Identification of the Magnesium−Histidine Stretching Vibration of the Bacteriochlorophyll Cofactors in Photosynthetic Reaction Centers via ^{15}N-Labeling of the Histidines

Kazimierz Czarnecki,[†] Veeradej Chynwat,[‡] Joy P. Erickson,[‡] Harry A. Frank,[‡] and David F. Bocian*,[†]

Department of Chemistry, University of California Riverside, California 92521
Department of Chemistry, University of Connecticut Storrs, Connecticut 06269

Received December 2, 1996

The reaction center (RC) is a membrane-bound protein responsible for the primary electron-transfer events in photosynthesis.[1] Bacterial RCs contain four bacteriochlorophyll (BChl) molecules. Two of the BChls exist as a dimer, designated the special pair, which serves as the primary electron donor.[1] The other two BChls, designated the accessory BChls, exist as monomeric species whose detailed function is still a subject of debate.[1] The central magnesium ion of each of the four BChl cofactors in RCs is ligated to a histidine residue which serves as the only covalent link to the protein.[2] Systematic spectroscopic studies aimed at elucidating the characteristics and/or functional importance of the magnesium−histidine linkage in photosynthetic proteins have only recently begun to appear.[3] These studies have been facilitated by both genetic and chemical engineering techiques which allow replacement of the ligated histidines[3a] or isotopic labeling of these residues,[3b,c] respectively. To date, vibrational techniques have not been used to examine the magnesium−histidine linkage, in large part because the magnesium−histidine stretching vibration, ν(MgN$_{his}$), has not been definitively identified in the vibrational spectrum of any photosynthetic protein or model complex.

As a first step toward a complete characterization of the low-frequency vibrational characteristics of the BChl cofactors in RCs, we recently reported near-infrared-excitation resonance Raman (RR) studies of RCs in which the BChl cofactors were labeled with ^{26}Mg or ^{15}N.[4] These studies revealed that four vibrational modes in the 50−425-cm^{-1} region are sensitive to ^{26}Mg substitution.[4b] These include RR bands of the special pair at 137, 179, 236, and 364 cm^{-1} and analog bands of the accessory BChls at 137, 190, 240, and 364 cm^{-1}. For the special pair, a single set of RR bands is observed because only one of the two cofactors in the dimer contributes to the spectrum.[5a] For the accessory BChls, both cofactors contribute to the RR spectrum; however, a single set of bands is observed because the frequencies of the analogous modes of the two cofactors are identical.[5] Of the four ^{26}Mg-sensitive modes, only the 179/190-cm^{-1} band of the special pair/accessory BChls is also sensitive to [^{15}N]pyrrole substitution. On the basis of the ^{26}Mg isotope shifts and the predictions of semiempirical normal coordinate calculations, the 137-cm^{-1} mode was assigned to the doming mode of the BChl macrocycle, γ(Mg$_{dome}$); the 179/190- and 364-cm^{-1} vibrations were assigned to other vibrations of the BChl core. The 236/240-cm^{-1} mode was tentatively identified as ν(MgN$_{his}$).[4b] A definitive assignment of ν(MgN$_{his}$) was not possible, however, because this assignment requires studies of RCs in which the histidine axial ligands are isotopically labeled.

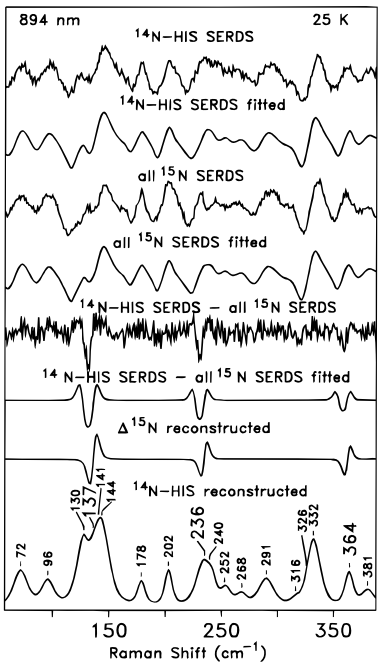

Figure 1. Low-frequency region of the near-infrared-excitation (λ_{ex} = 894 nm) RR spectrum of the special pair obtained at 25 K. The individual traces are discussed in the text.

In this communication, we report low-frequency, near-infrared-excitation RR spectra of bacterial RCs in which the histidine residues of the protein are selectively labeled with ^{15}N. For practical reasons, the studies were conducted by comparing the vibrational signatures of RCs in which ^{15}N was universally incorporated (all cofactors and all protein residues) (designated all-^{15}N RCs) with those in which [^{14}N]histidine was introduced as a reverse label (designated ^{14}N-His RCs) into the all ^{15}N-labeled RCs.[6] The studies of the histidine-labeled RCs reveal that the vibrational characteristics of the BChl core are far more complicated than originally anticipated. These results have clear implications for the photoexcitation dynamics of the BChls in RCs and may also have significant consequences for the dynamics of exogenous ligand binding to heme-based oxygen carriers.

The low-frequency regions (60−400 cm^{-1}) of the near-infrared-excitation RR spectra of the special pair (λ_{ex} = 894 nm) and the accessory BChls (λ_{ex} = 805 nm) in RCs from *Rhodobacter sphaeroides* 2.4.1 are shown in Figures 1 and 2, respectively.[7] Owing to the fact that the isotope shifts are relatively small (∼2 cm^{-1} or less) and the RR spectra ride on a fluorescence background, the RR spectra of the individual isotopomers were acquired using the shifted-excitation Raman difference spectroscopic (SERDS) method.[8] The SERDS

[†] University of California.
[‡] University of Connecticut.

(1) For an overview, see: Deisenhofer, J., Norris, J. R., Eds. *The Photosynthetic Reaction Center*; Academic: San Diego, CA, 1993; Vol. II.

(2) (a) Ermler, U.; Fritzsch, G.; Buchanan, S.; Michel, H. *Structure* **1994**, *2*, 925−936. (b) Deisenhofer, J.; Epp, O.; Sinning, I.; Michel, H. *J. Mol. Biol.* **1995**, *246*, 429−457.

(3) (a) Goldsmith, J. O.; King, B.; Boxer, S. G. *Biochemistry* **1996**, *35*, 2441−2428. (b) Zysmilich, M. G.; McDermott, A. *J. Am. Chem. Soc.* **1996**, *118*, 5867−5873. (c) Mac, M.; Tang, X.-T.; Diner, B.; McCracken, J.; Babcock, G. T. *Biochemistry* **1996**, *35*, 13288−13293.

(4) (a) Czarnecki, K.; Diers, J. R.; Chynwat, V.; Erickson, J. P.; Frank, H. A.; Bocian, D. F. In *Proceedings of the XVth International Conference on Raman Spectroscopy*; Asher, S. A., Stein, P. B., Eds.; Wiley: Chichester, U.K., 1996; pp 300−301. (b) Czarnecki, K.; Diers, J. R.; Chynwat, V.; Erickson, J. P.; Frank, H. A.; Bocian, D. F. *J. Am. Chem. Soc.* **1997**, *119*, 415−426.

(5) (a) Palaniappan, V.; Schenck, C. C.; Bocian, D. F. *J. Phys. Chem.* **1995**, *99*, 17049−17058. (b) Cherepy, N. J.; Holzwarth, A.; Mathies, R. A. *Biochemistry* **1995**, *34*, 5288−5293.

(6) (a) *Rb. sphaeroides* 2.4.1 was grown in a modified Hunter's (minimal) medium in the absence of caseamino acids.[6b] The incorporation of ^{15}N and ^{15}N in conjunction with the [^{14}N]histidine reverse label was accomplished as previously described.[3b] For both preparations, ^{15}NH$_4$Cl (^{15}N, 98%+), obtained from Cambridge Isotopes (Andover, MA), was used to introduce the label. The RCs were isolated and purified as previously described.[6c] The chemically reduced RCs used for the RR experiments were prepared by adding a slight excess of $Na_2S_2O_4$. (b) Cohen-Bazire, G.; Sistrom, W. R.; Stanier, R. Y. *J. Cell. Comp. Physiol.* **1957**, *49*, 25−68. (c) McGann, W. J.; Frank, H. A. *Biochim. Biophys. Acta* **1985**, *807*, 101−109.

S0002-7863(96)04107-8 CCC: $14.00

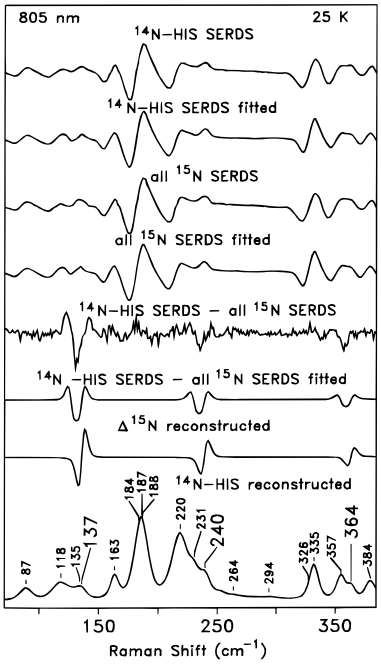

Figure 2. Low-frequency region of the near-infrared-excitation (λ_{ex} = 805 nm) RR spectrum of the accessory BChls obtained at 25 K. The individual traces are discussed in the text.

method and its application to RR studies of RCs are discussed in detail elsewhere.[5,8] In Figures 1 and 2, the first and third traces are the SERDS data for the ^{14}N-His and all-^{15}N RCs, respectively. The second and fourth traces are fits of the SERDS data. The isotope-sensitive RR bands are identified by subtracting the raw SERDS data sets of the two isotopomers without any smoothing or other manipulation of the data. These double-difference data (^{14}N-His SERDS − all-^{15}N SERDS) are shown in the fifth traces of each figure. The sixth traces are the fitted double-difference spectra. The seventh traces are the $\Delta^{15}N_{his}$ isotope-difference spectra reconstructed from the double-difference data. The protocols used to obtain and fit the double-difference spectra and to extract the isotope shifts are identical to those used in our previous RR studies of RCs in which only the cofactors were labeled.[4] The bottom traces in Figures 1 and 2 are the RR spectra of the [^{14}N]His RCs which were reconstructed from the SERDS data. In these spectra, the bands sensitive to ^{15}N-histidine substitution are labeled in bold. It should be noted that the RR spectra of the special pair and accessory BChls in the ^{14}N-His RCs were found to be identical to those previously obtained for RCs in which only the cofactors are ^{15}N-labeled.[4b] These results indicate that ^{15}N-labeling of the amino acid residues of the protein (other than the histidines) has no effect on the RR spectra of the cofactors.

Inspection of Figures 1 and 2 reveals that three of the four ^{26}Mg-sensitive low-frequency modes of the special pair/accessory BChls are sensitive to ^{15}N-labeling of the histidines. These include the 137-cm^{-1} band previously identified as γMg_{dome} ($\Delta^{15}N_{his} \sim 2.0$ cm^{-1}), the 236/240-cm^{-1} band tentatively assigned to $\nu(MgN_{his})$ ($\Delta^{15}N_{his} < 2$ cm^{-1}), and the 364-cm^{-1} band attributed to another core vibration ($\Delta^{15}N_{his} < 1$ cm^{-1}).[4b] The most surprising result is that both the 137-cm^{-1} and 236/240-cm^{-1} bands exhibit substantial [^{15}N]histidine shifts and that the shift of the former band is somewhat larger than that of the latter. The observed pattern of [^{15}N]histidine shifts can only be accounted for if $\nu(MgN_{his})$ and $\gamma(Mg_{dome})$ are very strongly mixed with one another and to a lesser extent with other motions of the BChl core. Collectively, these observations indicate that the internal-coordinate descriptors $\nu(MgN_{his})$ and $\gamma(Mg_{dome})$ are not representative of the actual normal modes of vibration of the BChl core.

The strong mixing of $\nu(MgN_{his})$ and $\gamma(Mg_{dome})$ has clear implications for the photoexcitation dynamics of the BChls in RCs. In particular, a number of static and time-resolved spectroscopic studies have shown that very low-frequency modes (30−150 cm^{-1}) are strongly coupled to the electronic transition of the special pair which initiates charge separation in RCs.[9,10] In contrast, none of the vibrational modes of the monomeric accessory BChls are strongly coupled to the Q_y transition. One of the strongly coupled special pair vibrations which has received particular attention is the so-called marker mode at ∼135 cm^{-1}.[9b] The marker mode is actually a cluster of several different vibrations of which the 137-cm^{-1} RR-active mode is one.[4b,5a] The fact that this latter vibration involves simultaneous motions of the magnesium−histidine linkage and BChl core provides a means by which photoinduced dynamical motions of the core could be much more efficiently transmitted to the protein matrix than if the vibration involved only motion of core. This cofactor−protein coupling could in turn influence the charge-separation process. In this regard, recent experiments have shown that the magnesium−histidine linkage is not essential for the relatively slow charge recombination events in RCs.[3a] It remains to be determined, however, whether the linkage has any influence on the ultrafast primary charge-separation process.

Finally, the strong mixing found for $\nu(MgN_{his})$ and $\gamma(Mg_{dome})$ may also have significant implications for the analogous motions associated with the iron−histidine linkage in heme proteins such as hemoglobin and myoglobin. For heme proteins, the extent of mixing between $\nu(FeN_{his})$ and $\gamma(Fe_{dome})$ has not been explicitly determined via isotopic substitution experiments such as those reported herein.[11] However, recent femtosecond impulsive studies explicitly show that motions nominally described as $\nu(FeN_{his})$ and $\gamma(Fe_{dome})$ are linked to the binding dynamics of exogenous ligands.[12] Any detailed dynamical model of ligand binding is predicated on an accurate description of the coupling between $\nu(FeN_{his})$ and $\gamma(Fe_{dome})$.

Acknowledgment. We thank Professor A. McDermott for providing the procedures for preparing the labeled RCs. This work was supported by Grants GM39781 (D.F.B) and GM30353 (H.A.F.) from the National Institutes of Health, the Cooperative State Research Service, USDA, Agreement No. 92-37306-7690 (H.A.F.), and the Human Frontier of Science Program (H.A.F.).

JA964107R

(7) (a) The RR measurements were made on optically dense (OD ∼ 1.8/mm at 800 nm; RC concentration ∼ 125 μM); snowy samples at 25 K contained in 1 mm i.d. capillary tubes. The sampling accessories, spectrometer, and laser systems have been previously described.[7b,c] The acquisition times for complete SERDS data sets were 7 h (λ_{ex} = 894 nm) and 4 h (λ_{ex} = 805 nm). The spectral resolution was ∼2 cm^{-1} at a Raman shift of 200 cm^{-1}. The laser powers were ∼750 μW. (b) Palaniappan, V.; Aldema, M. A.; Frank, H. A.; Bocian, D. F. *Biochemistry* **1992**, *31*, 11050−11058. (c) Palaniappan, V.; Martin, P. C.; Chynwat, V.; Frank, H. A.; Bocian, D. F. *J. Am. Chem. Soc.* **1993**, *115*, 12035−12049.

(8) (a) Shreve, A. P.; Cherepy, N. J.; Franzen, S.; Boxer, S. G.; Mathies, R. A. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 11207−11211. (b) Shreve, A. P.; Cherepy, N. J.; Mathies, R. A. *Appl. Spectrosc.* **1992**, *46*, 707−711. (c) Cherepy, N. J.; Shreve, A. P.; Moore, L. J.; Franzen, S.; Boxer, S. G.; Mathies, R. A. *J. Phys. Chem.* **1994**, *98*, 6023−6029.

(9) (a) Middendorf, T. R.; Mazzola, L. T.; Gaul, D. F.; Schenck, C. C.; Boxer, S. G. *J. Phys. Chem.* **1991**, *95*, 10142−10151. (b) Jankowiak, R.; Hayes, J. M.; Small, G. J. *Chem. Rev.* **1993**, *93*, 1471−1502. (c) Klevanik, A. V.; Ganago, A. O.; Shkuropatov, A. Y.; Shuvalov, V. A. *FEBS Lett.* **1988**, *237*, 61−64.

(10) (a) Vos, M. H.; Jones, M. R.; Hunter, C. N.; Breton, J.; Lambry, J.-C.; Martin, J.-L. *Biochemistry* **1994**, *33*, 6750−6757. (b) Stanley, R. J.; Boxer, S. G. *J. Phys. Chem.* **1995**, *99*, 859−863.

(11) (a) For a discussion of heme-histidine isotopic labeling of deoxymyoglobin see, Wells, A. V.; Sage, J. T.; Morikis, D.; Champion, P. M.; Chiu, M. L.; Sligar, S. G. *J. Am. Chem. Soc.* **1991**, *113*, 9655−9660. (b) For discussions of coupling between $\nu(FeN_{his})$ and $\gamma(Fe_{dome})$, see: Rosenfeld, Y. B.; Stavrov, S. S. *Chem. Phys. Lett.* **1994**, *229*, 457−464. Palaniappan, V.; Bocian, D. F. *J. Am. Chem. Soc.* **1994**, *116*, 8839−8840.

(12) (a) Zhu, L.; Li, P.; Huang, M.; Sage, J. T.; Champion, P. M. *Phys. Rev. Lett.* **1994**, *72*, 301−304. (b) Zhu, L.; Sage, J. T.; Champion, P. M. *Science* **1994**, *266*, 629−632. (c) Zhu, L.; Zhong, G.; Unno, M.; Sligar, S. G.; Champion, P. M. *Biospectroscopy* **1996**, *2*, 301−309.