detectable infrared bands which are sensitive to H/D exchange, such as Cys, might be used as site-specific probes (Arkin et al., 1996). It was found recently that at least one Cys residue that may be buried in the membrane interior produces a signal in the FTIR difference spectrum during Meta II formation (Rath et al., 1994). Cysteine residues introduced by genetic engineering with links to various probes might also be useful for monitoring site-specific H/D exchange upon photoactivation of rhodopsin. In this regard, cysteine residues linked to ESR labels have revealed regions of structural activity in rhodopsin during Meta II formation and decay (Farahbakhsh et al., 1993).

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