Site-Directed Isotope Labeling and FT-IR Spectroscopy: The Tyr 185/Pro 186 Peptide Bond of Bacteriorhodopsin Is Perturbed during the Primary Photoreaction

Sanjay Sonar,† Xiao-Mei Liu,† Chan-Ping Lee,§ Matthew Coleman,† Yi-Wu He,† Sandra Pelletier,‡ Judith Herzfeld,‡ Uttam L. RajBhandary,§ and Kenneth J. Rothschild*,†

Physics Department and Molecular Biophysics Laboratory Boston University, Boston, Massachusetts 02215 Department of Chemistry, Brandeis University Waltham, Massachusetts 02254 Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

Received June 16, 1995

FT-IR difference spectroscopy can provide detailed information about structure and conformational changes in proteins.[1] This approach is greatly enhanced by site-directed isotope labeling (SDIL).[2−4] In contrast to earlier methods employing site-directed mutagenesis, SDIL allows vibrational band assignments to be made in an essentially unaltered system through the replacement of single amino acid residues with their isotopic analogs.

In this work, we have used FT-IR/SDIL to probe protein conformational changes which occur during the primary photochemical reaction of bacteriorhodopsin (bR), a light-driven proton pump from *Halobacterium salinarium*. While it is known that this initial step involves an *all-trans* to 13-*cis* isomerization of the retinylidene chromophore,[5] little is known about the interactions which occur between the retinal and protein during this step or how these interactions eventually facilitate proton transport.[6] We have probed the response to chromophore isomerization of three specific tyrosine amide carbonyl groups residing in the retinal binding pocket (Tyr 57, 83, and 185),[7] as well as Tyr 147 outside of this pocket. Our results show that out of the 11 tyrosine residues in bR, only the amide carbonyl group of Tyr 185 is perturbed by chromophore isomerization.

Four SDIL analogs of bR containing L-[1-^{13}C]Tyr at positions 57, 83, 147, and 185 were constructed using methods described previously.[2,3,8] This was accomplished by introduction of amber codons into the synthetic bacterioopsin gene, enzymatic amino acylation of *Escherichia coli* tyrosine suppressor tRNA with L-[1-^{13}C]Tyr, cell-free synthesis of bacterioopsin in wheat germ extract in the presence of the amino acylated *E. coli* suppressor tRNA, and finally regeneration of functional bR in native lipids. Earlier studies have established that bR and SDIL bR analogs expressed in cell-free systems exhibit properties almost identical to those of the native system.[2,3] bR containing [1-^{13}C]Tyr at all of the tyrosines was produced as previously described.[3]

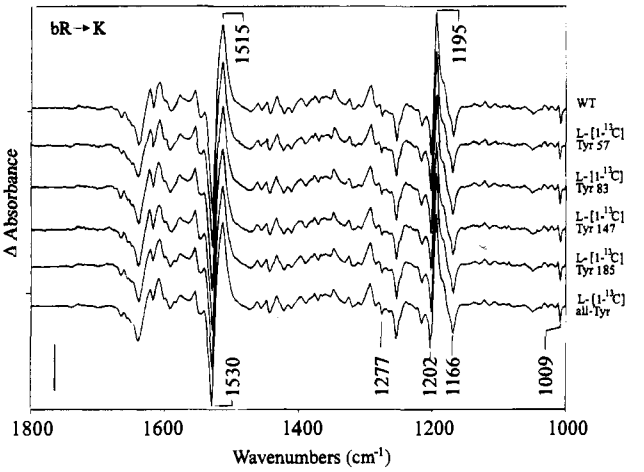

Figure 1. Comparison of FT-IR difference spectra for bR→K transition of bR and SDIL analogs of bR. Each spectrum was recorded at 2 cm^{-1} resolution and at 80 K under conditions described earlier.[24] Each spectrum consists of 30 averages of 2000 scans each. The scale bar (1 × 10^{-3} au) corresponds to the FT-IR difference spectrum of WT.

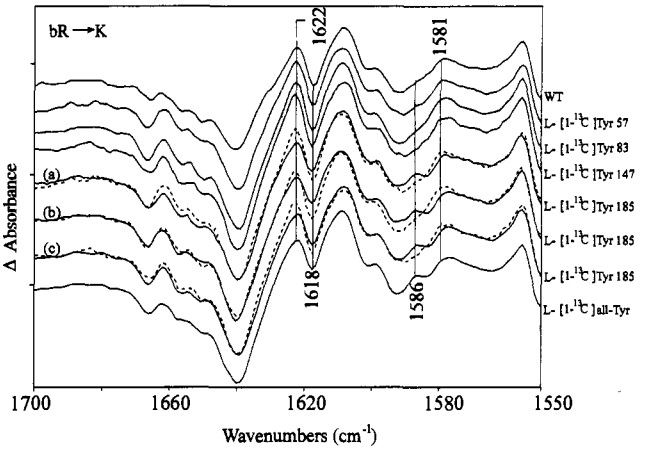

Figure 2. Expansion in selected regions of the data shown in Figure 1. Three independently recorded spectra of L-[1-^{13}C]Tyr 185 are shown and compared to (- - -) (a) [1-^{13}C]Tyr 57, (b) [1-^{13}C]Tyr 83, and (c) [1-^{13}C]Tyr 147.

Figure 1 shows the low-temperature (80 K) bR→K FT-IR difference spectra of cell-free expressed bR (wild-type, WT) along with the four SDIL bR analogs [1-^{13}C]Tyr 57,[9] [1-^{13}C]-Tyr 83, [1-^{13}C]Tyr 147, [1-^{13}C]Tyr 185, and bR containing [1-^{13}C] at all of the tyrosines, [1-^{13}C]-all-Tyr. All of these spectra are similar and reflect mainly the *all-trans* to 13-*cis* isomerization of the retinal chromophore. For example, negative bands previously assigned to the *all-trans* chromophore of light-adapted bR (bR_{570})[5] appear at 1530 cm^{-1} (ethylenic C=C stretch), 1202 and 1166 cm^{-1} (C—C stretch), and 1009 cm^{-1} (CH_3 bend). Positive bands characteristic of the K chromophore with a 13-*cis* configuration are also found at 1515 cm^{-1} (ethylenic C=C stretch) and 1195 cm^{-1} (C—C stretch).

The 1600−1700 cm^{-1} amide I region (Figure 2) should reflect any changes in the C=O stretching mode of peptide backbone groups that occur during the bR→K transition. While the [1-^{13}C]Tyr 57, [1-^{13}C]Tyr 83, and [1-^{13}C]Tyr 147 spectra are similar to those of WT, a distinct drop in the intensity of the

† Boston University.
‡ Brandeis University.
§ Massachusetts Institute of Technology.

(1) Rothschild, K. J. *J. Bioenerg. Biomembr.* **1992**, *24*, 147−167.
(2) Sonar, S.; Lee, C.-P.; Coleman, M.; Marti, T.; Patel, N.; Liu, X.; Khorana, H. G.; RajBhandary, U. L.; Rothschild, K. J. *Nature Struct. Biol.* **1994**, *1*, 512−517.
(3) Ludlam, C. F. C.; Sonar, S.; Lee, C.-P.; Coleman, M.; Herzfeld, J.; RajBhandary, U. L.; Rothschild, K. J. *Biochemistry* **1995**, *34*, 2−6.
(4) Liu, X.-M.; Sonar, S.; Lee, C.-P.; Coleman, M.; RajBhandary, U. L.; Rothschild, K. J. *Biophys. Chem.* **1995**, *56*, 63−70.
(5) Braiman, M.; Mathies, R. *Proc. Natl. Acad. Sci. U.S.A.* **1982**, *79*, 403−407.
(6) Rothschild, K. J.; Sonar, S. In *CRC Handbook of Organic Photochemistry and Photobiology*; Horspool, W. M., Song, P.-S., Eds.; CRC Press, Inc.: London, 1995; pp 1521−1544.
(7) Henderson, R.; Baldwin, J. M.; Ceska, T. A.; Zemlin, F.; Beckmann, E.; Downing, K. H. *J. Mol. Biol.* **1990**, *213*, 899−929.
(8) Noren, C. J.; Anthony-Cahill, S. J.; Griffith, M. C.; Schultz, P. G. *Science* **1989**, *244*, 182−188.

(9) [1-^{13}C]Tyr XX defines the SDIL analog of bR, wherein out of 11 tyrosines in bR, only the tyrosine at position XX is replaced with a tyrosine ^{13}C labeled at the carbonyl carbon. [1-^{13}C]-all-Tyr represents bR labeled by ^{13}C at the C1 position at all 11 tyrosine positions.

0002-7863/95/1517-11614$09.00/0

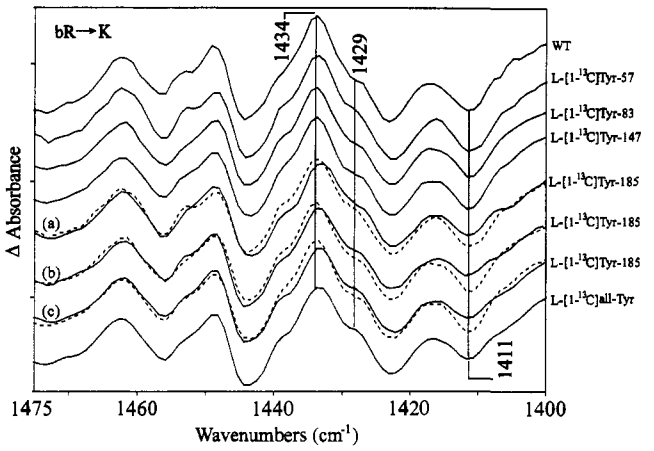

Figure 3. Expansion in selected regions of the data shown in Figure 1. (see Figure 2 caption).

bands near 1622 (+) and 1618 (−) cm^{-1} is seen in only the [1-^{13}C]Tyr 185 and [1-^{13}C]-all-Tyr samples.[10] In addition, a positive band appears near 1586 (+) cm^{-1}, and there is a drop in intensity at 1581 cm^{-1} in these samples.[11] These changes are consistent with a downshift of a set of (+/−) bands from 1622 and 1618 cm^{-1} to 1586 and 1581 cm^{-1}. This frequency shift is in accord with studies on synthetic polypeptides[12,13] and the membrane protein phospholamban[14] which show that the amide I band shifts ~30−40 cm^{-1} due to ^{13}C labeling of the carbonyl group.[15] Since this shift occurs only in [1-^{13}C]Tyr 185 and [1-^{13}C]-all-Tyr samples, we can assign the tyrosine carbonyl group that is changing during the bR→K transition to Tyr 185. In addition, the similarity of bR→K difference spectra of [1-^{13}C]Tyr 185 and [1-^{13}C]-all-Tyr samples indicates that no other tyrosine carbonyl groups contribute to the bR→K difference spectrum of WT bR. Thus, we conclude that out of 11 tyrosines, only the peptide carbonyl group of Tyr 185 is significantly perturbed by chromophore isomerization during the primary phototransition of bR.

We also observe isotope effects for the [1-^{13}C]Tyr 185 and [1-^{13}C]-all-Tyr samples (but not for the other SDIL bR analogs) in the region between 1420 and 1440 cm^{-1} (Figure 3). The 1434 cm^{-1} positive band is downshifted by 1 cm^{-1} with a lower intensity, and the negative band near 1410 cm^{-1} has a lower intensity in three independent sets of spectra shown.[11] It was previously found that this region of the bR→K difference spectrum is sensitive to uniform proline labeling with both L-[^{15}N]Pro and L-[$^{2}H_7$]Pro.[16] On this basis and studies of proline model compounds,[17] it was concluded that the stretch mode of the Xaa-Pro C−N bond of one or more proline residues was structurally active during this transition. Our current results indicate that at least one of these proline residues is Pro 186, since a ^{13}C label placed in the carbonyl group of Tyr 185 should cause an isotope effect for the C−N stretch mode of the Tyr 185/Pro 186 peptide bond but not for other Xaa-Pro C−N bonds in bR. As expected, uniform L-[^{15}N]Pro labeling also causes a drop in intensity and a small frequency shift of the 1622 cm^{-1} band assigned to an amide I mode of an Xaa-Pro peptide group.[18,19] These results, however, do not exclude other prolines such as Pro 51 and Pro 91 from participating in a structural change during the bR→K transition.

The finding that the Tyr 185/Pro 186 peptide bond is perturbed during the bR→K transition suggests that its environment or structure is altered due to retinal isomerization during the primary phototransition. Since Pro 186 is close to the β-ionone ring of the retinal (3−4 Å),[7] chromophore isomerization might exert forces on this group which are relieved by movement around the Tyr 185/Pro 186 region of the backbone. This model would help explain why the substitution of Pro 186 with residues Gly, Ala, and Val results in alterations of the structure, conformational changes, and function of bR.[20] Alternatively, the perturbation we observe may reflect a vibrational Stark effect[21] due to a change in the local electric field near the Tyr 185/Pro 186 peptide bond.

The peptide carbonyl group of Tyr 185 appears to undergo a change not only during the bR→K transition but also during subsequent steps in the photocycle (X. M. Liu, S. Sonar, and K. J. Rothschild, unpublished data), including the M→N transition.[3] This last step has been found to involve structural perturbations of membrane-embedded α-helical structure[22] consistent with a net reorientation of the cytoplasmic portion of the F and C helices.[23] Thus, these results suggest that the Tyr 185/Pro 186 region may function as a type of "hinge" which responds to the initial chromophore isomerization by undergoing gradually increasing structural changes that culminate during the M→N transition.

Acknowledgment. This work was supported by grants from the NIH (GM47527) and the NSF (MCB 9106017) to K.J.R., the Army Research Office (ARO) (DAAL03-92-G-0172) to K.J.R. and U.L.R., the NIH (GM17151) to U.L.R., and the NIH (GM36810) to J.H. We gratefully acknowledge the help of H. G. Khorana and T. Marti in preparation of the Tyr 185→am construct.

JA9519593

(10) These bands are also observed in picosecond infrared spectra of bR recorded at ambient room temperature (Diller, R.; Iannone, M.; Bogomolni, R.; Hochstrasser, R. *Biophys. J.* **1991** *60*, 286−289).

(11) Note that three independently measured spectra of [1-^{13}C]Tyr 185 are shown and compared with those of other SDIL analogs to demonstrate the reproducibility of these shifts. The signal/noise ratio is estimated to be 3:1 near the 1586 cm^{-1} band. The absence of water vapor bands at 1717, 1696, and 1684 cm^{-1} indicates that the 1586 cm^{-1} band in the [1-^{13}C]Tyr 185 spectra is not due to water vapor.

(12) Tadesse, L.; Nazarbaghi, R.; Walters, L. *J. Am. Chem. Soc.* **1991**, *113*, 7036−7037.

(13) Martinez, G. V.; Fiori, W. R.; Millhauser, G. *Biophys. J.* **1994**, *66*, A65.

(14) Arkin, I. T.; Rothman, M. S.; Ludlam, C. F. C.; Aimoto, S.; Engelman, D. M.; Rothschild, K. J.; Smith, S. O. *J. Mol. Biol.* **1995**, *248*, 824−834.

(15) Dwivedi, A. M.; Krimm, S. *Biopolymers* **1984**, *23*, 923−924.

(16) Rothschild, K. J.; He, Y. W.; Gray, D.; Roepe, P. D.; Pelletier, S. L.; Brown, R. S.; Herzfeld, J. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 9832−9835.

(17) Miyazawa, T.; Blout, E. R. *J. Am. Chem. Soc.* **1961**, *83*, 712−719.

(18) Krimm, S.; Bandekar, J. *Advances in Protein Chemistry*; Academic Press, Inc.: 1986; pp 181−365.

(19) Gerwert, K.; Hess, B.; Engelhard, M. *FEBS Lett.* **1990**, *261*, 449−454.

(20) Rothschild, K. J.; He, Y. W.; Mogi, T.; Marti, T.; Stern, L. J.; Khorana, H. G. *Biochemistry* **1990**, *29*, 5954−5960.

(21) Maiti, S.; Walker, G. C.; Cowen, B. R.; Pippenger, R.; Moser, C. C.; Dutton, P. L.; Hochstrasser, R. M. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 10360−10364.

(22) Rothschild, K. J.; Marti, T.; Sonar, S.; He, Y. W.; Rath, P.; Fischer, W.; Bousche, O.; Khorana, H. *J. Biol. Chem.* **1993**, *268*, 27046−27052.

(23) Subramaniam, S.; Gerstein, M.; Oesterhelt, D.; Henderson, R. *Embo J.* **1993**, *12*, 1−8.

(24) Rothschild, K. J.; Roepe, P.; Ahl, P. L.; Earnest, T. N.; Bogomolni, R. A.; Das Gupta, S. K.; Mulliken, C. M.; Herzfeld, J. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 347−351.