Conformational Changes Detected in a Sensory Rhodopsin II-Transducer Complex*

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Sensory rhodopsins (SRs) are light receptors that belong to the growing family of microbial rhodopsins. SRs have now been found in all three major domains of life including archaea, bacteria, and eukaryotes. One of the most extensively studied sensory rhodopsins is SRII, which controls a blue light avoidance motility response in the halophilic archaean *Natronobacterium pharaonis*. This seven-helix integral membrane protein forms a tight intermolecular complex with its cognate transducer protein, HtrII. In this work, the structural changes occurring in a fusion complex consisting of SRII and the two transmembrane helices (TM1 and TM2) of HtrII were investigated by time-resolved Fourier transform infrared difference spectroscopy. Although most of the structural changes observed in SRII are conserved in the fusion complex, several distinct changes are found. A reduction in the intensity of a prominent amide I band observed for SRII indicates that its structural changes are altered in the fusion complex, possibly because of the close interaction of TM2 with the F helix, which interferes with the F helix outward tilt. Deprotonation of at least one Asp/Glu residue is detected in the transducer-free receptor with a pK_a near 7 that is abolished or altered in the fusion complex. Changes are also detected in spectral regions characteristic of Asn and Tyr vibrations. At high hydration levels, transducer-fusion interactions lead to a stabilization of an M-like intermediate that most likely corresponds to an active signaling form of the transducer. These findings are discussed in the context of a recently elucidated x-ray structure of the fusion complex.

Sensory rhodopsin (SR) I is a seven-helix integral membrane protein found in halophilic archaea that functions as a light receptor for negative phototaxis (1). Together with sensory rhodopsin I (SRI), which is a dual photoattractant/photorepellent receptor (2); bacteriorhodopsin (BR), a light-driven proton pump; and halorhodopsin, a light-driven chloride pump, these proteins belong to a widespread family of photoactive retinylidene proteins or microbial rhodopsins (3). Microbial rhodopsins have several common features including an all-trans-retinylidene chromophore covalently attached to the protein via a protonated Schiff base linkage. Light absorption results in an all-trans – 13-cis-isomerization of the chromophore that triggers subsequent conformational changes associated with a photocycle characteristic of each protein. Members of the microbial rhodopsin family have recently been found in diverse organisms including marine proteobacteria (4), cyanobacteria (5), and lower eukaryotes such as *Neurospora crassa* (6) and *Chlamydomonas reinhardtii* (7).

Sensory rhodopsin II transmits the photorepellent signal to the cell cytoplasm by activating the transmembrane domain of its cognate transducer HtrII (8), which is tightly bound to the receptor helices F and G (9). The photocycle of SRII comprises several photointermediates (10–13), including the blue-shifted M and red-shifted O intermediates, which are likely to be receptor signaling states (14).

Several recent studies have explored various aspects of the receptor-transducer interaction primarily in SRII from *Natronobacterium pharaonis*. Photocycle kinetics in the detergent-solubilized SRII-HtrII complex was measured by Sudo et al. (15), who concluded that the decay of the M intermediate is slowed in the presence of transducer. On the other hand, no change in the photocycle rate was observed for the same complex reconstituted in lipids (16, 17). Measurements of transient voltage and pH changes in membranes containing transducer-free SRII demonstrated that the receptor (but not the receptor-transducer complex) functions as a light-driven proton pump, although with less efficiency than BR (17, 18). SRI also exhibits light-driven proton pumping (19) inhibited by its cognate transducer HtrI by closure of its cytoplasmic proton-conducting channel (20). These findings suggest that the conformational change that opens the cytoplasmic channel, believed to involve an outward tilting of helix F, is hindered by transducer interaction in both sensory rhodopsins (1).

High resolution x-ray crystallographic structures have been obtained of the receptor dark state (21, 22), and more recently a fusion complex formed between SRII and a truncated form of HtrII consisting of the two transmembrane helices, TM1 and TM2, and a short membrane-proximal region (9). The latter study reveals close interaction between helices F and G of the receptor and TM2 of the transducer, including a bond formed between Tyr199 (helix G) and Asn74 (TM2). Current models of signal transduction in this system predict that an outward tilting of helix F, as observed in BR, causes a rotation, piston-like motion or other movement of TM2 that is propagated toward the more distal methyl-accepting and kinase regions of...
the transducer (9, 23). In this process, the Tyr<sup>199</sup>–Asn<sup>74</sup> bond may be disrupted. This possibility is consistent with photocycle studies utilizing the T199F mutant of SRII (24). EPR studies of spin-labeled SRII and its complex with HtrII also indicate that outward tilting of the receptor helix F displaces transmembrane helices of the transducer HtrII from their initial positions (25).

Since its original application to BR (26, 27), FTIR difference spectroscopy has been successfully applied to a variety of membrane proteins (28) including transducer-free sensory rhodopsin II (29–33). We report here an extension of this approach to study protein-protein interactions in a SRII-HtrII fusion complex. FTIR difference spectra were obtained of transducer-free SRII and SRII fused to the transmembrane helices (TM1 and TM2) and truncated cytoplasmic portion of the transducer. Our findings reveal bands associated with the interaction of the transducer and receptor including the deprotonation of one or more Asp/Glu residues and a possible disruption of the Tyr<sup>199</sup>–Asn<sup>74</sup> interaction observed in the resting state of the SRII-HtrII complex. In contrast to some models that predict an increase in structural rearrangement of the protein backbone, we observed an overall decrease in structural changes as reflected by bands in the amide I region. In addition, at high hydration levels, the presence of the transducer stabilizes the receptor during the photocycle in the late M intermediate, which is likely to be a signal transducing form of the complex.

**EXPERIMENTAL PROCEDURES**

**Plasmid Construction**—In construct JS008 the sopII gene from *N. pharaonis* strain DSM2160 was placed under control of the bop promoter in a pBJ301-derivative, as described by Kunji et al. (34). JS018 encodes a fusion protein in which full-length SRII and the 147 N-terminal residues of HtrII are joined by the flexible linker ASASNGASA. The fusion gene was also placed in pKJ301 under control of the bop promoter.

**Purification of His-tagged SRII and SRII-HtrII Complex**—Purification of His-tagged proteins was performed by a modification of the procedure previously described (35). Briefly, two 2-liter flasks containing 1.8 liters of complex medium and mevinolin (1.0 μg/ml) were inoculated with 5.5 ml of liquid culture of the transformant strains JS008 and JS018. The cultures were grown to stationary phase (A<sub>660</sub> = −0.7) under illumination at 37 °C with 240 rpm rotary shaking. The cells were harvested by centrifugation and resuspended in 120 ml of sonication buffer (25 mM NaCl, pH 6.8) in the presence of 40 mg of DNase (Sigma). The cells were disrupted by sonication, and the unbroken cells were removed by low speed centrifugation. The membranes were collected by centrifugation (39,000 × g for 60 min), and the pellets were resuspended in a total combined volume of 36 ml of solubilization buffer (50 mM MES, 300 mM NaCl, pH 6.0) with 1.0% w/v octylglucoside. His-tagged protein was purified by Ni<sup>2+</sup>-affinity chromatography by adding 1.0 ml of His-Bind resin to 36 ml of solubilized sample and shaken at 4 °C for at least 3 h. The bound resin was applied to a 50-cm chromatography column (Bio-Rad). The packed His-tagged SRII-bound resin was washed with 10 ml of solubilization buffer followed by 10 ml of the wash buffer E50 (50 mM Tris-HCl, 300 mM NaCl, 50 mM imidazole, 1% octylglucoside, 0.02% sodium azide, pH 7.5). The protein was eluted by the addition of elution buffer E150 (50 mM Tris-HCl, 300 mM NaCl, 150 mM imidazole, 1% octylglucoside, 0.02% sodium azide, pH 7.5). The eluted protein was concentrated to the final concentration of 1.4 mg/ml for SRII and 0.2 mg/ml for SRII-HtrII.

**Proteoliposome Reconstitution**—Purified His-tagged SRII and the fusion complex were reconstituted in phospholipids by the dialysis procedure previously described (35). In the work reported here, the protein-to-lipid ratio was 1.65 w/w. Extraction of halobacterial phospholipids used in the reconstitution procedures was performed as previously described (30).

**Rapid Scan Time-resolved FTIR Difference Spectroscopy**—A stock suspension of membrane (10 mg/ml) containing SRII or the SRII-HtrII complex in 25 mM Tris-HCl, 50 mM NaCl, pH 8.0, or in 25 mM MES, 50 mM NaCl, pH 6.0, was kept in the refrigerator prior to use. The protein films were prepared by depositing 5–10 μl of this suspension onto a polished 2-mm-thick, 25-mm-diameter BaF<sub>2</sub> window (Wilmad, Buena, NJ) and then placing the sample in a dry box for 1 h. The films were rehydrated via the vapor phase and then sealed in a temperature-controlled infrared cell (model TFC, Harrick Scientific Corp., Ossining, NY) using a second BaF<sub>2</sub> window. Alternatively, the samples were saturated with water by gently washing dry films with ~100 μl of the buffer solution and then removing the excess solution. The level of hydration was monitored by the absolute absorption IR spectra. The amount of water in samples with complete water saturation was at least 50% higher compared with the samples hydrated via the vapor phase as estimated from the increase of the absorption bands near 3400 and 1640 cm<sup>−1</sup> because of the bulk water. For H<sub>2</sub>O → D<sub>2</sub>O exchange, the dry protein films were rehydrated with 50 μl of D<sub>2</sub>O allowed to dry and then rehydrated in the infrared cell as described above with D<sub>2</sub>O. The spectra were recorded with a BRUKER IFS 66 v/s FTIR spectrometer (Bruker Optics) as described previously (36) at a 4 cm<sup>−1</sup> spectral resolution and 240 kHz scanner velocity corresponding to the data acquisition window of 18 ms. Four individual spectra collected within 85 ms after initiation of the photocycle were averaged to produce the final spectrum.

**RESULTS**

**The Transducer-free SRII Receptor**

Fig. 1 (top) shows the FTIR difference spectrum of SRII reconstituted in *Halobacterium salinarum* phospholipids at pH 8 and 298 K. The spectrum was recorded at a 4 cm<sup>−1</sup> resolution using rapid scan FTIR (37) and averaged over the first 85 ms of the photocycle. The recovery rate of the protein dark state was estimated by measuring the decay of intensity of the negative ethylenic C=CH stretching band of the chromophore at 1545 cm<sup>−1</sup> (Ref. 38; see Ref. 36 for details). A best fit single exponential curve was obtained using regression analysis, and the exponential time decay constant was determined as between 200 and 250 ms, somewhat faster than the slowest time constant obtained by flash photolysis experiments (340 ms) (12).
The spectrum shown in Fig. 1 is very similar to the late M intermediate spectra (101-ms half-time) of SRII recorded using step-scan FTIR at 37 °C and 8 cm⁻¹ resolution (32). In addition to the band at 1545 (−) cm⁻¹, negative bands corresponding to the C=C stretch of the chromophore in the dark state SRII appear at 1242, 1200, and 1163 cm⁻¹ (38). In contrast, a positive band at 1570 (+) corresponds to the C=C stretching mode of the M intermediate (38). An additional, small positive band appears near 1530 cm⁻¹ assigned to the C=C stretch mode of the O intermediate (32, 38). In general, the O and possibly N photointermediates have been found to be present in dynamic equilibrium with the M state (13) and therefore may not be easily separated from the M state by varying the pH or temperature.

In addition to vibrations of the retinylidene chromophore, several bands in the C=O carboxylic acid stretch region (1700–1800 cm⁻¹) and the amide I region (1600–1700 cm⁻¹) can be assigned to protein vibrations. For example, the positive band at 1764 cm⁻¹ arises from the protonation of the Schiff base counterion (Asp75) (33) in analogy with a similar band found near this frequency in almost all other microbial rhodopsins including BR (26) and NOP-1 (36). A previously unreported negative band also appears at 1724 cm⁻¹ that becomes more intense for spectra recorded at pH 6 (Fig. 1, bottom). Because no positive component is seen, this band most likely reflects the deprotonation of an as yet unidentified Asp/Glu residue that has a pKₐ at or below 7. A similar band is also observed in the low temperature spectrum of transducer-free SRII at 243 K (data not shown). Its assignment to an Asp/Glu group is also indicated by the disappearance of this band in D₂O (Fig. 1, inset) and its possible downshift to 1719 cm⁻¹. An additional positive/negative pair of bands at 1707/1701 cm⁻¹ has been assigned using site-directed mutagenesis to Asn105 in the K and M intermediates of SRII (33). Interestingly, the corresponding residue, Asp115, in BR also responds to photoisomerization as early as the K intermediate (39). As seen in Fig. 1 (inset), these bands are not appreciably affected by H₂O exchange. Bands also appear in the amide I region at 1686 (−), 1664 (+), 1633 (−), and 1618 (+) cm⁻¹ in agreement with earlier studies (29, 32, 33). A small shoulder at 1657 cm⁻¹ (Fig. 1, inset) most likely corresponds to the C=N stretch of the protonated Schiff base, which downshifts as expected to near 1633 cm⁻¹ in D₂O (32). The negative band at 1664 cm⁻¹ is of particular interest because earlier studies on BR using polarized FTIR associate a negative band at a slightly higher frequency (1669 cm⁻¹) to a small outward tilting (away from the membrane normal) of the α-helical structure (40).

The SRII-HtrII Transducer Complex

Time-resolved FTIR difference spectra were also recorded of the SRII-HtrII fusion complex (Fig. 2) under identical conditions as described above for the transducer-free SRII. Although the transducer-free and fusion complex exhibit similar difference spectra, several distinct highly reproducible alterations were found and are discussed below.

Chromophore—Negative peaks characteristic of the dark-adapted SRII at 1545, 1242, 1200, and 1163 cm⁻¹ (see above) and of the M intermediate at 1570 cm⁻¹ are still present in the fusion complex. However, the intensity of the 1545 (−) cm⁻¹ band is somewhat reduced compared with SRII. This phenomenon might be due to the formation of an N-like intermediate that exhibits a positive band with a slightly upshifted ethylenic stretch frequency that cancels the negative 1545 cm⁻¹ band in analogy to a similar effect in BR (41). In fact, such an upshifted band is evident in fusion samples studied under conditions of high humidity as described below (see Fig. 4). Alternatively, a positive band could arise from contributions of the amide II mode. Contributions of the O intermediate, which gives rise to a band at 1530 cm⁻¹, are slightly reduced at pH 8 in the fusion complex (Fig. 2, top) compared with the receptor alone and thus could not account for the reduced intensity of the 1545 (−) cm⁻¹ band.

Asp/Glu Carboxylic Acid Groups—The region above 1700 cm⁻¹ undergoes several distinct changes. At both pH 6 and 8, the band at 1724 cm⁻¹ is absent, indicating that this group is no longer undergoing a deprotonation in the fusion complex. One possibility is that this band arises from Asp193 in helix G, which corresponds to the proton release group, Glu204 in BR (see “Discussion”). In addition, a new negative band appears near 1715 cm⁻¹ in the fusion complex in H₂O, but not D₂O, indicating it originates from a carboxylic acid group. It is possible that this band is simply downshifted from the 1724 cm⁻¹ band observed in the transducer-free receptor. However, it appears to be present at both pH 8 and 6 (Fig. 2) and thus is likely to arise from a completely different group. It may reflect the deprotonation of an Asp/Glu group on the transducer such as Glu43 on TM1, which is hydrogen-bonded to Thr189 on helix F (9). In contrast to the 1724 cm⁻¹ peak, the positive band at 1764 cm⁻¹ caused by Asp75 appears at the same frequency and undergoes an identical 12 cm⁻¹ downshift in D₂O in both spectra (Fig. 3). Because the C=O stretching frequency and extent of its D₂O-induced shift are sensitive to the hydrogen bonding strength (42), the environment of Asp75 seems to be unchanged by the presence of transducer.

Asn/Glu C=O Groups—The positive/negative band at 1707/
1701 cm\(^{-1}\) assigned to Asn\(^{105}\) (33) is replaced by a more intense negative doublet at 1701/1688 cm\(^{-1}\) (Fig. 2, insets). Interactive subtraction of the two spectra reveals that the 1707/1701 cm\(^{-1}\) feature is still present but superimposed on an additional negative band located near 1694 cm\(^{-1}\) (Fig. 3, inset). Such a feature would be consistent with an additional Asn or Glu residue undergoing a structural change. One possibility is Asn\(^{74}\) on TM2 which hydrogen bonds with Tyr\(^{199}\) of the receptor (9). A second possibility is that this band, which falls in the amide I region, reflects alterations in secondary structure of the transducer. The 1701/1688 (\(-\) cm\(^{-1}\)) doublet is also present in the D\(_2\)O spectra (Fig. 3), although the intensity of the 1701 cm\(^{-1}\) band is reduced, possibly because of the downshift of the 1694 (\(-\) cm\(^{-1}\)) band observed in the H\(_2\)O double difference spectrum to near 1689 cm\(^{-1}\) upon the D\(_2\)O exchange. A frequency downshift caused by the H\(_2\)O → D\(_2\)O exchange is compatible with assignment of this band to either an Asn or amide I vibration (43, 44).

Tyrosine Groups—Alterations are observed in the 1250–1280 cm\(^{-1}\) region (Fig. 2, insets), which is typical for the vibration modes of tyrosine such as previously observed for the case of BR and assigned to Tyr\(^{185}\) (45, 46). One possibility is that these changes arise from Tyr\(^{199}\). Alternatively, vibration of a methionine residue has also been assigned in this region for the case of BR on the basis of selenomethionine substitution (47).

Amide I Bands—The pattern of amide I bands from 1600 to 1700 cm\(^{-1}\) is largely unchanged in the fusion complex compared with the transducer-free receptor with the exception of the negative band near 1664 cm\(^{-1}\), which appears to have a reduced intensity (Fig. 2, insets). This reduction is unlikely to arise from the strong absorption caused by water (near 1640 cm\(^{-1}\)) that is observed in the absolute absorption spectrum because the effect is still present in the case of D\(_2\)O (Fig. 3). This indicates that the secondary structural changes occurring in the receptor may be suppressed in the fusion complex. A possible explanation is that the interaction of TM2 with F helix restrains its movement.

Fig. 3 also shows that the overall pattern of band shifts caused by D\(_2\)O exchange in the amide I region is similar for the receptor and complex. There is a decreased intensity near 1657 (\(-\) cm\(^{-1}\)) and an increase of the 1633 (\(-\) cm\(^{-1}\)) band, which is likely to reflect the expected downshift of the C=N Schiff base stretching vibration assigned at 1650 cm\(^{-1}\) and 1627 cm\(^{-1}\) in H\(_2\)O and D\(_2\)O, respectively (38). Additional changes near 1618 (\(+\) cm\(^{-1}\)) and 1600 (\(-\) cm\(^{-1}\)) may reflect the D\(_2\)O induced downshift of a tyrosine ring mode near 1620 cm\(^{-1}\) as previously observed in bacteriorhodopsin (48).

Comparison of Late Photocycles of Receptor and Fusion Complex

Fig. 4 (upper panel) compares the time-resolved spectra from SRII and the SRII-HtrII fusion complex recorded under conditions of complete water saturation (see “Experimental Procedures”) during the late photocycle (20–300 ms). Several of the features discussed above that distinguish the fusion complex
from the receptor are evident in this time series including: (i) the increased negative intensity near 1694 cm\(^{-1}\) (Asn/Gln or amide I); (ii) reduction in negative intensity near 1664 cm\(^{-1}\) (amide I); and (iii) reduction in intensity of negative band at 1545 cm\(^{-1}\) (ethylenic stretch of chromophore) along with the appearance of a shoulder at 1555 cm\(^{-1}\), which might reflect the presence of the N intermediate.

These spectra also reveal that unlike SRII alone, which exhibits a gradual increase in the level of the O intermediate, the fusion complex appears to remain almost exclusively in an M-like state throughout its decay back to the dark state. In particular, the receptor difference spectra display a shift from 1764 to 1757 cm\(^{-1}\) and an increase in intensity at 1530 cm\(^{-1}\) and at 1190 and 998 cm\(^{-1}\) (not shown), which have all previously been associated with formation of the O intermediate in BR. In contrast, none of these features are observed in the fusion complex. Instead, all of the spectral time slices when scaled are very similar. In addition, very little O intermediate is present as indicated by the weak intensity at 1530 cm\(^{-1}\) as well as other spectral features discussed above. This could occur for example if the decay of the M intermediate is prolonged relative to the O intermediate for the fusion complex.

Similar measurements performed in D\(_2\)O (Fig. 4, lower panel) result in a higher level of O intermediate, which gradually appears during the late photocycle for both SRII and the SRII-HtrII complex. This alteration is not surprising because D\(_2\)O is known to alter the kinetics of SRII (13). However, most of the features that distinguish the receptor and the fusion complex are still present, including the increase intensity in the region near 1690 cm\(^{-1}\), reduction in the negative band at 1664 cm\(^{-1}\), and the appearance of a pronounced shoulder near 1555 cm\(^{-1}\). The lower intensity of the 1664 cm\(^{-1}\) band in the fusion complex is particularly significant because it indicates that this effect observed in H\(_2\)O is not an artifact caused by the high absorption of water near 1640 cm\(^{-1}\), since this band downshifts to near 1220 cm\(^{-1}\) in D\(_2\)O.

**Discussion**

FTIR difference spectroscopy was used for the first time in this work to explore the molecular interactions and conformational changes that occur during the photocycle between a receptor (SRII) and its cognate transducer, HtrII. Complementing earlier FTIR studies that investigated the conformational changes of the isolated receptor (29, 33), the SRII-HtrII fusion complex allows changes to be detected that are associated with the activation of the transducer. Although the fusion complex did not include the entire cytoplasmic domain of HtrII, earlier studies have shown that the interaction between receptor and transducer is localized to their membrane and membrane-proximal regions (25, 49). In addition, the light-driven proton transport by SRII is abolished in the presence of a similar truncated HtrII (17, 18), indicating that the transmembrane domain of the transducer interacts with the receptor in a manner similar to that of the intact transducer. Furthermore, the similarly truncated HsHtrI confers normal photochemical reactions to its cognate receptor HsSRI, which is greatly perturbed in the absence of the transducer interactions (50).

Recent x-ray crystallographic studies show that the receptor resting state remains largely unperturbed by the HtrII interaction (9). Thus, conformational changes we detect in the FTIR difference spectrum of the fusion complex should reflect changes from the resting (dark) state of the receptor alone plus any changes occurring in the transducer. An important conclusion of the current study is that the net structural changes involving the \(\alpha\)-helical transmembrane structure do not increase in the fusion complex. In fact, bands in the region from 1655 to 1665 cm\(^{-1}\) typical of the amide I mode in \(\alpha\)-helices appear to undergo a reduction in intensity in the fusion complex compared with the receptor alone. The only change observed in the amide I region that is new in the fusion complex (and might be attributable to the transducer) occurs above 1686 cm\(^{-1}\), which is more typical of \(\beta\)-turns or \(\beta\)-sheet structure.

These results place tight constraints on the possible models of secondary structural changes that occur in the receptor and transducer complex. For example, an outward tilting of the F helix in SRII, which is expected on the basis of structural studies in BR (61), is likely to cause alterations in the amide I region. In fact, as discussed previously (22), a prominent negative band at 1669 cm\(^{-1}\) in the BR to N FTIR difference spectrum is consistent with the reorientation of the F helix. Assuming that the negative 1664 cm\(^{-1}\) band in SRII originates also from this F helix tilting motion, its reduction in intensity might be interpreted as a suppression of this motion (Fig. 5). This might occur, for example, because of the tight packing between the F helix and TM2. The results also effectively rule out large changes in the orientation of the transducer helices TM1 and TM2 because these motions would also be expected to contribute to intensity in the \(\alpha\)-helix region. On the other hand, these results are consistent with a rotation or piston motion of TM2, which has been proposed (9) in analogy with a similar motion deduced for the aspartate chemotaxis receptor (53) and also consistent with EPR measurements with SRII and HtrII (25). We cannot exclude a motion that involves outward tilting of the F helix away from the membrane normal and inward tilting of a combination of TM1 and TM2 toward the membrane normal, which results in a partial cancellation of positive and negative bands in the \(\alpha\)-helix amide I region.

Our results also reflect local structural changes involving individual groups in the receptor and/or transducer complex. We cannot at this stage unambiguously assign bands to individual groups without further studies utilizing site-directed mutagenesis and/or isotope labeling. However, several conclusions can be drawn.

(i) The pattern of protonation changes involving the Asp/Glu group is different in the isolated receptor and fusion complex. This is indicated by alterations in the carboxylic acid C=O region. Although the band at 1764 cm\(^{-1}\) reflecting the proto-
nation of the Schiff base counterion, Asp$^{75}$, remains the same, changes are observed at 1724 and 1715 cm$^{-1}$. The negative 1724 cm$^{-1}$ band, which appears only in the transducer-free receptor at pH 6, may reflect the deprotonation of Asp$^{93}$. This residue corresponds to Glu$^{204}$ in BR, which is located on the F helix near the extracellular surface and functions as part of the extracellular proton release mechanism (54). In the SRII reconstituted in liposomes, Asp$^{93}$ has a pK$_a$ near pH 7 (55), significantly lower than the pK$_a$ estimated for Glu$^{204}$ in BR (pK$_a$ = 10) (56). One likely explanation for this shift in pK$_a$ is that Arg$^{27}$ is closer to Asp$^{93}$ relative to BR where the analogous residue (Arg$^{95}$) is located closer to the Schiff base and two counterions Asp$^9$ and Asp$^{12}$ (57). Hence, only at pH below 7 would we expect to observe a deprotonation signal arising from Asp$^{93}$, which is associated with the proton pumping form of SRII. In the fusion complex, this signal is not observed at pH 6 and 8, indicating that the environment of Asp$^{93}$ is altered. An intriguing possibility is that the movement of Arg$^{27}$ toward the extracellular side caused by Schiff base counterion protonation and its ultimate role in proton ejection, as predicted originally by an early spectroscopic model of BR (39), also retains some role in signal transduction via Arg$^{27}$ and Asp$^{93}$. Additional information should be available once these bands are assigned using site-directed mutagenesis.

In contrast to the band at 1724 cm$^{-1}$, which is present only in the transducer-free SRII (at low pH), the 1715 cm$^{-1}$ band is present only in the fusion complex at both pH 6 and 8. Hence, it might reflect deprotonation of an Asp/Glu residue located on the transducer. One candidate is Glu$^{43}$ located on TM1 near the extracellular side of the membrane that hydrogen bonds with Thr$^{189}$ on helix G in the receptor. A disruption of this interaction might occur as a consequence of rearrangements in the packing of helix F and G on the receptor and TM1 and TM2 of the transducer. Alternatively, the bands at 1724 and/or 1715 cm$^{-1}$ might reflect carbohydrate groups located on the F or G helix near the cytoplasmic side of the membrane. The crystal structure of the receptor-transducer complex shows that the transducer TM2 is nestled in the groove formed by the receptor helices F and G (9). Although the crystal model does not resolve residues on the cytoplasmic end of helix F, this region is predicted to likely interact functionally with the TM2 of HtrII (23). Alterations of such an interaction are in a good position to support a signal relay mechanism in which photoactivation of SRII causes TM2 rotation (25). A potential residue in this region is Asp$^{214}$ located on helix G outside of the membrane.

(ii) Bands are detected that may reflect disruption of the Tyr$^{199}$-Asn$^{74}$ receptor/transducer interaction. As discussed under “Results,” these bands are detected in the receptor-transducer complex but not the receptor alone, which can be assigned to changes in the hydrogen bonding of an Asn/Gln residue. In addition, the bands associated with a tyrosine residue in the receptor are altered in the receptor-transducer complex. A potential candidate to account for the altered tyrosine signal is Tyr$^{199}$ on helix G. This residue was first predicted to interact with the transducer because without such an interaction the phenolic moiety would point into the hydrophobic interior of the membrane (21). In the structure of the receptor-transducer complex, Tyr$^{199}$ was found to hydrogen bond to Asn$^{74}$ on TM2 (9). Furthermore, the predicted rotation of TM2 might be expected to disrupt this interaction, thereby giving rise to both Tyr and Asn signals in the difference spectrum of the complex.

In summary, initial FTIR analysis of the SRII-HtrII fusion complex reveals a set of altered bands that reflect the receptor-transducer interaction as well as placing tight constraints on the type of structural changes that the receptor and transducer each undergo in the complex. Further probes should be possible by combining FTIR with methods of band assignment including isotope labeling and site-directed mutagenesis. In addition, it should be possible to apply specialized FTIR techniques including polarization (40, 58) and attenuated total reflection (52) to probe changes in orientation of specific groups under well defined aqueous environment.
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