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Protonation states of membrane-embedded carboxylic acid groups in rhodopsin and metarhodopsin II: A Fourier-transform infrared spectroscopy study of site-directed mutants

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ABSTRACT **A method was developed to measure Fourier-transform infrared (FTIR) difference spectra of detergent-solubilized rhodopsin expressed in COS cells. Experiments were performed on native bovine rhodopsin, rhodopsin expressed in COS cells, and three expressed rhodopsin mutants with amino acid replacements of membrane-embedded carboxylic acid groups: Asp-83 → Asn (D83N), Glu-122 → Gln (E122Q), and the double mutant D83N/E122Q. Each of the mutant opsins bound 11-*cis*-retinal to yield a visible light-absorbing pigment. Upon illumination, each of the mutant pigments formed a metarhodopsin II-like species with maximal absorption at 380 nm that was able to activate guanine nucleotide exchange by transducin. Rhodopsin versus metarhodopsin II-like photoproduct FTIR-difference spectra were recorded for each sample. The COS-cell rhodopsin and mutant difference spectra showed close correspondence to that of rhodopsin from disc membranes. Difference bands (rhodopsin/metarhodopsin II) at 1767/1750 cm^{-1} and at 1734/1745 cm^{-1} were absent from the spectra of mutants D83N and E122Q, respectively. Both bands were absent from the spectrum of the double mutant D83N/E122Q. These results show that Asp-83 and Glu-122 are protonated both in rhodopsin and in metarhodopsin II, in agreement with the isotope effects observed in spectra measured in ^2H_2O. A photoproduct band at 1712 cm^{-1} was not affected by either single or double replacements at positions 83 and 122. We deduce that the 1712 cm^{-1} band arises from the protonation of Glu-113 in metarhodopsin II.**

Rhodopsin is a member of the superfamily of seven-transmembrane-helix, G protein-coupled receptors. The rhodopsin chromophore 11-*cis*-retinal is covalently bound to the protein via a protonated Schiff base linkage (1) to a lysine residue (Lys-296 in bovine rhodopsin) (2, 3). After photoisomerization of the chromophore, thermal relaxation leads to an active conformation, R*, which binds the G protein transducin and thereby couples photon absorption to the visual signal transduction cascade. It has been shown by chemical modifications of Lys-296 in bovine rhodopsin that the deprotonation of the Schiff base is a prerequisite for R* formation (4, 5). Spectroscopically, this state is designated metarhodopsin II (MII) and characterized by a visible absorption maximum (λ_{max}) at 380 nm, indicative of the unprotonated Schiff base of all-*trans*-retinal. Biochemical studies (6–10) and resonance Raman spectroscopy (11) of recombinant rhodopsins have shown that the positive charge at the Schiff base nitrogen in rhodopsin is stabilized by Glu-113, which acts as a Schiff base counterion in the transmembrane domain of the opsin.

To investigate the protonation states and possible protonation changes of membrane-embedded carboxyl groups in rhodopsin and its MII photoproduct, we have performed Fourier-transform infrared (FTIR) difference spectroscopy on recombinant pigments. This form of vibrational spectroscopy has been shown to be a powerful method for studying the molecular changes occurring in rhodopsin, from the formation of bathorhodopsin through the decay of MII (12–16). From previous experiments on bovine rhodopsin, it is known that three C=O stretching vibrations of membrane-embedded protonated carboxyl groups are affected during the thermal relaxation to the MII state (12, 17–19). These groups have been postulated to include Asp-83, Glu-113, and Glu-122 because these residues are located within the hydrophobic interior of the protein as deduced from hydrophobicity plots and biochemical studies of mutant pigments (6, 8, 20). We have used site-directed mutagenesis in combination with FTIR difference spectroscopy to identify the absorbance changes associated with these carboxylic acid groups.

We prepared rhodopsin mutants with single and double amino acid replacements at positions 83 and 122 and recorded their FTIR spectra. Comparisons of these spectra have allowed the assignments of the C=O stretching vibrations of these groups. The results show that Asp-83 and Glu-122 are protonated in both rhodopsin and MII. The rationale for the construction of a double mutant was to allow also band assignments for Glu-113 by abolishing contributions from the other membrane-embedded carboxyl groups. This approach circumvents problems caused by the pH-dependent heterogeneity of FTIR samples obtainable from relevant Glu-113 mutants. In addition, since the mutants chosen for FTIR studies can form MII-like species and can activate transducin, a putative proton transfer to Glu-113 should still occur in these mutants. The results strongly suggest that Glu-113 acts as the proton acceptor for the net proton transfer from the Schiff base to the opsin that occurs upon formation of MII.

METHODS

Materials. Sources of materials and oligonucleotide preparation have been reported (21–24).

Preparation of Rhodopsin and Rhodopsin Mutants. Rod outer segment (ROS) membranes were prepared (25). Site-directed mutagenesis was performed by using restriction-fragment-replacement "cassette mutagenesis" (26) in a synthetic gene for bovine rhodopsin (23, 27). Mutant E122Q was prepared by replacement of a 69-bp *Rsr* II–*Spe* I restriction fragment with a synthetic duplex containing the desired codon alteration. Mutant D83N was prepared by replacement

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Abbreviations: R*, light-activated rhodopsin; MII, metarhodopsin II; FTIR, Fourier-transform infrared; ROS, rod outer segment(s); D_2O, ^2H_2O.

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of a 98-bp *Hin*dIII–*Nco* I restriction fragment. The double mutant D83N/E122Q was assembled from the single mutants by using the single intervening *Rsr* II site. The nucleotide sequence of all cloned synthetic duplexes described was confirmed by DNA sequencing by using deoxyadenosine 5′-[α-(^{35}S)thio]triphosphate on purified plasmid DNA (28). The mutant genes were expressed in COS-1 monkey kidney cells and regenerated with chromophore (6, 29, 30). The pigment purification was based on the immunoaffinity procedure of Oprian *et al.* (29), modified as described (22, 30) except that a 1 mM sodium phosphate, pH 6.8/0.02% dodecyl maltoside buffer was used for wash and elution steps. The purified pigment from 120–160 culture dishes (100 mm) of COS cells was concentrated by filtration (Centricon-30, Millipore). The octadecapeptide used for elution from the immunoaffinity resin was removed from the sample by passing the concentrated pigment solution over a prepacked Sephadex G-50 column (Pharmacia). The void-volume fractions were collected and reconcentrated so that the final sample had an absorbance at the visible λ_{max} of >1 OD unit (>23 μM) in a volume of 0.1–0.2 ml.

Biochemical Characterization of Rhodopsin Mutants. UV–visible absorption spectroscopy was performed as described (21, 22). Determination of the λ_{max} value was based on at least three independent sample preparations. Values for ε (extinction coefficient) were determined by acid denaturation of each mutant pigment (6). The long-pass filters used for pigment illumination were as follows: D83N, 495 nm; E122Q, 475 nm; D83N/E122Q, 475 nm. Transducin activation assays were carried out as described (22, 31).

FTIR Spectroscopy of Rhodopsin Mutants. Approximately 1.2 nmol of pigment in solution (pH adjusted to 5.5 with potassium phosphate buffer) was dried under a stream of N_2 onto an AgCl window. The film was rehydrated and immediately sealed with a Ge window to protect it from radiation of the He–Ne laser controlling the interferometer. For ^2H_2O (D_2O) measurements, about 0.5 μl of D_2O was added to the sample chamber. All samples were cooled to 0°C. For measurements of native rhodopsin in disc membranes, ≈2.5 nmol of rhodopsin in a suspension of ROS fragments was used.

A homemade FTIR spectrophotometer equipped with an IFS 88 interferometer (Bruker, Billerica, MA) and a Hg/Cd/Te detector (EG & G Judson, Salem, MA) was used. After sample equilibration for 1–2 hr within the FTIR instrument, four blocks of 64 scans each were accumulated before and after illumination for 1 min with a 150-W slide projector and fiber optics, fitted with a 495-nm long-pass filter. Spectral resolution was 2 cm^{-1}. Difference spectra were formed as described (17, 32). With the exception of native ROS rhodopsin, the spectra were normalized to the retinal band intensity at 1238 cm^{-1}.

RESULTS

Preparation and Biochemical Characterization of Mutant Pigments. Genes for opsin single-replacement mutants D83N and E122Q and double-replacement mutant D83N/E122Q were prepared and expressed in COS cells. After regeneration with 11-*cis*-retinal, each of the mutants formed a visible-absorbing pigment. The λ_{max} values were as follows: D83N, 495 nm; E122Q, 480 nm; D83N/E122Q, 475 nm (Fig. 1). Upon illumination, a MII-like photoproduct absorbing at 380 nm was obtained for each of the pigments (Fig. 1). Each of the photoactivated mutant pigments was able to catalyze nucleotide exchange by transducin (Fig. 2). Similar results were reported previously for mutants D83N and E122Q (6, 33).

Comparison of FTIR Spectra of ROS and COS-Cell Rhodopsin. FTIR difference spectra were obtained from ROS rhodopsin, purified COS-cell rhodopsin, and each of the mutant pigments (Fig. 3). Chromophore absorption bands are

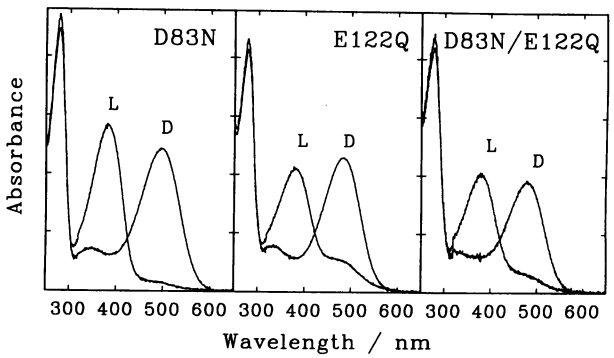

FIG. 1. UV–visible absorption spectra of mutant pigments. Spectra of each mutant pigment regenerated with 11-*cis*-retinal and purified in dodecyl maltoside detergent are presented. A dark spectrum (D) is shown for each mutant. The λ_{max} values for the pigments were as follows: D83N, 495 nm; E122Q, 480 nm; D83N/E122Q, 475 nm. COS-cell rhodopsin prepared under the same conditions had a λ_{max} of 500 nm (data not shown). A spectrum after illumination (L) is shown for each pigment.

observed mainly between 1100 cm^{-1} and 1300 cm^{-1}. They are caused by C—C stretching and C—H bending vibrations which couple to each other. The coupling renders these bands sensitive to the isomeric state of the retinal (16, 34, 35). The close correspondence of the difference bands in the range 1000–1300 cm^{-1} between COS-cell rhodopsin and ROS rhodopsin demonstrates the formation of a native chromophore binding site in the COS-cell pigment and is consistent with previous biochemical and resonance Raman studies (6, 11, 29). The frequency range 1300–1500 cm^{-1} covers many protein vibrations for which chemical assignments are not available. However, the good agreement between COS-cell and ROS spectra in Fig. 3 shows that essentially the same conformational changes that affect this spectral region occur in both preparations upon photoconversion to MII. This finding is consistent with previous biochemical comparisons of ROS rhodopsin with purified COS-cell rhodopsin (28, 30).

Protein backbone absorption bands are observed around 1650 cm^{-1} (amide I) and 1550 cm^{-1} (amide II). The difference bands caused by the rhodopsin → MII conversion in ROS membranes are all reproduced in the purified COS-cell

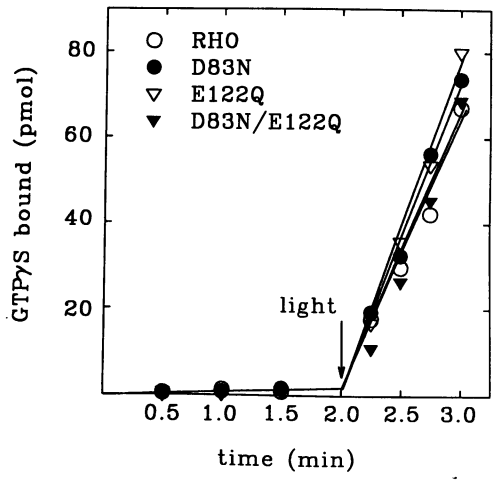

FIG. 2. Rhodopsin-catalyzed nucleotide exchange by transducin. The abilities of COS-cell rhodopsin and the mutant pigments to activate transducin were evaluated by a guanosine 5′-[γ-thio]triphosphate (GTPγS) filter-binding assay. Results of a single experiment are shown. Each reaction mixture contained 2 μM transducin, 5 nM pigment, and 20 μM GTPγS. Under the conditions of the assay, each of the mutant pigments activated transducin at about the same rate as rhodopsin.

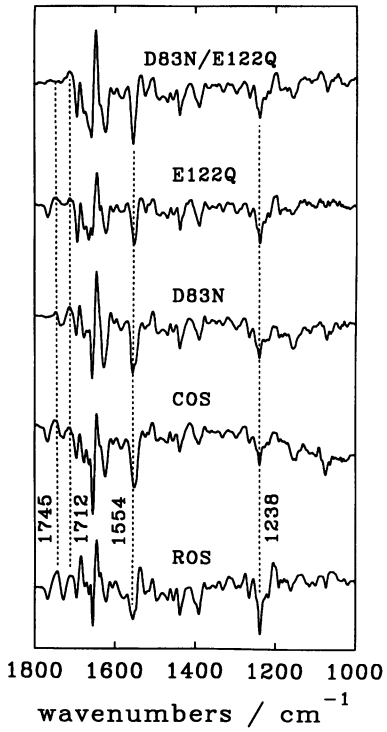

FIG. 3. FTIR difference spectra of rhodopsin in washed ROS membranes (ROS), recombinant COS-cell rhodopsin purified in the presence of dodecyl maltoside (COS), mutant D83N, mutant E122Q, and double mutant D83N/E122Q (from bottom to top). Absorption bands of rhodopsin are pointing downward; those of photoproducts point upward. Spectra of recombinant pigments are scaled to identical intensity of the chromophore vibration at 1238 cm^{-1}, to compensate for different amounts of material. Spectra were obtained at 0°C.

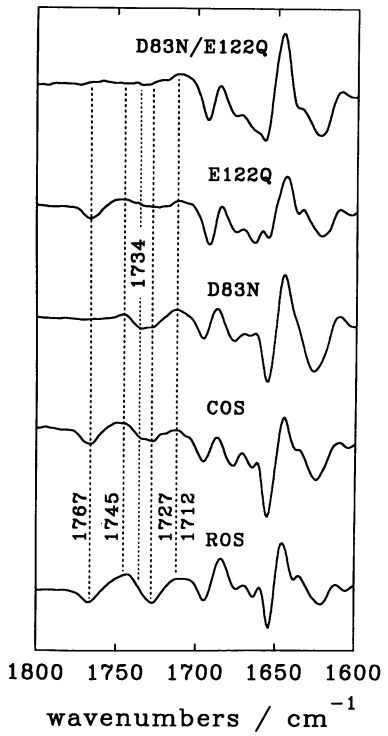

FIG. 4. FTIR difference spectra in the spectral range of the C=O stretching vibration of protonated carboxyl groups. The same spectra are shown as those in Fig. 3, using identical scaling factors.

rhodopsin → MII conversion. However, the relative intensities of these bands are slightly altered. This difference is best appreciated between 1530 and 1555 cm^{-1}, where the C=C stretching band of the retinal is also located. Such differences are expected, since the recombinant pigment was prepared in detergent solution. ROS disc membranes display anisotropy because of partial orientation of the membrane fragments parallel to the window during preparation of the IR sample. Detection of IR bands that have their transition dipole moments oriented either perpendicular or parallel to the membrane fragments is highly sensitive to the sample anisotropy. The amide I and amide II bands of transmembrane α-helices are oriented preferentially perpendicular and parallel to the membrane bilayer, respectively. Therefore, detection of amide II bands in transmembrane α-helices is enhanced in the ROS samples. The solubilized COS-cell rhodopsin molecules are randomly oriented and the amide I vibrations become relatively more pronounced. This has been observed with samples of detergent solubilized ROS rhodopsin as well (data not shown). Despite these deviations, the COS-cell rhodopsin spectrum is in quite good agreement with the ROS spectrum. Thus, by FTIR criteria, COS-cell rhodopsin prepared in dodecyl maltoside forms a MII species that is structurally similar to MII in ROS membranes.

The band pattern between 1700 and 1800 cm^{-1} results from C=O stretching vibrations of protonated carboxyl groups (Fig. 4). This frequency range is almost free of contributions from other vibrations. The negative band at 1767 cm^{-1} is caused by the C=O stretch of a protonated carboxylic acid in rhodopsin, and the two positive bands at 1745 and 1712 cm^{-1} are characteristic of protonated carboxyl groups in the MII photoproduct. These bands are present in COS-cell rhodopsin as well as in ROS rhodopsin. However, the spectrum of COS-cell rhodopsin displays an additional negative band at 1734 cm^{-1}. Although caused by a carboxylic acid group (see below), the appearance of this band is due to a detergent effect, since control spectra of bovine rhodopsin purified in dodecyl maltoside exhibit the 1734 cm^{-1} band as well (data not shown).

FTIR Spectra of Mutants D83N and E122Q. FTIR difference spectra were obtained from mutant pigments D83N and E122Q (Fig. 3). All difference bands between 1000 and 1660 cm^{-1} agree with those of COS-cell rhodopsin. The small differences seen between 1675 and 1660 cm^{-1} are likely to be caused by the C=O band of Asn/Gln that replaced the respective carboxyl group or by small structural differences causing alterations of amide I bands. The mutations affect predominantly the band pattern between 1700 and 1800 cm^{-1} (Fig. 4). The spectrum of D83N clearly shows that the single amino acid exchange causes the disappearance of the negative (rhodopsin) band at 1767 cm^{-1}. Simultaneously, the high-frequency part of the positive MII band at 1745 cm^{-1} is absent. Therefore, Asp-83 causes a difference band located at 1767/1750 cm^{-1} (rhodopsin/MII) in COS-cell rhodopsin, demonstrating that Asp-83 is protonated both in rhodopsin and in MII (C=O stretches of ionized carboxyl groups absorb outside the depicted frequency range). Because of its high C=O stretching frequency in rhodopsin, we conclude that Asp-83 is not hydrogen bonded in this state, whereas it becomes weakly hydrogen bonded in MII as evidenced by the downshift of the C=O stretching frequency.

The replacement of Glu-122 by Gln (Fig. 3) causes spectral alterations similar to those described for mutant D83N. The two negative (rhodopsin) bands at 1734 cm^{-1} and 1727 cm^{-1} and the low-frequency part of the positive (MII) band at 1745 cm^{-1} are absent from the difference spectrum of mutant E122Q (Fig. 4). As was the case for Asp-83, the spectra show that Glu-122 is protonated in rhodopsin and remains protonated in MII. However, the carbonyl group of Glu-122 becomes less hydrogen bonded in the MII-like form than in

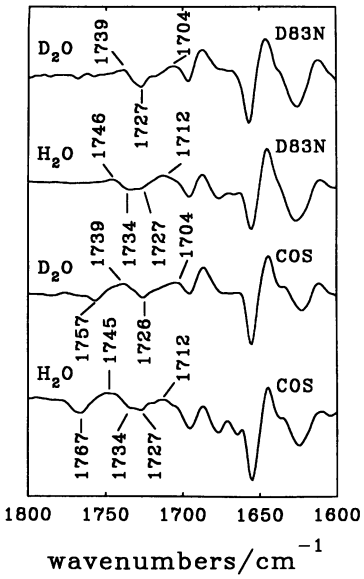

FIG. 5. Effect of D_2O/H_2O exchange on carbonyl vibrational frequencies.

rhodopsin, as evidenced by the upshift of its C=O stretching frequency. In contrast to the effect of replacing Asp-83, two negative bands are abolished by the replacement of Glu-122, and it is not clear whether the absorptions at 1734 and 1727 cm^{-1} can both be assigned to a single C=O group.

The rhodopsin MII difference spectra of the recombinant pigments were measured in D_2O to further characterize the negative bands at 1734 and 1727 cm^{-1}. Carbonyl stretching vibrations of protonated carboxylic acids in bovine rhodopsin and MII have been identified by previous FTIR studies on samples prepared in D_2O (12, 13, 18, 35, 36). The absorption at 1727 cm^{-1} was assigned to an amide I vibration in rhodopsin, based on a lack of D_2O sensitivity and on results obtained using retinal analogs (35). The D_2O effects in COS-cell rhodopsin and in mutant D83N are shown in Fig. 5. The spectra clearly show that all bands, with the exception of the negative band at 1727 cm^{-1}, exhibit the expected isotope shift. The band at 1734 cm^{-1} shifts into that at 1727 cm^{-1}. Therefore, the band at 1727 cm^{-1} in the COS-cell rhodopsin spectrum and in the D83N spectrum behaves as expected from previous studies of ROS rhodopsin. An explanation for the band at 1727 cm^{-1} is presented below. The negative band at 1734 cm^{-1} can be assigned to the C=O stretching vibration of protonated Glu-122.

In summary, the single amino acid replacements at positions 83 and 122 show localized effects in the difference spectra, demonstrating that global structural changes in the rhodopsin → MII transition are not caused by the mutations. The only C=O stretching vibration not affected by the single mutations is located at 1712 cm^{-1}.

FTIR Spectra of Double Mutant D83N/E122Q. FTIR difference spectra were recorded of the double mutant D83N/E122Q to verify the absence of any direct or indirect contributions of Asp-83 or Glu-122 to the band at 1712 cm^{-1} not affected by the single mutations. In the double mutant, all carbonyl bands are absent with the exception of the positive (MII) band at 1712 cm^{-1} (Fig. 4). Therefore, the results of the single mutations are confirmed and their spectral influences are additive. These additive effects also suggest that the mutations did not cause global structural perturbations.

DISCUSSION

IR absorption bands of rhodopsin and its MII photoproduct have been assigned to individual amino acids by studying site-directed mutant pigments. The recombinant pigments were able to activate transducin after photoconversion to a form absorbing at 380 nm (Figs. 1 and 2). The FTIR difference spectra of COS-cell rhodopsin exhibited all of the vibrational features typical of the rhodopsin → MII transition observed in rhodopsin from ROS. Therefore, the difference spectrum of COS-cell rhodopsin in detergent could serve as a reference spectrum for band assignments using rhodopsin mutants.

The D83N and E122Q replacements were motivated by the fact that deprotonation of the Schiff base and proton uptake from the bulk water phase are known to accompany the formation of R*. Therefore, carboxyl groups in the transmembrane domain of opsin may be involved in proton transfer reactions that are necessary for R* formation. The spectral effects resulting from the single amino acid replacements D83N and E122Q have allowed the characterization of both residues in terms of their protonation states and hydrogen-bonding strength in rhodopsin and in MII.

Replacement of Asp-83 or Glu-122 abolished difference bands at 1767/1750 cm^{-1} and 1734/1745 cm^{-1}, respectively (Fig. 4), without preventing transducin activation (Fig. 2). These bands are typical of a frequency shift of a C=O stretching vibration in a protonated carboxyl group, indicating that Asp-83 and Glu-122 are protonated in rhodopsin as well as in MII. The absorption at 1734 cm^{-1} assigned to Glu-122 is observed in ROS rhodopsin only in the MI transition. However, the 1734 cm^{-1} band is also observed when MII of ROS rhodopsin is measured in the presence of dodecyl maltoside detergent (data not shown). Since the COS-cell pigments otherwise display the typical MII band pattern throughout the remaining spectral range, a contribution of a MI photoproduct to the spectra is unlikely. This indicates that a structural constraint for Glu-122 that is transiently changed in MI and becomes restored in MII is still altered in the MII state of the recombinant pigments. Therefore, the frequency shift of the C=O stretching vibration from 1734 cm^{-1} to 1745 cm^{-1} in MII may be related to the lack of membrane pressure on rhodopsin in detergent as compared with that in the native lipid environment.

Interestingly, mutation of either Asp-83 or Glu-122 leaves the difference band of the other unaffected, demonstrating that the two groups probe different protein environments in rhodopsin as well as in MII. In particular, our data exclude a direct interaction between Asp-83 and Glu-122 through a common hydrogen bond in rhodopsin as well as in MII. Since no protonation change occurs in the side chain of Asp-83, it does not seem to play a role as an acceptor or donor group in the MII state.

A puzzling result is that replacing Glu-122 by Gln abolishes not only the negative band at 1734 cm^{-1} but also the one at 1727 cm^{-1}. If the previous assignment of the absorbance at 1727 cm^{-1} to an amide I band in rhodopsin is correct, we have to conclude that the replacement of Glu-122 causes a localized structural perturbation that prevents the alteration of a peptide bond usually occurring during MII formation. The particular amide groups would exhibit an unusually high amide I frequency. Glu-122 is preceded by two Gly residues known to destabilize α-helical secondary structure. The hydrogen bond of Glu-122 may influence the nature of the secondary structure imposed on the neighboring Gly residues. This could explain the influence of the replacement of Glu-122 on the absorption at 1727 cm^{-1}. An alternative explanation would be to assign the entire band structure 1734–1727 cm^{-1} to Glu-122. Such an assignment would imply that Glu-122 can form two hydrogen bonds and, thus, that the population of this particular recombinant rhodopsin is heterogeneous. With this assignment, the larger integral intensity of the negative (rhodopsin) band at 1727 cm^{-1} as compared with the positive (MII) band at 1745 cm^{-1} would

suggest a partial deprotonation of this residue upon formation of MII.

The double mutant D83N/E122Q was designed to remove the C=O stretching vibrations of these amino acids from the difference spectra and thereby isolate the putative C=O stretching absorption of the remaining membrane-embedded carboxylic acid group, Glu-113. Of the three C=O stretching vibrations of MII from COS cells at 1750 cm^{-1} (Asp-83), 1745 cm^{-1} (Glu-122), and 1712 cm^{-1}, only the band at 1712 cm^{-1} is present in the difference spectrum of the double mutant (Fig. 4). Although its low position indicates strong hydrogen bonding, the band at 1712 cm^{-1} can be assigned to an internal carboxyl group since an external group would show a much wider bandwidth. The assignment to an internal group is supported by the fact that the protonated carboxyl group causing the band at 1712 cm^{-1} in MII is not titratable from the bulk water phase in the range pH 5.5–8 as shown by time-resolved studies on MII decay (37). For these reasons, carboxylic acid residues at transmembrane helix borders (Glu-33, Glu-134, and Glu-201) are not likely to be assignable to the band at 1712 cm^{-1}. Mutation of Glu-134, located at the cytoplasmic border of helix C, also did not affect this band (data not shown). These results strongly suggest that the band at 1712 cm^{-1} is caused by the C=O stretching vibration of protonated Glu-113 in MII.

Previous studies have shown that Glu-113 is ionized in rhodopsin and serves as the counterion to the protonated Schiff base (6–9, 11). Therefore, the FTIR spectroscopy results suggest that the Schiff base counterion is also the acceptor group for the net proton transfer from the Schiff base to opsin in MII. This protonation is supported by measurements in the presence of D_2O, since the 1712 cm^{-1} band is sensitive to the isotope but has no negative D_2O-sensitive counterpart (Fig. 5).

Since the protonation step causing the band at 1712 cm^{-1} still occurs in mutant D83N/E122Q, neither of these residues is necessary for the net proton transfer from the Schiff base to opsin. In particular, the lack of any influence of mutations at positions 83 and 122 on the band at 1712 cm^{-1} excludes Asp-83 and Glu-122 as hydrogen-bonding partners for the protonated form of Glu-113 in MII. This argues that the Schiff base proton is either directly transferred to Glu-113 in MII or takes a pathway that includes an intermediary different from Asp-83 or Glu-122. The strong hydrogen bond exhibited by Glu-113 indicates that a water molecule may also serve as the intermediary.

In a time-resolved FTIR study of native ROS rhodopsin, the band pattern in the 1700–1800 cm^{-1} range was shown to decay with the MII → MIII transition (37). Accordingly, it was suggested that the amino acid side chains responsible for these bands were specifically involved in transducin activation. The present results of FTIR and transducin activation assays argue against the direct involvement of Asp-83 and Glu-122 in intramolecular proton transfer during photoactivation and in transducin activation. However, an indirect role of both residues cannot be ruled out.

In summary, we have used a combination of site-directed mutagenesis and FTIR difference spectroscopy to assign absorption bands to specific carboxylic acid residues. Asp-83 and Glu-122 are protonated in both dark rhodopsin and MII. Glu-113 is the only membrane-embedded carboxylic acid to which the observed protonation of a carboxyl group in MII can be assigned. Such an assignment is supported by the identification of Glu-113 as the counterion to the Schiff base in rhodopsin. In general agreement with studies on constitutively active rhodopsin mutants (38, 39), the neutralization of Glu-113 by proton uptake in MII as deduced from the FTIR data seems to be a crucial step in light activation of rhodopsin.

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