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Helical and Reverse Turn Changes in the BR  $\rightarrow$  N Transition of Bacteriorhodopsin<sup>†</sup>Tzvetana Lazarova<sup>‡</sup> and Esteve Padrós\*

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**ABSTRACT:** Fourier transform infrared deconvoluted spectra of bacteriorhodopsin and the N intermediate were compared with the N/BR infrared difference spectrum. In the amide I, clear changes in the bands at 1666 cm<sup>-1</sup>, assigned to  $\alpha_{II}$  helices, 1659 cm<sup>-1</sup>, assigned to  $\alpha_I$  and  $\alpha_{II}$  helices, and 1652 cm<sup>-1</sup>, assigned to both  $\alpha_I$  helices and unordered structures, were found. These changes could arise from conversion of some  $\alpha_{II}$  into  $\alpha_I$  helices. Variations in the bands at 1692 and 1683 cm<sup>-1</sup>, corresponding to reverse turns, were also detected. The side chains of Tyr (band at 1517 cm<sup>-1</sup>) and Phe (band at 1498 cm<sup>-1</sup>) were found to change in going from BR to N. In the carboxylate region, no band was detected at 1737 cm<sup>-1</sup> in the deconvoluted spectra that could correspond to the peak observed in the difference spectrum. It is argued that resolution-enhancement methods used along with difference spectra provide more detailed insights into the conformational changes occurring between photocycle intermediates.

Bacteriorhodopsin (BR)<sup>1</sup> is a photoactive protein of the purple membrane of *Halobacterium salinarum*. It contains the chromophore retinal, covalently linked to Lys216 via a protonated Schiff base [for a recent review, see Lanyi (1993)]. The absorption of light by retinal initiates the photocycle of BR, which contains several spectroscopically distinct species. The simplest photocycle scheme contains five intermediates: BR- K- L- M- N- O -BR.<sup>2</sup> They have been identified by their absorption maxima and kinetic parameters (Lozier *et al.*, 1975; Birge, 1990; Lanyi, 1992). It is generally accepted that during M formation the Schiff base becomes deprotonated and a proton is released into the extracellular medium (Lanyi, 1992; Oesterhelt *et al.*, 1992). The decay of M is accompanied by the reprotonation of the Schiff base and the uptake of a proton from the aqueous phase. The key role of Asp85 as a proton acceptor during M formation and of Asp96 as a proton donor during N formation has been shown by site-directed mutagenesis and FTIR difference spectroscopy [for a recent review, see Rothschild (1992) and references cited therein].

During the BR photocycle, structural changes in both retinal and the protein occur. It seems that great protein changes appear in the N intermediate. Evidence for significant changes in the secondary protein structure during the BR to N transition was reported using different approaches:

protein modification (Rothschild *et al.*, 1993), site-directed isotope labeling (Ludlam *et al.*, 1995), or conditions which favor the N intermediate (Pfefferlé *et al.*, 1991; Ormos *et al.*, 1992). Exchange of the 1660 and 1670 cm<sup>-1</sup> intensity peaks in the FTIR difference spectra as well as an increase of peak intensities at 1692(-), 1670(-), and 1650(+) cm<sup>-1</sup> has been found to start in the M intermediate and continue during the M/N transition (Braiman *et al.*, 1991). On the other hand, on the basis of a significant reduction in intensity of the peaks at 1558(+), 1650(+), and 1670(-) cm<sup>-1</sup> during O formation, a reversal of much of the protein change that occurs up to N formation was proposed (Bousché *et al.*, 1992).

In the present study, we utilize resolution-enhanced FTIR spectroscopy to identify the nature of protein structural changes involved in the BR/N transition. We show that this approach, used in parallel with infrared difference spectroscopy, provides a more detailed insight on the conformational changes appearing in the N intermediate. A similar approach on the rhodopsin/metarhodopsin II transition has been published recently (Garcia-Quintana *et al.*, 1995).

## MATERIALS AND METHODS

**Sample Preparation.** Purple membrane was isolated from *H. salinarum* strain S9 according to literature protocols (Oesterhelt & Stoekenius, 1974). For IR experiments, films of PM suspended in 10 mM carbonate–bicarbonate buffer, 3 M KCl, pH 10.3, were prepared following the method described by Pfefferlé *et al.* (1991). After centrifuging twice, the small amount of pellet was placed on a CaF<sub>2</sub> window, and was dried overnight under vacuum. The resulting PM film was then rehydrated prior to insertion into a sealed transmittance homemade cell. The water content of the samples was checked following Braiman *et al.* (1987).

**Acquisition of Spectra.** Before FTIR measurements, the PM film was light-adapted by illumination of the sample with light from a 250 W tungsten lamp passing through a 15 cm path length water heat filter and a 530 nm long-pass filter for 5 min at 275 K. The N intermediate was accumulated by illumination of the PM film as above at 275

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<sup>1</sup> Abbreviations: BR, bacteriorhodopsin; PM, purple membrane; N, N intermediate of the photocycle of bacteriorhodopsin; M, M intermediate of the photocycle of bacteriorhodopsin; IR, infrared; FTIR, Fourier transform infrared; FWHH, full width at half-height.

<sup>2</sup> Depending upon the pH, some of the intermediates can contain subspecies. Moreover, equilibrium between two different intermediates or subspecies has been described (Lanyi, 1993).

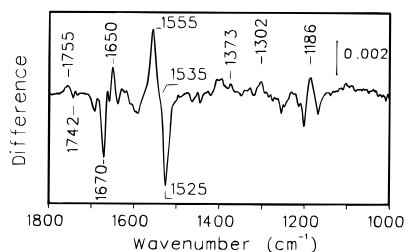


FIGURE 1: Steady-state FTIR difference spectrum of N minus BR at pH 10.3 in the 1800–1000  $\text{cm}^{-1}$  region. The BR and N spectra were recorded at 275 K with 2  $\text{cm}^{-1}$  resolution. 2560 scans were accumulated for each 1 of 3 independent samples, and were averaged together to give the displayed spectrum. The N intermediate was obtained by irradiation with  $>530$  nm light for 1 min. The vertical solid line represents the scale bar in absorbance units.

K for 1 min. To obtain the spectra of BR and N, first the spectrum of the BR ground state was collected, and then the sample was illuminated to accumulate the N intermediate, and the resulting spectrum was collected again. In one cycle, 320 interferograms were co-added and Fourier-transformed. This procedure was repeated, and at least 8 cycles were averaged (i.e., a total of at least 2560 interferograms per spectrum were accumulated). Three spectra corresponding to independent samples were obtained, and an N/BR difference spectrum was calculated by subtracting N minus BR for each sample.

FTIR spectra were collected using a Mattson Polaris spectrometer equipped with an MCT detector, at 2  $\text{cm}^{-1}$  resolution. The temperature was controlled and maintained at 275 K using a homemade cell holder. Absorption spectra were Fourier self-deconvoluted using the Kauppinen algorithm (Kauppinen *et al.*, 1981) of the program GRAMS (Galactic Industries Inc.). Apart from the bandshape, which is usually set to Lorentzian, the deconvolution algorithm needs the input of the band half-width (FWHH) and the band-narrowing factor  $k$  (Moffatt & Mantsch, 1992). Deconvolution in the amide region was done using an FWHH of 14  $\text{cm}^{-1}$  and a  $k$  factor of 2.5 (Cladera *et al.*, 1992). In the 1700–1800  $\text{cm}^{-1}$  region, an FWHH of 8  $\text{cm}^{-1}$  and a  $k$  factor of 1.5 were used. These lower values of FWHH and  $k$  led to less-resolved spectra, but were found to be sufficient to reveal differences between BR and N. They were required by the low absorbances and hence low signal-to-noise ratios, and also by the low bandwidths in this region. If higher values of deconvolution were used, periodic noise started to appear.

## RESULTS

**FTIR Difference Spectrum of the N Intermediate.** The N/BR difference spectrum shown in Figure 1 displays all characteristic peaks of “pure” N intermediate. They are in very close agreement with those reported before (Pfefferlé *et al.*, 1991; Ormos *et al.*, 1992; Sasaki *et al.*, 1992; Hessling *et al.*, 1993; Ludlam *et al.*, 1995). In the region above 1700  $\text{cm}^{-1}$ , the spectrum shows a broad well-defined positive peak at 1755  $\text{cm}^{-1}$ , a smaller one at 1737  $\text{cm}^{-1}$ , and a negative one at 1742  $\text{cm}^{-1}$ . It has been shown that the peak centered at 1755  $\text{cm}^{-1}$  results from a shift of the 1762  $\text{cm}^{-1}$  peak assigned to protonated Asp85 in the M intermediate, due to environmental changes (Braiman *et al.*, 1991; Pfefferlé *et al.*, 1991; Sasaki *et al.*, 1992). The positive peaks at 1555, 1373, 1302, and 1186  $\text{cm}^{-1}$ , attributed to the ethylenic stretching mode of the chromophore, have the same frequen-

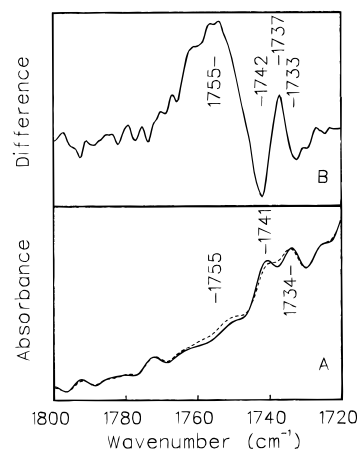


FIGURE 2: Deconvoluted spectra of BR (—) and N (---) (panel A) and the difference spectrum of N minus BR (panel B) in the 1800–1720  $\text{cm}^{-1}$  region. The deconvolution parameters used were FWHH = 8  $\text{cm}^{-1}$  and  $k = 1.5$ .

cies in our N/BR difference spectrum as in Pfefferlé *et al.* (1991) or in Sasaki *et al.* (1992), and they are at very similar frequencies compared with the Raman spectrum of the N intermediate (1548, 1376, 1302, and 1186  $\text{cm}^{-1}$ ; Fodor *et al.*, 1988).

**1800–1700 Spectral Region of C=O Stretching Vibrations of COOH Groups.** Figure 2 shows an expanded view of the 1800–1720  $\text{cm}^{-1}$  spectral region of the N/BR difference spectrum (panel B), in comparison with BR and N deconvoluted spectra (panel A). Although in this region some of the bands can have a contribution from incompletely compensated water vapor bands, it is apparent that the maxima and minima in the difference spectrum have a correspondence with changes in the deconvoluted spectra. Thus, the broad positive peak at 1755  $\text{cm}^{-1}$  in the N/BR difference spectrum, assigned to protonated Asp85, corresponds to an increase in intensity around 1755  $\text{cm}^{-1}$  in the N deconvoluted spectrum. Likewise, the negative peak at 1742  $\text{cm}^{-1}$ , assigned to the deprotonation of Asp96 during the M–N transition (Braiman *et al.*, 1991; Maeda *et al.*, 1992), corresponds to a decrease and shifting of the 1741  $\text{cm}^{-1}$  band of BR. The band at 1734  $\text{cm}^{-1}$  in BR slightly shifts to 1735  $\text{cm}^{-1}$  in the N deconvoluted spectrum, which corresponds to the appearance of the negative peak around 1733  $\text{cm}^{-1}$  in the difference spectrum. On the other hand, the positive peak at 1737  $\text{cm}^{-1}$  seen in the difference spectrum does not seem to correspond to any real band in the deconvoluted spectra. It most likely arises from the simultaneous changes of the 1742 and 1734  $\text{cm}^{-1}$  bands of BR.

**Amide I Spectral Changes.** Negative/positive peaks at 1692  $\text{cm}^{-1}$  and 1686  $\text{cm}^{-1}$  found in the N/BR difference spectrum correspond to slight changes between the BR and N absorption spectra, which are incremented by deconvolution (Figure 3). It is apparent that the band at 1692  $\text{cm}^{-1}$  is less intense and hardly resolved in N (Figure 3A). The band at 1683  $\text{cm}^{-1}$  in the BR deconvoluted spectrum becomes slightly more broad in N. As these bands have been assigned to reverse turns in BR (Cladera *et al.*, 1992), it seems reasonable to conclude that there are small but measurable conformational changes in some reverse turns in going from BR to N.

A particular feature of the N minus BR difference spectrum is the intense negative peak at 1670  $\text{cm}^{-1}$ . Comparison of BR and N deconvoluted spectra reveals the bands at 1675

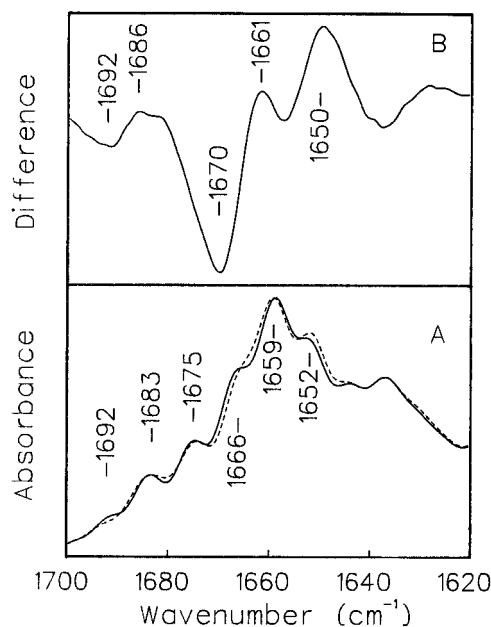


FIGURE 3: Deconvoluted spectra of BR (—) and N (---) (panel A) and the difference spectrum N minus BR (panel B) in the 1700–1620  $\text{cm}^{-1}$  region. The deconvolution parameters used were FWHH = 14  $\text{cm}^{-1}$  and  $k = 2.5$ .

and 1666  $\text{cm}^{-1}$  resolved in this region. The 1675  $\text{cm}^{-1}$  band does not change appreciably upon N formation, while the band at 1666  $\text{cm}^{-1}$  in the BR deconvoluted spectrum undergoes a slight downshift and also probably broadening, giving rise to a not well-resolved band in N. Therefore, the 1670  $\text{cm}^{-1}$  negative peak in the N/BR difference spectrum actually arises from changes in the band centered at 1666  $\text{cm}^{-1}$ , assigned to the  $\alpha_{\text{II}}$  helical structure of BR (Krimm & Dwivedi, 1982; Earnest *et al.*, 1990; Cladera *et al.*, 1992; Torres *et al.*, 1995). On the other hand, the band at 1659  $\text{cm}^{-1}$  in BR undergoes a small, but reproducible upshift to 1660  $\text{cm}^{-1}$  in the N deconvoluted spectrum (three independent samples), which corresponds to the positive peak at 1661  $\text{cm}^{-1}$  in the N/BR difference spectrum. Likewise, the intensity increase and widening of the 1652  $\text{cm}^{-1}$  band in the N deconvoluted spectrum correspond to the appearance of the positive peak around 1650  $\text{cm}^{-1}$  in the N/BR difference spectrum.

**Amide II Spectral Changes.** Figure 4B displays the difference and Figure 4A the deconvoluted spectra in the amide II region. The intense positive peak at 1555  $\text{cm}^{-1}$  in the difference spectrum corresponds mainly to changes in the 1558  $\text{cm}^{-1}$  band, which is seen as a shoulder in the BR deconvoluted spectrum and gains in intensity, but shows a less clear shoulder in the N spectrum. The main band at 1548  $\text{cm}^{-1}$  and the shoulder at 1541  $\text{cm}^{-1}$  do not change significantly during N formation. Thus, changes in the band at 1558  $\text{cm}^{-1}$ , attributed to the protein moiety (Pfefferlé *et al.*, 1991), have the main contribution in the appearance of the positive peak at 1555  $\text{cm}^{-1}$  in the N/BR difference spectrum. A shoulder at 1535  $\text{cm}^{-1}$  found in N/BR difference spectra most probably originates from an intensity increase of the band centered at 1534  $\text{cm}^{-1}$  in the deconvoluted spectrum, which corresponds to the stretching mode of C=C in the N chromophore (Fodor *et al.*, 1988). Likewise, the changes in the band at 1527  $\text{cm}^{-1}$ , which is slightly shifted and less intense in the N deconvoluted spectrum, correspond to the intense negative peak at 1525  $\text{cm}^{-1}$  in the N/BR difference spectrum. This peak has been

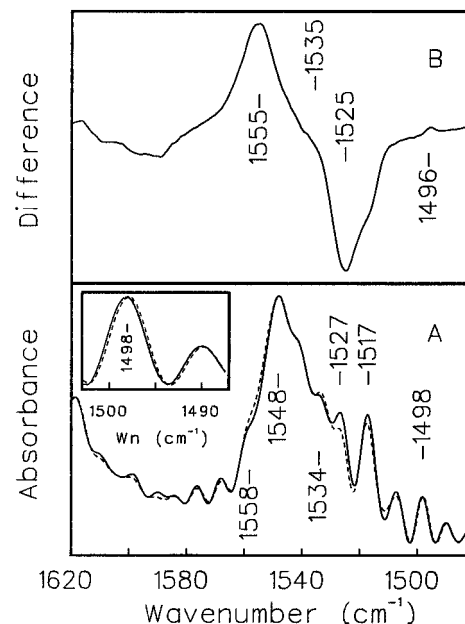


FIGURE 4: Deconvoluted spectra of BR (—) and N (---) (panel A) and the difference spectrum N minus BR (panel B) in the 1620–1480  $\text{cm}^{-1}$  range. The deconvolution parameters used were the same as in Figure 3. The inset shows the zone around the 1498  $\text{cm}^{-1}$  band expanded, to show the changes more clearly.

assigned to the C=C ethylenic stretching mode of retinal in BR, and it has been shown to be sensitive to C<sub>15</sub> deuteration (Smith *et al.*, 1987; Pfefferlé *et al.*, 1991).

The well-resolved band at 1517  $\text{cm}^{-1}$  in the deconvoluted spectra, which is attributed to Tyr side chains (Roepe *et al.*, 1987; Earnest *et al.*, 1987), slightly decreases in intensity in the N deconvoluted spectrum, giving rise to a shoulder at about 1517  $\text{cm}^{-1}$  in the difference spectrum. The small but reproducible positive peak at 1496  $\text{cm}^{-1}$  in the difference spectrum, which is seen in all published N difference spectra, corresponds to a slight downshift of the band at 1498  $\text{cm}^{-1}$ . Note that in this case, the band does not change in intensity or bandwidth (see the insert to Figure 4A), but only a small shift is detected, sufficient to produce a feature in the difference spectrum at just the point of maximum slope. This band has been reported to be due to Phe side chains (Earnest *et al.*, 1987; Venyaminov *et al.*, 1990). Finally, the small band at 1590  $\text{cm}^{-1}$  in BR virtually disappears in N, and those at 1599 and 1611  $\text{cm}^{-1}$  decrease significantly, a fact that is seen in the difference spectrum as several minima appearing in this region. Some of these bands could correspond to the COO<sup>-</sup> group of Asp or Glu side chains (Chirgadze *et al.*, 1975).

## DISCUSSION

**Analysis of the N Intermediate by FTIR Deconvoluted Spectra.** In this work, we investigate the changes occurring in BR due to the formation of the N intermediate using Fourier self-deconvolution methods in the infrared region. It is well-known that FTIR difference spectroscopy, alone or in combination with isotope labeling or site-directed mutagenesis, has provided a vast amount of information about the changes that occur in the BR photocycle (Rothchild, 1992). However, difference spectra are in general difficult to interpret because the observed features can be due to several types of alterations in the absorption bands. For example, the increase in intensity, broadening, or shifting or a change in the shape of the absorbing band will produce

features in the difference spectrum. Moreover, simultaneous changes in two contiguous bands can produce an apparent peak in the difference spectrum. On the other hand, it is known that the use of deconvolution methods can introduce errors in the bands if the intrinsic bandwidth is different from that used in the deconvolution (Mantsch *et al.*, 1988). Nevertheless, this is of no relevance in the present study, since only relative changes between two similar states are quantified. Thus, all changes observed must be due to real changes in the absorbing bands.

Another aspect to be taken into account concerns the increase in the intensity of the "bands" due to noise or to water vapor, brought about by the deconvolution process. This also is of little concern in our case, because a high signal-to-noise ratio has been obtained by accumulating a minimum of 2500 interferograms for each sample. Concerning the water vapor bands, only in the 1700–1800  $\text{cm}^{-1}$  region can these bands have a comparable intensity to those of the protein. On the other hand, clear evidence for pure N intermediate is the presence of the positive peak at 1186  $\text{cm}^{-1}$ , which is not seen in the M intermediate (Ormos *et al.*, 1992; Rothschild *et al.*, 1993). It has been assigned to a C–C stretching mode of the chromophore, and represents the appearance of the 13-*cis*-retinal configuration with a protonated SB (Fodor *et al.*, 1988). Thus, we can deduce that we accumulated mainly the N intermediate under our conditions. As we show in this work, analysis of BR and N deconvoluted spectra provides additional information about alterations in the protein secondary structure and allows us to get a more detailed picture of these changes.

**The Carboxylate Spectral Region.** Above 1700  $\text{cm}^{-1}$ , and notwithstanding the low absorption intensity of IR bands in this region, the signal-to-noise ratio of the spectra was sufficient to obtain reproducible changes in the deconvoluted spectra of several independent samples. Comparison of the deconvoluted spectra shows clearly that the bands at 1741 and 1734  $\text{cm}^{-1}$  undergo changes upon N formation, giving rise to the peaks at 1742, 1737, and 1733  $\text{cm}^{-1}$  in the difference spectrum. Although on the basis of our data alone it is not possible to assign the bands to individual carboxylic acid residues, it is wise to take into account that the positive peak at 1737  $\text{cm}^{-1}$  in the difference spectrum could arise from changes in the contiguous bands at 1741 and 1734  $\text{cm}^{-1}$ , or from changes only in the 1734  $\text{cm}^{-1}$  band. If this were the case, no real absorption band would correspond to the peak at 1737  $\text{cm}^{-1}$ . Our interpretation agrees with the recent assignment of both 1737 and 1733  $\text{cm}^{-1}$  peaks to changes in the Asp115 environment (Sasaki *et al.*, 1994).

**Structural Changes in the Amide I.** Our data give evidence for significant changes of  $\alpha$  helical structure during the BR to N transition. We established that the peaks at 1670/1661  $\text{cm}^{-1}$  in the N/BR difference spectrum arise from frequency and intensity changes of the bands resolved at 1666 and 1659  $\text{cm}^{-1}$  in the deconvoluted spectra. These bands have been attributed to two different  $\alpha$  helical conformations,  $\alpha_I$  and  $\alpha_{II}$  (Krimm & Dwivedi, 1982; Earnest *et al.*, 1990; Cladera *et al.*, 1992; Reisdorf & Krimm, 1995). Although the existence of the  $\alpha_{II}$  component is still under debate, recently published data give support to the proposal for at least two different  $\alpha$  helical components in BR (Torres & Padrós, 1995; Torres *et al.*, 1995). Our results clearly show changes of these two bands upon N formation, which can be due to changes in the orientation of some transmembrane  $\alpha$  helices like those described for the M intermediate (Subramaniam

*et al.*, 1993), or to a true conformational helical change (Reisdorf & Krimm, 1995).

Likewise, the peak at 1650  $\text{cm}^{-1}$  in the difference spectrum arises from changes in the band at 1652  $\text{cm}^{-1}$  in the deconvoluted spectrum. This band has been assigned to both  $\alpha_I$  helix and nonordered structures in nearly equal amounts (Cladera *et al.*, 1992). We cannot decide at this moment which of these structures is undergoing a conformational change, although it is possible that both change. Nonordered structures, located in the extramembranous space, could undergo changes in a similar fashion to those envisaged below for reverse turns located in the loops.

Considering the changes observed in the 1666 and 1652  $\text{cm}^{-1}$  bands, a possibility for a conformational change would be the conversion of the  $\alpha_{II}$  helix (1666  $\text{cm}^{-1}$  band) in BR to  $\alpha_I$  (1652  $\text{cm}^{-1}$  band) in N. Torres *et al.* (1995) have recently described that BR solubilized in SDS presents a unique  $\alpha$ -helical band at 1655  $\text{cm}^{-1}$ , with no evidence for  $\alpha_{II}$  helix at 1666  $\text{cm}^{-1}$ . This 1655  $\text{cm}^{-1}$  band in SDS was interpreted as the result of loss of helix–helix interactions. In the bleached membrane, where the helical bundle appears somewhat relaxed, the 1666  $\text{cm}^{-1}$  band is also slightly less intense than in BR (Cladera *et al.*, 1996). Thus, the decrease of  $\alpha_{II}$  in N could reflect the postulated opening of the protein conformation at the cytoplasmic channel (Lanyi, 1993), resulting in a decreased helix–helix interaction.

The peaks at 1692 and 1686  $\text{cm}^{-1}$  in the N/BR difference spectrum originate from small changes in the bands resolved at 1692 and 1683  $\text{cm}^{-1}$  in the deconvoluted spectra, which have been assigned to reverse turns in BR (Cladera *et al.*, 1992). Thus, the changes occurring in the helices are propagated to the extramembranous segments of BR, where reverse turns are most likely located. This conformational change in the loops can be a reminiscent behavior of the mode of activation of the G-protein coupled receptors, where a conformational change in the cytoplasmic loops upon activation is postulated to account for the G-protein activation (Strader *et al.*, 1994).

**Structural Changes in the Amide II.** In the amide II region, we found that the peak at 1555  $\text{cm}^{-1}$  is mainly due to changes in protein structure. This peak is one of the characteristic features of the N intermediate, and it is not found in K, L, or M intermediates (Braiman *et al.*, 1991; Ormos *et al.*, 1992). The peak at 1555  $\text{cm}^{-1}$  has been assigned to the protein N–H stretching mode or the ethylenic (C=C) stretching mode of the chromophore (Pfefferlé *et al.*, 1991). Experiments with  $\text{C}_{15}$ -deuterated BR and N-deuterated BR showed that the peak at 1555  $\text{cm}^{-1}$  is composed of two peaks at 1558 and 1544  $\text{cm}^{-1}$ . The peak at 1558  $\text{cm}^{-1}$  has been assigned to the protein, while that at 1544  $\text{cm}^{-1}$  to the C=C stretching mode of the chromophore (Pfefferlé *et al.*, 1991). The peak at 1548  $\text{cm}^{-1}$  was seen in the resonance Raman spectra of the N intermediate (Fodor *et al.*, 1988). Our data demonstrate that from the three bands resolved in this frequency region, only the band at 1558  $\text{cm}^{-1}$  changes during N formation. Thus, on the basis of these data, we can confirm that the peak at 1555  $\text{cm}^{-1}$  in the N/BR difference spectrum is mainly due to protein changes, which could have the same origins as the changes seen in the  $\alpha$  helical bands

<sup>3</sup> If the 1527  $\text{cm}^{-1}$  band can be assigned solely to the chromophore, the area decrease gives an approximate percentage of the N intermediate obtained under our conditions, of about 31% as a mean of three experiments.

in the amide I region. This explanation is also supported by H/D exchange data (Rothschild *et al.*, 1993).

Apart from bands corresponding to secondary structures, in the 1500–1600  $\text{cm}^{-1}$  region there are other bands in the deconvoluted spectra that show changes in the transition from BR to N. The most prominent change corresponds to the band at 1527  $\text{cm}^{-1}$ , assigned to the *all-trans* chromophore in BR (Smith *et al.*, 1987). This band decreases in intensity upon N formation, giving rise to the negative peak at 1525  $\text{cm}^{-1}$  in the N/BR difference spectrum.

As indicated under Results, changes in the band at 1517  $\text{cm}^{-1}$  provide evidence of ionization of some Tyr residues or of changes in the environment of Tyr residues between BR and N. From FTIR data alone, it is not possible to determine the nature of these changes. However, they indicate that the secondary/tertiary structural changes detected in the amide I and II regions are also found at the level of one or more Tyr residues. Similarly, the small peak at 1496  $\text{cm}^{-1}$  is due to changes in the environment of Phe side chains (Earnest *et al.*, 1987; Venyaminov *et al.*, 1990). Taking into account previous reports that showed changes in Trp side chains in M (Sabés *et al.*, 1984; Roepe *et al.*, 1988), which probably also persist in N, we can conclude that one or more side chains of each of Phe, Tyr, and Trp suffer changes in environment upon N formation.

**Concluding Remarks.** As a whole, our work confirms the existence of conformational changes in the BR/N transition, which not only involve the helices, but also some reverse turns and aromatic amino acids. Taking into account that only a limited percentage of the BR molecules initiate the photocycle and convert to the N intermediate,<sup>3</sup> the changes observed in the deconvoluted spectra are relatively intense. This is in accordance with the view that the conformational changes in the N intermediate are probably the most extensive in the BR photocycle (Pfefferlé *et al.*, 1991; Ormos *et al.*, 1992; Rothschild *et al.*, 1993; Ludlam *et al.*, 1995). From a methodological point of view, we found that use of FTIR deconvoluted spectra gives new information as compared to difference spectroscopy. It allows a more accurate determination of spectral changes, whether they are due to intensity, bandwidth, or bands shape changes, or band shift. Thus, it would be interesting to compare the conformational changes in N with those of the M and O intermediates.

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