Probing secondary, tertiary, and quaternary structure along with protein-cofactor interactions for a helical transmembrane protein complex through 1H spin diffusion with MAS NMR spectroscopy

Ganapathy, S.; Gammeren, A.J. van; Hulsbergen, F.B.; Groot, H.J.M. de

J|A|C|S
COMMUNICATIONS

Published on Web 01/20/2007

Probing Secondary, Tertiary, and Quaternary Structure along with Protein−Cofactor Interactions for a Helical Transmembrane Protein Complex through ^{1}H Spin Diffusion with MAS NMR Spectroscopy

Swapna Ganapathy, Adriaan J. van Gammeren, Frans B. Hulsbergen, and Huub J. M. de Groot*

Leiden Institute of Chemistry, Gorlaeus Laboratories, Leiden University, P.O. Box 9502, 2300 RA Leiden, The Netherlands

Received September 6, 2006; E-mail: ssnmr@chem.leidenuniv.nl

Magic angle spinning (MAS) NMR is rapidly developing as a technique to resolve structure in biological systems.[1−3] An important step in solving structure with solid-state NMR is the detection of distance constraints, in addition to the chemical shift assignment.[4−10] For small molecules that form ordered aggregates, this is becoming routine.[11] For proteins or amyloid systems having β-sheet motifs, detection of correlations between backbone carbons from adjacent chains is sometimes possible using ^{13}C spin diffusion.[12−14] Membrane proteins are generally comprised of α-helical segments with narrow shift dispersion, and the inter-helical distances between adjacent segments are too large to be bridged by conventional ^{13}C spin diffusion. Contacts between a ligand and side chains have been detected, however, without a specific assignment.[15] In addition, resolving structure from secondary shifts is not always possible, and the lack of access to distance restraints is a severe limitation.[10]

Here we demonstrate for a helical transmembrane protein complex that it is possible to get access to four classes of distance constraints: (i) along the helix for assignment of signals, (ii) between helix side chains and cofactors, (iii) between amino acids of two subunits that form the monomer, and (iv) between amino acids of different monomers, paving the way for access to structure of complexes, such as secondary, tertiary, and quaternary structure, as well as contacts between the protein and cofactors or other ligands. The model system we use is the photosynthetic light-harvesting 2 (LH2) protein complex from the anaerobic *Rhodopseudomonas* (*Rps.*) *acidophila* strain 10050 purple bacterium.[16−18] A sequence-specific assignment of the NMR response was recently obtained for 76 of the 94 residues of the monomeric unit, and getting a structure from shifts is not possible.[10] The LH2 complex comprises a circular aggregate of nine identical monomeric units, each monomer a complex of two α-helical membrane spanning segments, that is, the α-subunit (53 residues) and β-subunit (41 residues) and enclosing three Bacteriochlorophyll *a* (BChl *a*) cofactors.

The profusion of protons which are in close proximity within the helix and more specifically between side chains in adjoining helical segments makes ^{1}H−^{1}H spin diffusion a viable option for identifying inter-helical constraints in the LH2 complex.[19] The favorable polarization properties of ^{1}H combined with the spectral resolution of ^{13}C nuclei in the 2D ^{13}C−^{13}C magic angle spinning (MAS) CHHC/CP^3 experiment have been used successfully in the past to resolve a model for the 3D stacking in self-aggregated, uniformly enriched chlorophyll *a*/H_2O and for the 3D structure determination of β-sheet polypeptides.[3,20−23] Recently, there has been a discussion going on as to whether or not long-range ^{1}H transfer between helical segments is truncated by relayed intra-helical transfer. In this study, we demonstrate that by using the CHHC/CP^3 experiment on uniformly ^{13}C labeled as well as biosynthetic site-specific ^{13}C pattern labeled samples of the LH2 complex at very short mixing times (200−350 μs) it is very well possible to detect through-space long-range intermolecular correlations between the two α-helical membrane spanning segments which make up the monomeric unit of the LH2 complex as well as through-space correlations between the amino acid residues and the labeled BChl *a* via ^{1}H−^{1}H spin diffusion.

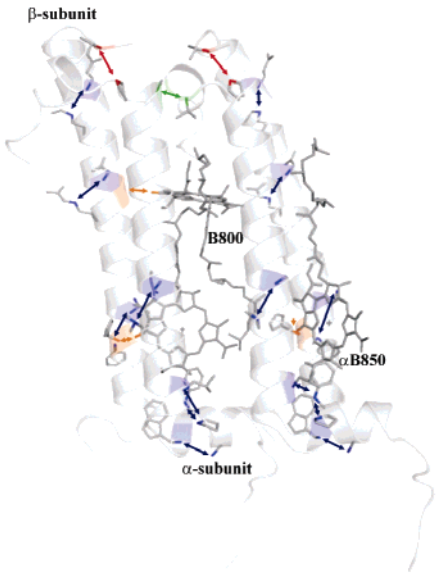

Figure 1. The structure of two monomeric units of the LH2 complex derived from the 1NKZ PDB file.[18] The βB850 cofactor is not shown. The red arrow indicates the pair, corresponding to an inter-helical inter-monomeric correlation between the α_1V10 and α_2A13 residues; the green arrow shows inter-helical intra-monomeric correlations between the βT2 and αP12 residues; the orange arrows indicate cofactor−residue contacts between the αB850 cofactor and the βH30 residue as well as the B800 cofactor and βG18 residue; and the remaining blue arrows point to inter-residue correlations along the helix.

The ^{13}C-enriched samples were obtained biosynthetically by growing the bacteria anaerobically in light at 30 °C on a defined medium. The pattern labeled ^{13}C LH2 sample (2.3-LH2) was prepared by using isotopically labeled [2,3-^{13}C]-succinic acid as the nutrient source in the expression medium. The procedures for the sample preparation and the labeling patterns of all the amino acids and the BChl *a* cofactors in the U-LH2 and 2.3-LH2 samples have been described in detail elsewhere.[24]

Two-dimensional CHHC/CP^3 (see ref 23) spectra were recorded at ^{1}H diffusion times of 200 and 325 μs for the U-LH2 sample and at 250 and 325 μs for the 2.3-LH2 sample. A detailed experimental section has been given in the Supporting Information. Characteristic datasets for the U-LH2 sample are shown in Figure 2 and in Figure S2 for the 2.3-LH2 sample.

For the CHHC/CP^3 ^{1}H spin diffusion experiment, an effective maximum ^{1}H−^{1}H transfer range d_{max} of ∼3.0 Å has been

10.1021/ja0664436 CCC: $37.00

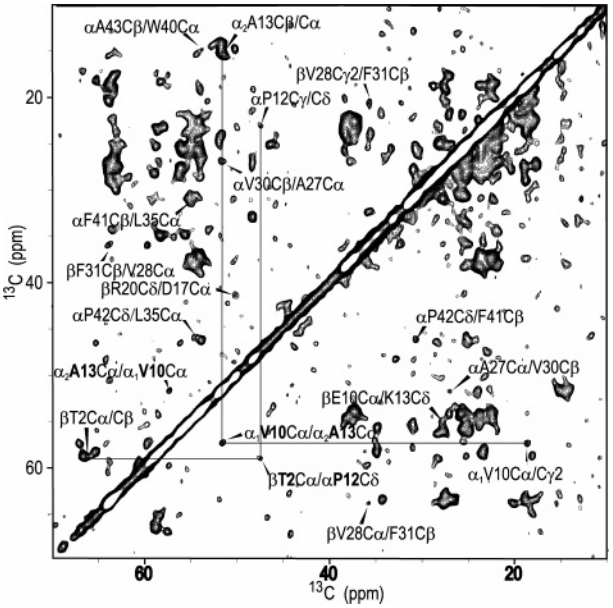

Figure 2. Aliphatic region of a CHHC/CP^3 dataset collected from the U-LH2 sample at $\omega_r/2\pi = 12\,000$ Hz and with 200 μs ^{1}H$-^1$H spin diffusion mixing time.

experimentally determined from a wide range of intra-residue ^{13}C$-^{13}$C cross-peaks using ^{1}H$-^1$H mixing times of 200$-$350 μs.[22,25,26] The CHHC/CP^3 spectra of the U-LH2 sample for a short mixing time of 200 μs give us over 30 additional unique cross-peaks when compared to the 2D ^{13}C$-^{13}$C proton-driven spin diffusion dataset published previously for a 50 ms mixing time.[10,27] A few of these new peaks can be assigned to intra-residue correlations, thereby giving us additional chemical shift information. Utilizing the estimated d_{max}, we have been able to assign an inter-helical distance constraint between α_1V10 and α_2A13 in the α-subunits of two adjacent monomeric units. They have the shortest ^{1}H$-^1$H distance of 2.60 Å. We have also detected another inter-helical transfer event over a 2.85 Å ^{1}H$-^1$H distance, between αP12 in the α-subunit and βT2 in the β-subunit of a single monomer. Due to the reduced labeling of the 2.3-LH2 sample, the CHHC/CP^3 spectrum is less crowded and better resolved for a short mixing time of 250 μs. By virtue of d_{max}, we are able to assign unique through-space cofactor$-$residue correlations between the αB850 cofactor and the βH30 residue as well as the B800 cofactor and βG18 residue in the spectrum (Supporting Information). Most of the predicted inter-subunit contacts from the X-ray structure that fall within d_{max} but whose cross-peaks are not seen in the CHHC/CP^3 spectrum are from C-terminal residues αQ46-αA53 in the α-subunit and βL40-βH41 in the β-subunit.[18] This indicates a flexible or disordered C-terminus. The remaining predicted contacts that could not be assigned involve methyl groups from side chains or aromatic side chains.[22]

The unique correlations that are observed between carbons from the BChl *a* cofactor and the LH2 protein provide structural information on the active site and on how the organized protein complex interacts with the cofactor. Only with access to such information may *de novo* design of self-assembled biomimetic nanomachines become reality. In conclusion, the CHHC/CP^3 experiment helps to discriminate between intra-helical and inter-helical constraints. This leads to the identification of new through-space distance constraints and an improved chemical shift assignment of a helical membrane protein complex, which is the next logical step toward determination of its secondary, tertiary, and quaternary structure, along with protein$-$cofactor interactions.

Acknowledgment. The support of J. Hollander is gratefully acknowledged. This research was supported by NWO-CW, EU Grant BIO4-CT97-2101, and Bruker.

Supporting Information Available: Experimental section as well as additional 1D and 2D spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Castellani, F.; van Rossum, B.; Diehl, A.; Schubert, M.; Rehbein, K.; Oschkinat, H. *Nature* **2002**, *420*, 98−102.
(2) Zech, S. G.; Wand, A. J.; McDermott, A. E. *J. Am. Chem. Soc.* **2005**, *127*, 8618−8626.
(3) Lange, A.; Becker, S.; Seidel, K.; Giller, K.; Pongs, O.; Baldus, M. *Angew. Chem., Int. Ed.* **2005**, *44*, 2089−2092.
(4) McDermott, A.; Polenova, T.; Bockmann, A.; Zilm, K. W.; Paulsen, E. K.; Martin, R. W.; Montelione, G. T. *J. Biomol. NMR* **2000**, *16*, 209−219.
(5) Pauli, J.; Baldus, M.; van Rossum, B.; de Groot, H.; Oschkinat, H. *Chembiochem* **2001**, *2*, 272−281.
(6) Bockmann, A.; Lange, A.; Galinier, A.; Luca, S.; Giraud, N.; Juy, M.; Heise, H.; Montserret, R.; Penin, F.; Baldus, M. *J. Biomol. NMR* **2003**, *27*, 323−339.
(7) Igumenova, T. I.; Wand, A. J.; McDermott, A. E. *J. Am. Chem. Soc.* **2004**, *126*, 5323−5331.
(8) Igumenova, T. I.; McDermott, A. E.; Zilm, K. W.; Martin, R. W.; Paulson, E. K.; Wand, A. J. *J. Am. Chem. Soc.* **2004**, *126*, 6720−6727.
(9) Franks, W. T.; Zhou, D. H.; Wylie, B. J.; Money, B. G.; Graesser, D. T.; Frericks, H. L.; Sahota, G.; Rienstra, C. M. *J. Am. Chem. Soc.* **2005**, *127*, 12291−12305.
(10) van Gammeren, A. J.; Hulsbergen, F. B.; Hollander, J. G.; de Groot, H. J. M. *J. Biomol. NMR* **2005**, *31*, 279−293.
(11) de Boer, I.; Matysik, J.; Amakawa, M.; Yagai, S.; Tamiaki, H.; Holzwarth, A. R.; de Groot, H. J. M. *J. Am. Chem. Soc.* **2003**, *125*, 13374−13375.
(12) Jaroniec, C. P.; MacPhee, C. E.; Bajaj, V. S.; McMahon, M. T.; Dobson, C. M.; Griffin, R. G. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 711−716.
(13) Heise, H.; Hoyer, W.; Becker, S.; Andronesi, O. C.; Riedel, D.; Baldus, M. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 15871−15876.
(14) Siemer, A. B.; Ritter, C.; Ernst, M.; Riek, R.; Meier, B. H. *Angew. Chem., Int. Ed.* **2005**, *44*, 2441−2444.
(15) Creemers, A. F. L.; Kiihne, S.; Bovee-Geurts, P. H. M.; DeGrip, W. J.; Lugtenburg, J.; de Groot, H. J. M. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 9101−9106.
(16) McDermott, G.; Prince, S. M.; Freer, A. A.; Hawthornthwaite-Lawless, A. M.; Papiz, M. Z.; Cogdell, R. J.; Isaacs, N. W. *Nature* **1995**, *374*, 517−521.
(17) Prince, S. M.; Papiz, M. Z.; Freer, A. A.; McDermott, G.; Hawthornthwaite-Lawless, A. M.; Cogdell, R. J.; Isaacs, N. W. *J. Mol. Biol.* **1997**, *268*, 412−423.
(18) Papiz, M. Z.; Prince, S. M.; Howard, T.; Cogdell, R. J.; Isaacs, N. W. *J. Mol. Biol.* **2003**, *326*, 1523−1538.
(19) Schmidt-Rohr, H. W.; Spiess, K. *Multidimensional Solid-State NMR and Polymers*; Academic Press: London, 1994.
(20) Wilhelm, M.; Feng, H.; Tracht, U.; Spiess, H. W. *J. Magn. Reson.* **1998**, *134*, 255−260.
(21) Mulder, F. M.; Heinen, W.; van Duin, M.; Lugtenburg, J.; de Groot, H. J. M. *J. Am. Chem. Soc.* **1998**, *120*, 12891−12894.
(22) de Boer, I.; Bosman, L.; Raap, J.; Oschkinat, H.; de Groot, H. J. M. *J. Magn. Reson.* **2002**, *157*, 286−291.
(23) Lange, A.; Luca, S.; Baldus, M. *J. Am. Chem. Soc.* **2002**, *124*, 9704−9705.
(24) van Gammeren, A. J.; Hulsbergen, F. B.; Hollander, J. G.; de Groot, H. J. M. *J. Biomol. NMR* **2004**, *30*, 267−274.
(25) van Rossum, B. J.; Schulten, E. A. M.; Raap, J.; Oschkinat, H.; de Groot, H. J. M. *J. Magn. Reson.* **2002**, *155*, 1−14.
(26) Lange, A.; Seidel, K.; Verdier, L.; Luca, S.; Baldus, M. *J. Am. Chem. Soc.* **2003**, *125*, 12640−12648.
(27) Szeverenyi, N. M.; Sullivan, M. J.; Maciel, G. E. *J. Magn. Reson.* **1982**, *47*, 462−475.

JA0664436