Fourier Transform Infrared Spectroscopy Indicates a Major Conformational Rearrangement in the Activation of Rhodopsin

David Garcia-Quintana,* Andrés Francesch,‡ Pere Garriga,* Ángel R. de Lera,‡ Esteve Padrós,* and Joan Manyosa*

*Unitat de Biofísica, Departament de Bioquímica i de Biologia Molecular, Facultat de Medicina, Universitat Autonòma de Barcelona, 08193 Bellaterra, Catalonia, Spain, and ‡Departamento de Química Orgánica, Facultad de Ciencias, Universidade de Santiago de Compostela, 15706 Santiago de Compostela, Spain

ABSTRACT The study of the structural differences between rhodopsin and its active form (metarhodopsin II) has been carried out by means of deconvolution analysis of infrared spectra. Deconvolution techniques allow the direct identification of the spectral changes that have occurred, which results in a significantly different view of the conformational changes occurring after activation of the receptor as compared with previous difference spectroscopy analysis. Thus, a number of changes in the bands assigned to solvent-exposed domains of the receptor are detected, indicating significant decreases in extended (β) sequences and in reverse turns, and increases in irregular/aperiodic sequences and in helices with a non-α geometry, whereas there is no decrease in α-helices. In addition to secondary structure conversions, qualitative alterations within a given secondary structure type are detected. These are seen to occur in both reverse turns and helices. The nature of this spectral change is of great importance, since a clear alteration in the helices bundle core is detected. All these changes indicate that the rhodopsin → metarhodopsin II transition involves not a minor but a major conformational rearrangement, reconciling the infrared data with the energetics of the activation process.

INTRODUCTION

Rhodopsin is the G protein-coupled receptor responsible for scotopic vision. G protein-coupled receptors constitute a large family of signal-transducing cell membrane receptors involved in a number of important physiological functions. Structural characterization of these receptors will not only result in an increased knowledge of the molecular mechanisms of cell communication, but should also allow the development of pharmacological strategies based on the design of agonist/antagonist ligands of specific action. The unusually high concentration of rhodopsin in the disc membranes of retina rod cells, where it is found in a virtually pure state, makes rhodopsin an ideal candidate for the structural investigation of G protein-coupled receptors in native membrane environment (Hargrave and McDowell, 1992).

An interesting feature of the characterization of the activation mechanism of the receptor is the determination of the conformational changes that distinguish the ligand-activated receptor from the inactive form. Of particular interest is the fact that the ligand binding pocket of the visual photoreceptors is already occupied by a pre-active form of the agonist: 11-*cis*-retinal chromophore, which is isomerized into all-*trans*-retinal by light around 500 nm. This triggers the conformational rearrangement in the protein moiety, leading through a sequence of still inactive intermediates to meta II, which is the active form capable of activating the G protein transducin (G_t) (Kühn et al., 1981; Kibelbek et al., 1991). Thus, activation depends only on light, i.e., a physical stimulus that can be administered synchronously to the pool of receptors in the sample. In this way, high concentrations of homogeneous intermediates can be obtained. In addition, the decay of meta II can be thermally blocked. In this manner, the spectroscopic characterization of the active form of rhodopsin is greatly facilitated, in comparison with other G protein-coupled receptors.

The changes between rhodopsin and meta II have been previously investigated by means of difference infrared spectroscopy (Rothschild et al., 1983; Siebert et al., 1983; De Grip et al., 1988). Difference spectra suggest that, whereas meta II is the most distorted intermediate, in view of the relatively low intensity of the difference peaks the conformational changes are apparently very limited, proportional to at most a few peptide bonds undergoing a secondary structural rearrangement (Rothschild et al., 1983; Rothschild et al., 1987; De Grip et al., 1988). Difference spectroscopy is a powerful technique allowing the detection of small spectral changes. However, it is difficult to interpret a difference trace, because a difference peak may be caused by different types of spectral phenomena, including a change in the intensity of a component band, a shift in the frequency of maximum absorbance, or a modification of width. Therefore, we have approached the analysis of the spectral changes between rhodopsin and meta II by means of deconvolution analysis of the absorbance spectra. Resolution enhancement techniques enable us to visualize the

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Address reprint requests to Dr. David Garcia-Quintana, Unitat de Biofísica, Edifici M, Univ. Autonoma de Barcelona, 08193 Bellaterra Catalonia, Spain. Tel.: 34-3-581-1177; Fax: 34-3-581-1907; E-mail: rhodopsin@cc.uab.es.

Abbreviations used: FTIR, Fourier transform infrared; FSD, Fourier self-deconvolution; HPLC, high-performance liquid chromatography; meta I, metarhodopsin I; meta II, metarhodopsin II; MLR, maximum likelihood restoration; NMR, nuclear magnetic resonance.

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component bands in each individual spectrum, thus allowing a more detailed, less ambiguous interpretation of the spectral changes occurring. We based our study on a previous work, dealing with the quantitative characterization of the structure of rhodopsin (Garcia-Quintana et al., 1993).

MATERIALS AND METHODS

Materials

Fresh bovine eyes were obtained and were kept in darkness for a ½ h to allow rhodopsin to regenerate. The retinas were dissected and the outer segments of rod cells were isolated in sucrose step gradient, as described (Papermaster and Dreyer, 1974). The plasma membrane of rod outer segments were lysed, and membranes were depleted of peripheral proteins by thorough washing in hypotonic, low ionic strength buffer (1.0 mM Pipes, 0.1 mM EDTA, 0.5 mM DTT, pH 7.0). Membranes were then washed in middle ionic strength buffer (70 mM potassium phosphate buffer, 1 mM $MgCl_2$, 0.1 mM EDTA, 0.5 mM DTT, pH 7.0). Protein purity was controlled by means of sodium dodecyl sulfate-polyacrylamide gel electrophoresis and the A_{280}/A_{500} ratio.

FTIR spectroscopy and deconvolution procedures

A detailed description of all spectroscopic and data processing methods has already been given (Garcia-Quintana et al., 1993), with the exception of the following. Membrane fragments were rinsed in 70 mM potassium phosphate buffer, 1 mM $MgCl_2$, 0.1 mM EDTA, pH 5.5. The infrared cell was thermostated at 2.0 ± 0.2°C. 1500 interferograms for 4 cm^{-1} resolution were coadded for each spectrum. The sample was then scanned again after having been illuminated for 10 s, by means of an optic fiber conducting the light from a Wratten 16-filtered (Eastman Kodak, Rochester, NY) tungsten lamp. Under these conditions, meta II was spectrophotometrically verified to be the sole species during the scanning time. This was repeated for up to 15 samples. To achieve a high signal-to-noise ratio (S/N) for subsequent deconvolution analysis, the spectra were coadded in groups of three. This yielded a total of five pairs of 4500-scan spectra, displaying a noise average always lower than 5×10^{-6} absorbance units (in the absorbance-free region above 1775 cm^{-1}), for an amide I intensity average slightly over 0.1 absorbance units. A variance test was performed on the deconvolution results from the five pairs of averaged spectra.

Spectra were resolved by means of Fourier self-deconvolution (FSD) (Kauppinen et al., 1981a); the chosen parameters were $k = 2.8$, $2\sigma = 18$ cm^{-1}, Lorentzian line shape, and Bessel apodization function. The high signal-to-noise ratio ensures that even the lower intensity component bands meet the $k < \log_{10}(S/N)$ condition to avoid enhancement of noise to a misleading level (Kauppinen et al., 1981b). Thus, no significant deconvolution-enhanced noise was evident in the neighboring non-absorbing regions. On the other hand, Pistorius and De Grip (1994) suggested that the additional component bands revealed in the amide I spectrum of rhodopsin by a narrowing factor $k = 2.8$ (Garcia-Quintana et al., 1993) as compared with a $k = 1.8$ analysis could be due to the effect of overdeconvolution-generated negative sidelobes. In this connection, both in our previous work and in the present report, it is worth noting the following. 1) No characteristic overdeconvolution sidelobes are present at the borders of the absorbing regions. 2) Apodization with a triangular squared function (small positive sidelobes upon overdeconvolution) yields deconvolution results identical to those presented below. Nevertheless, Bessel function has been preferred, because it yields the best compromise between high resolution and low generation of periodic noise and sidelobes (see Kauppinen et al., 1981b). 3) The spectral differences between rhodopsin and meta II presented below (see Results) are already evident after a conservative deconvolution ($k = 2.0$, $2\sigma = 12$ cm^{-1}), in which attribution of the resolved features to overdeconvolution sidelobes is highly improbable. However, such a low deconvolution is of no use in estimating the magnitude of the spectral changes, because component bands remain highly overlapped with non-evident widths. This is an important point since, as is well known, curve-fitting to poorly component-defined or undeconvolved contours has an unrestricted number of solutions, making a reliable quantification unviable. 4) Additional support to FSD results is provided by analysis of the spectra by means of Fourier derivative and maximum likelihood restoration (MLR) (performed as described in Garcia-Quintana et al., 1993). This latter procedure calculates the most likely composition in component bands with no subjective choice of a band narrowing factor. Significantly, results are identical to those obtained by FSD (see Results). 5) The overdeconvolved spectrum obtained with $k = 2.8$ by Pistorius and De Grip (1994), is more probably due to the characteristics of their spectrum, which is not the original absorbance spectrum, but a synthetic one constructed by the addition of Lorentzian bands. The point is that most of the chosen synthetic bands are narrower at half-height (11–16 cm^{-1}) than the chosen deconvolution 18 cm^{-1} estimate; as is well known, this leads to overdeconvolution, i.e., the appearance of undesired sidelobes (Kauppinen et al., 1981a).

Quantitative analysis was performed by means of curve-fitting of the FSD spectra, as described elsewhere (Garcia-Quintana et al., 1993). The significance of the spectral variations between rhodopsin and meta II (band frequency, band width at half-height, and relative band area) were tested by means of variance analysis (Fisher-Snedecor test); a stringent interval of confidence was chosen ($\alpha = 0.01$).

Synthesis of retinals and regeneration of rhodopsin

Synthesis of [15-^{13}C]11-*cis*-retinal started with the readily available intermediate β-ionilideneacetaldehyde (Dugger and Heathcock, 1980). Aldol reaction between β-ionilideneacetaldehyde and acetone in basic media (Pardoen et al., 1986) at room temperature afforded known C18-ketone in 75% yield. A Horner-Emmons reaction, using an improved procedure for the in situ generation of the phosphonate reagent (from two equivalents of lithium diisopropylamide and one equivalent of [1-^{13}C]CH_3CN, followed by the addition of 1 equivalent of diethylchlorophosphate), was used for the incorporation of the label at C_{15} (Gebhard et al., 1990). Addition of the phosphonate to the C18-ketone gave the C20-nitrile in 71% yield. Finally, diisobutylaluminum hydride reduction of the C20-nitrile to the aldehyde provided a 73:27 mixture (43% yield) of [15-^{13}C]all-*trans*-retinal and [15-^{13}C]13-*cis*-retinal as described (Pardoen et al., 1984), which were separated by HPLC. Proof of the location of the label at C15 in [15-^{13}C]all-*trans*-retinal was obtained from the ^{1}H-NMR spectrum, which shows an H15 signal with a J_{HC} of 169.7 Hz in the range of the aldehyde-CH bond.

[15-^{13}C]11-*cis*-retinal was generated from a dilute solution of [15-^{13}C]all-*trans*-retinal in acetonitrile, which was irradiated for 90 min using a 200 W tungsten lamp. The photostationary state mixture was separated by HPLC to provide, among other isomers, the desired [15-^{13}C]11-*cis*-retinal (Spijker-Assink et al., 1988). Structural assignment for the 11-*cis* geometry was based on the coupling constant for H_{11} in the ^{1}H-NMR spectrum.

Unlabeled 11-*cis*-retinal was also synthesized to carry out the controls of the regeneration process.

Opsin to be regenerated was obtained from rhodopsin bleached with light of wavelengths longer than 520 nm (Wratten 16 filter), and allowed to decay at 37°C. Opsin was mixed with a 1.2-fold molar excess of either [15-^{13}C]11-*cis*-retinal or unlabeled 11-*cis*-retinal in ethanol, at such concentrations that <4 μl was added to a total 1 ml volume. Incubation was allowed at 20°C, under dry N_2 atmosphere, until the original absorbance at 498 nm was recovered.

RESULTS

The FSD amide I infrared spectra of rhodopsin and meta II are shown in Fig. 1. The difference between the two original infrared absorption spectra is shown as a control in Fig. 2; this difference trace coincides with those previously re-

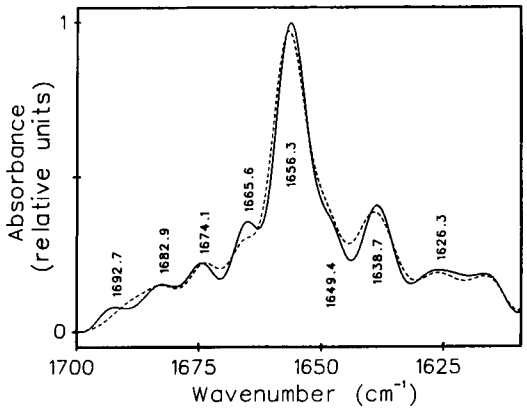

FIGURE 1 FSD amide I spectra of rhodopsin (——) and meta II (----) in membrane fragments suspended in 70 mM potassium phosphate buffer, 1 mM $MgCl_2$, 0.1 mM EDTA, pH 5.5. The indicated frequencies refer to the spectrum of rhodopsin.

ported by independent groups (Rothschild et al., 1983; Siebert et al., 1983; Klinger and Braiman, 1992; Fahmy et al., 1993).

Assessment of the reliability of the deconvolution analysis

FSD is a well established mathematical method for the resolution of highly overlapped component bands, and for many years has been applied to infrared spectroscopy of proteins. However, some aspects may seriously affect the reliability of the results obtained. 1) A high signal-to-noise ratio is required. FSD greatly reduces the S/N ratio; this may lead to the erroneous identification of noise as a real component band. The use of highly sensitive detectors, together with coaddition of a reasonable number of scans per spectrum, yield S/N ratios safely below the risk threshold. 2) Overdeconvolution can occur. This is due to an erroneous estimation of the component band width (rather than to the choice of a high narrowing factor); if a component band is narrower than the estimation, its relative contribution to the deconvolved spectrum is significantly enhanced. In addition, sidelobes are generated that may yield spurious bands, or alter neighboring real bands. Control work designed to rule out overdeconvolution is summarized in the Materials and Methods section. A final control of the accuracy of the deconvolution result is given by the full matching between the difference trace of the absorbance amide I spectra, and the difference trace of the deconvolved amide I spectra (Fig. 2, *inset*). 3) FSD preserves the relative integral component band areas only when all the component bands have an identical width at half-height. Because of this, a degree of uncertainty is to be admitted in a secondary structure characterization. Nevertheless, this is of no relevance in the present study, since only relative changes between two states are quantified.

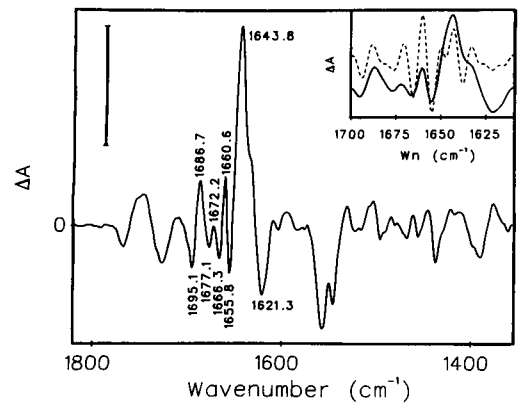

FIGURE 2 The difference trace between the original infrared absorbance spectrum of meta II minus that of rhodopsin. The bar indicates 1% of the amide I original absorbance. (*Inset*) The same trace (——) has been expanded in the amide I region for comparison with the difference trace between the deconvolved spectra (----); all the peaks in the deconvolved spectra difference trace coincide with peaks or obvious shoulders, now resolved thanks to the band narrowing produced by FSD, in the absorbance spectra difference trace. This is conclusive additional evidence that all the component bands revealed by FSD have a spectral basis, and are not a result of overdeconvolution.

The FSD result has been contrasted by means of both MLR and Fourier derivative (shown in Fig. 3). MLR is a nonlinear method that estimates the most probable components of the spectrum by looking for the parent set that maximizes the probability of the recorded spectrum. The usefulness of the complementary use of FSD and MLR must be emphasized, since the weak points of the two methods are different. 1) FSD gives an exact solution, but this solution includes noise or, at least, the noise within the interferogram region not truncated by the apodization function. 2) MLR acknowledges the presence of noise, and yields the maximum resolution consistent with it (i.e., there is no subjective choice of a band narrowing factor). Its weakness is that it gives only one—the most probable—of many possible solutions; therefore, small variations in the

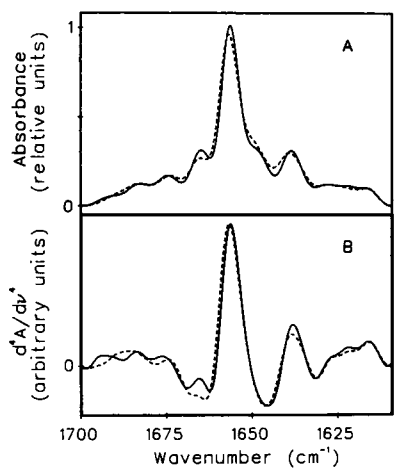

FIGURE 3 The same amide I spectra of rhodopsin (——) and meta II (----) as in Fig. 1, but resolved by means of (*A*) MLR and (*B*) Fourier derivative.

input may result in significant alterations in the output. We therefore introduce MLR as a method only to contrast the FSD solution. The absolute coincidence of the deconvolution result from these two methods provides solid support for the accuracy of the analysis presented.

Analysis of the conformational changes in the rhodopsin → meta II transition

The component bands in the amide I spectra of rhodopsin and meta II under the described conditions have been quantified by curve-fitting of the FSD spectra. Differences in frequency, area, and width of the fitted component bands have been tested for significance by means of variance analysis.

In this way, both a significant shift to higher frequency and a significant widening are verified for the band at 1656.3 cm^{-1}, in the characteristic range of α-helices. In view of its low accessibility to solvent, this band was previously assigned to the helical sequences that constitute the seven hydrophobic membrane spanning segments (Garcia-Quintana et al., 1993). Absorbance of the protonated Schiff base in rhodopsin is also expected in this region. The 11-*cis*-retinal chromophore is bound to the opsin moiety by means of a protonated Schiff base linkage; its C=N bond stretching vibration mode absorbs by 1659 cm^{-1} in rhodopsin (Ganter et al., 1988), whereas the unprotonated bond in meta II is expected to undergo a shift to lower frequency. Because of this, the spectral contribution of such a shift was investigated. Opsin was regenerated with [15-^{13}C]11-*cis*-retinal. $^{12}C \rightarrow {}^{13}C$ isotopic substitution shifts the protonated Schiff base C=N bond stretching vibration frequency in rhodopsin from 1659 cm^{-1} to 1636.5 cm^{-1} (Ganter et al., 1988). Labeled rhodopsin/meta II spectra are indistinguishable from the corresponding spectra in Fig. 1, and from control rhodopsin regenerated with unlabeled 11-*cis*-retinal (not shown). In consequence, the observed alterations in the 1656.3 component band are indicative of a conformational change in the protein. A dichroism artifact due to a change in the tilt of the helices can be discarded, since 1) care has been taken to avoid arraying of the membrane fragments that would result in anisotropic order; and 2) identical results are obtained with rhodopsin solubilized in dodecyl maltoside (not shown), where anisotropical arraying is suppressed. Thus, the nature of a shift to higher frequency reflects, in principle, a weakened pattern of hydrogen bonding (Krimm and Bandekar, 1986), suggesting distortion in any of the helices (Chirgadze et al., 1976). With respect to the verified widening, quantification analysis indicates that the deconvolved component band width at half-height increases by 0.6 cm^{-1} in the meta II spectrum, i.e. a 1.7 cm^{-1} estimate in the non-narrowed component band. Since this widening does not accompany an increase in integral band area, it rather reflects an increase in the heterogeneity of the α-helices. Widening of bands from α-helices has also been associated with distortions of the polypeptide backbone (Chirgadze et al., 1976). Finally, no decrease in α-helix is detected. The negative difference peak at 1655.8 cm^{-1} (see Fig. 2) actually results from the shift to higher frequency of the component band centered at 1656.3 cm^{-1}, as does the positive difference peak at 1660.6 cm^{-1}.

A significant shift and widening are seemingly verified for the band at 1638.7 cm^{-1}, although the widening is accompanied in this case by an increase in band area, proportional to 13 additional peptide C=O bonds. This band has been assigned to helices with a non-α-geometry (Garcia-Quintana et al., 1993), and its spectral behavior, parallel to that of the band characteristic of α-helices, furnishes further support for a helical assignment. On the other hand, a significant increase in band area, proportional to seven peptide C=O bonds, is also verified to occur for the band at 1649.4 cm^{-1}, assigned to solvent-exposed irregular/aperiodic sequences (Garcia-Quintana et al., 1993). It is the simultaneous width increase in these two component bands that yields the most intense positive peak in the difference spectrum, at 1643.8 cm^{-1}, which is now seen to correspond to no real band.

Other changes are observed. A significant decrease is verified in the 1620–1630 cm^{-1} region. This frequency range is characteristic of the amide C=O bonds in β-strands, although contributions from turns cannot be dismissed (Garcia-Quintana et al., 1993). The area decrease is calculated to be proportional to 10 peptide C=O bonds. This decrease was partially counterbalanced in the difference spectrum, on the higher frequency side, by the widening and area increase of the neighboring band at 1638.7 cm^{-1} (it should be borne in mind that the original component bands are much wider and, thus, more densely overlapped than the deconvolution-narrowed ones in Fig. 1). Secondary structure prediction shows that the fourth cytoplasmic loop of rhodopsin is likely to fold in a β-strand (Pappin et al., 1984); this is one of the domains involved in the interaction with the G_t protein (Konig et al., 1989).

Changes are also evident in the region over 1660 cm^{-1}, which corresponds to the absorptions of reverse turns (Garcia-Quintana et al., 1993), indicating that turn structures are affected in the rhodopsin → meta II conversion. A global decrease in integral band area is verified, mainly because of the intensity decrease of the band at 1665.6 cm^{-1}, which is calculated to be proportional to six amino acid residues leaving their former conformation. This change was partially masked in the difference spectrum because of the concomitant shift to higher frequency of the intense band at 1656.3 cm^{-1}. In addition, a significant shift is observed for the band at 1692.7 cm^{-1}; the vibrational frequency of amide C=O groups in reverse turns is highly sensitive to modifications in dihedral angles of the peptide backbone (Krimm and Bandekar, 1986). This shift is therefore suggestive of additional, qualitative alteration in turns. Reverse turns in rhodopsin appear to be mostly solvent-exposed (Garcia-Quintana et al., 1993), and so they are expected to be in the external loops of the receptor. A reverse turn is predicted in both the second and third cytoplasmic loops (Ovchinnikov,

1982), which are involved in the interaction with the G_t protein (Konig et al., 1989).

DISCUSSION

From the deconvolution analysis of the infrared amide I region, a significantly different vision of the conformational changes occurring in the activation of rhodopsin arises, regarding both the nature and the extent of the changes involved, as will be discussed later. The judicious use of FSD, combined with the contrast value of MLR, has allowed a more precise interpretation of the spectral changes involved in the process, which until now had been investigated only by means of difference. Although difference spectroscopy is a sensitive technique able to detect small spectral changes, these are difficult to interpret unambiguously: a difference peak may equally arise from diverse spectral phenomena (an intensity change of a band; a band shift; the change of a bandwidth), each one having a different conformational interpretation. Moreover, it is difficult to interpret complex cases on the basis of the difference trace (e.g., a combination of changes in one of the component bands; or changes in two neighboring component bands, which may combine or cancel each other out in the difference trace, or yield a spurious peak). Thus, regarding the nature of the spectral changes that occur, the shift of the band assigned to α-helices (1656.3 cm^{-1}) obviously results in a negative difference peak corresponding to the frequency of maximum absorbance. It is also shown that the positive difference peak at 1643.8 cm^{-1} is indeed due to the concomitant widening and area increase of two neighboring bands. These two difference peaks, taken together, might be interpreted as indicative of a partial conversion of α-helices into extended structures. Thus, an unfolding of the receptor (affecting a few residues) had been concluded to be the most relevant conformational change in the rhodopsin $\rightarrow$ meta II transition (Rothschild et al., 1987), a conclusion that is now discarded. Regarding the extent of the spectral changes occurring, it is not fully apparent in the difference trace, because some of them are compensated by changes in neighboring component bands. Some examples may be seen from a detailed comparison of the deconvolved spectra (Fig. 1) and the difference trace of the absorbance spectra (Fig. 2). The intensity decrease of the component band at 1665.6 cm^{-1}, e.g., is significantly counteracted in the absorbance spectra by the shift and widening of the bands at 1656.3 and 1674.1 cm^{-1} (it should be borne in mind that the original component bands are narrowed in the deconvolved spectrum). Seemingly, the intensity increase of the component band at 1649.4 cm^{-1} is compensated in the difference trace by the shift of the 1656.3 cm^{-1} band; the intensity decrease of the 1626.3 cm^{-1} band is compensated by the widening and area increase of the 1638.7 cm^{-1} band. In summary, the difference trace is apparently indicative of a very limited rearrangement of the receptor upon photoactivation.

Results from curve-fit quantitative analysis indicate that, in the decay from rhodopsin to meta II (disregarding potential interconversions that might further increase this number), the global secondary structure change is proportional to ~36 peptide C=O bonds (i.e., ~10% of total). This means that about half this number (18 residues, 5% of total) would change the secondary structure type in which they are involved: decreases in extended (β) sequences and in reverse turns give rise to increases in irregular/aperiodic sequences and in helices with a non-α geometry. Such a major rearrangement is fully compatible with the energetics of the process. Assuming the most adverse case, in which all the detected secondary structure changes occur by means of the break and the reforming of the hydrogen bonds involved, around 18 hydrogen bonds would break and reform. Considering that +12.5 kJ $\times$ mol^{-1} are required to break an average C=O $\cdots$ H-N hydrogen bond in the protein backbone (Schulz and Schirmer, 1979), then as much as +225 kJ $\times$ mol^{-1} should be required for the rhodopsin $\rightarrow$ meta II transition. Although no precise estimation of the activation energy involved in the rhodopsin $\rightarrow$ meta II transition is available, data referring to partial sequences are sufficiently indicative. The activation energies in the batho $\rightarrow$ lumi $\rightarrow$ meta I $\rightarrow$ meta II sequence have been estimated to involve a total around +300 kJ $\times$ mol^{-1} (Cooper, 1981). More recently, Arnis and Hofmann (1993) calculate $\Delta H^{\neq a}$ = 156 kJ $\times$ mol^{-1}, and $\Delta H^{\neq a}$ = 61 kJ $\times$ mol^{-1}, respectively, for the meta I $\rightarrow$ meta IIa $\rightarrow$ meta IIb sequence alone (i.e., a total +217 kJ $\times$ mol^{-1}). Also, the high positive entropy of activation for the meta I $\rightarrow$ meta II transition has been considered larger than that for denaturation of many proteins (Liebman et al., 1987). The results here presented reconcile for the first time the extent of the conformational rearrangement estimated by means of infrared spectroscopy, with the energetics data associated with the activation of rhodopsin.

In summary, from the deconvolution analysis of the infrared amide I spectra, a different vision of the conformational changes occurring upon the activation of rhodopsin arises. The changes in secondary structure are seen to correspond to residues in solvent-exposed domains, whereas there is no decrease in α-helical structure. These conformational changes are more extensive than was apparent from the difference trace. The extent is larger if the qualitative structural alterations within a given secondary structure type are taken into account. These are seen to occur in both reverse turns and helices. The nature of this latter spectral change is of greater importance, since an alteration in the hydrophobic helical core of the receptor (suggesting distortion in the helical sequences which results in an enhanced irregularity of the polypeptide backbone) is clearly detected. The rearrangement in the helix bundle should result in the alteration of the external loops, making then possible the interaction with the G_t protein. The observed changes, in their entirety, account for a major rearrangement of the receptor.

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