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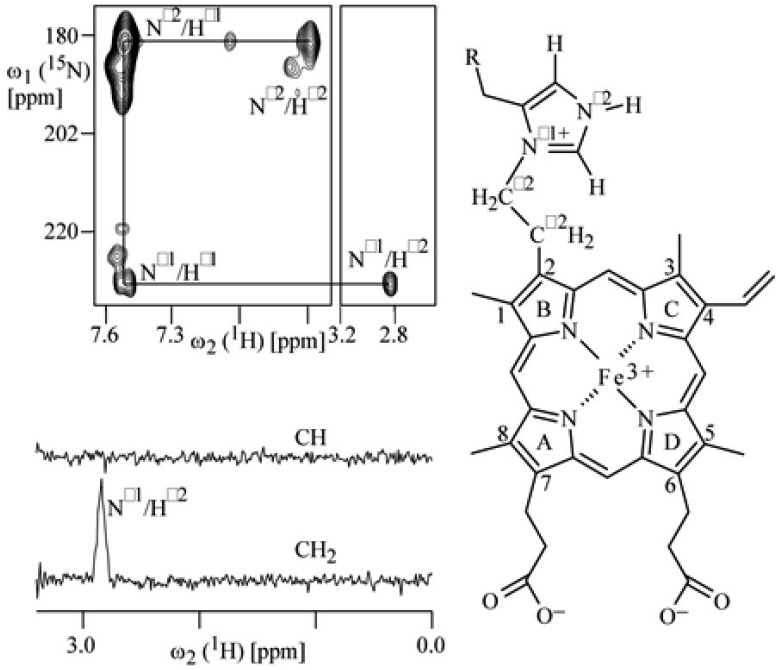

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Unusual Heme−Histidine Bond in the Active Site of a Chaperone

Donghan Lee,[†,§] Konstantin Pervushin,*[,†] Daniela Bischof,[‡] Martin Braun,[‡] and Linda Thöny-Meyer[‡]

Laboratory of Physical Chemistry and Institute of Microbiology, ETH Hönggerberg, CH-8093 Zürich, Switzerland

Received September 3, 2004; E-mail: kope@bionmr.phys.chem.ethz.ch

Heme is the redox-active cofactor of cytochromes involved in respiratory and photosynthetic electron transfer, the prosthetic group of numerous proteins, like hemoglobins, catalases, peroxidases, guanylate cyclase, and NO synthase,[1] or the sensor of regulatory pathways for O_2-, CO-, or NO-dependent gene expression.[2] Transport and delivery of heme to the proteins that carry it are scarcely understood. Recently, it was found that a bacterial heme chaperone, CcmE, binds heme in the periplasm and delivers it to cytochromes of the *c* type.[3] It thus acts as an intermediate of a heme delivery pathway during cytochrome *c* biogenesis. It has also been shown that CcmE forms an unusual transient, yet covalent, bond between an essential histidine, H130, and heme.[4] Moreover, the solution structure of the soluble domain of apo-CcmE in combination with site-directed mutagenesis revealed a hydrophobic surface as the putative heme binding site.[5] When heme was positioned optimally to that surface, the 2-vinyl of heme was within binding distance to H130. Here, we report on the discovery of the chemical structure of this bond by NMR, where the heme 2-vinyl or 4-vinyl is cross-linked at the β carbon to the $N^{\delta 1}$ of H130.

A C-terminally truncated version of soluble CcmE lacking the last 19 amino acid residues still bound heme at levels similar to those of wild-type CcmE. This version, CcmE30-140, was histidine-tagged with six C-terminal histidines. A quantity of 28 mg of apo-/holo-CcmE was purified from 2l ^{13}C, ^{15}N-labeled cells grown in Celtone CN medium (Martek). After digestion with trypsin, the peptide heme-HDENYTPPEVEHHHHHH with a mass of 2925.45 (*m*/*z*), suggesting that isotope labeling had occurred to 96% (calculated mass of ^{15}N, ^{13}C-doubly labeled heme-CcmE peptide is 2931 Da), was purified by nickel affinity chromatography and its mass confirmed by MALDI-TOF mass spectroscopy (Applied Biosystems Voyager-DE Elite). The isolated histidine-tagged heme peptide was stable after formation of a cyanide complex and was thus suitable for NMR analysis, whereas the dithionite-reduced peptide formed aggregated.

All NMR measurements were recorded on a Bruker DRX 600 spectrometer with Cryo Probe equipped with shielded z-gradient coils at 25 °C and pH 7.2. A comparison of the [^1H,^{13}C] HSQC spectra of the biscyano heme−CcmE peptide complex and biscyano complex of free iron−protoporphyrin IX[6,7] demonstrates chemical modifications of one of two vinyl groups in the heme−peptide which is recruited for the covalent bond with the H130 of CcmE. The resolved heme methyl resonances in the heme−peptide are shifted compared to those in bis-[CN] iron−protoporphyrin IX (see Supporting Information), which might indicate some other coordination rather than bis-[CN][6−11] and calling for a complete assignment of the whole peptide. This task is compounded by the problem of unusually fast transverse relaxation of all spins in the heme−peptide, both problems are probably due to the presence of the His-tag and are relegated to future work.

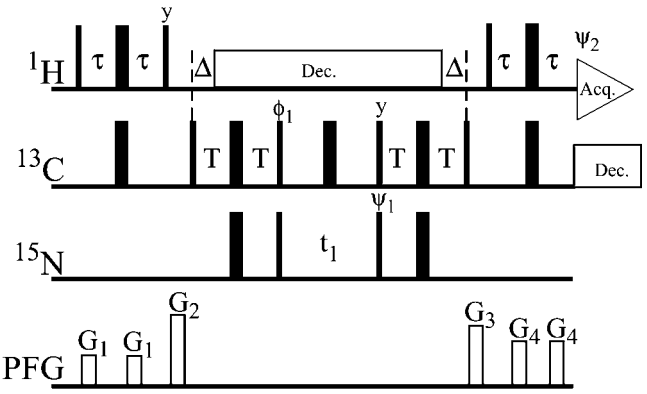

Figure 1. Pulse scheme for the TROSY-HCN to establish spin topology in CH and CH_2 groups. The radio frequency pulses on ^1H, ^{13}C, and ^{15}N are applied at 4.7, 100, 205 ppm, respectively. Narrow and wide bars stand for nonselective pulses. The line marked PFG indicates the duration and strength of pulsed field gradients applied along the *z*-axis: G_1 500 μs, 50 G/cm; G_2 500 μs, 80 G/cm; G_3 500 μs, 70 G/cm; G_4 500 μs, 60 G/cm. GARP decoupling on ^{13}C was used during the acquisition. The delays $\tau = 1.3$ ms and $T = 7.14$ ms were adjusted for optimal sensitivity. For the CH and CH_2 group selection experiment, the delay Δ was selected to $1/(2\ ^1J_{CH})$ and $1/(4\ ^1J_{CH})$ for the CH and CH_2 group selections, respectively, in addition to DIPSI-2 decoupling on ^1H. For the TROSY-HCN experiment, the decoupling on ^1H was omitted. The phase cycles are $\phi_1 = \{y, y, -y, -y\}$, $\psi_1 = \{x, -x\}$, $\psi_2 = \{x, -x, -x, x\}$, and $\{x\}$ otherwise. Quadrature detection in the $^{15}N(t_1)$ dimension is achieved by the States−TPPI method applied to the phase ψ_1.

To identify the nature of the covalent bond, the 2D TROSY-HCN[12] spectra of the heme−CcmE peptide complex were measured (Figure 1A). In the experiment, one and up to three cross-peaks corresponding to $N^{\epsilon 2}-C^{\epsilon 1}H$, $N^{\epsilon 2}-C^{\delta 2}H$, and $N^{\delta 1}-C^{\epsilon 1}H$ connectivities are expected, depending on the tautomeric states of the histidine side chain.[13] In addition to the signals from the histidine side chain, an upfield cross-peak was observed, suggesting an additional covalent connection ($N^{\delta 1}$ to aliphatic carbon), in agreement with the formation of a bond with the modified heme vinyl group. In principle, both the α and β carbons of the heme vinyl could form a bond with the $N^{\delta 1}$ of H130 of the CcmE peptide. The two possibilities can be distinguished because in one case, the α carbon becomes a CH group and the β carbon into a CH_3 group, whereas in the other case, both carbons become CH_2 groups. Since the magnetization transfer from ^{13}C to ^1H in a CH_2 group is twice as fast as that in a CH group, the isomers can be easily distinguished in the 2D TROSY-HCN experiment by choosing the different transfer time.[14] The one-bond scalar coupling between the ^{13}C and ^1H of the vinyl was determined with the 2D TROSY-HCN experiment by omitting ^{13}C decoupling during the acquisition period ($^1J_{CH} = 140$ Hz).

For distinguishing CH/CH_2 isomers, the conventional 2D TROSY-HCN experiment was modified with an additional magnetization transfer period, in which the antiphase magnetization of ^{13}C refocuses to the in-phase magnetization with the transfer rate

[†] Laboratory of Physical Chemistry.
[‡] Institute of Microbiology.
[§] Present address: NCI-Frederick, NIH, Frederick, MD 21702.

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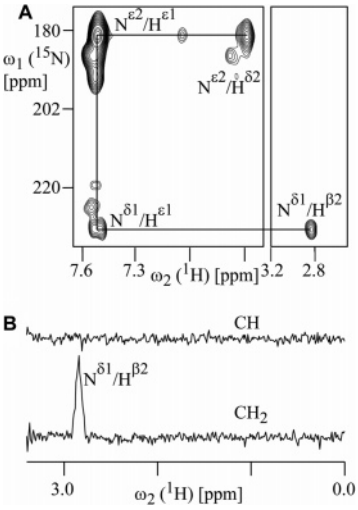

Figure 2. (A) Two-dimensional TROSY-HCN spectra of the covalent CcmE−heme complex. Connectivities suggesting the covalent bonding are indicated. (B) One-dimensional traces of 2D TROSY-HCN spectra with the selection of CH (upper) and CH_2 (lower) groups, showing that the β carbon of the modified heme 2-vinyl is attached to the histidine side chain of CcmE.

depending on the number of attached protons. By choosing this transfer time to $1/(2\ {}^1J_{CH})$, only the CH group is expected to give a signal, while the square root and one-half of signal intensity for CH and CH_2 groups, respectively, are expected when setting the transfer time to $1/(4\ {}^1J_{CH})$. The results show that an upfield cross-peak is present only in the CH_2 group selection but not in the CH group selection (Figure 2B). Thus, the β carbon of the heme vinyl group makes a covalent bond with $N^{\delta 1}$ of the histidine, representing a novel type of heme−histidine complex (Scheme 1).

The involvement of a heme vinyl group in the formation of the covalent heme−histidine complex was postulated previously.[4,5,15] Normally, covalent heme adducts are formed at the α carbon according to the Markovnikov rule[16] by an electrophilic addition to the vinyl group. For a covalent bond with the β carbon, radicals are involved in the reaction mechanism following the anti-Markovnikov rule.[16] Thus, the formation of the CcmE−heme adduct may require a radical mechanism. Recently, a covalent heme−histidine complex was found in a cyanobacterial hemoglobin; however, in that case, the $N^{\epsilon 2}$ of the histidine was bound to the α carbon of the heme 2-vinyl.[17] The novel heme derivative presented here has unique features that apparently allow the formation of a transient and yet covalent complex with the CcmE heme chaperone. Our work reveals proof of the covalent nature of the heme−H130 bond in the heme chaperone; absolute assignment to the 2- or 4-vinyl group will be addressed in future work.

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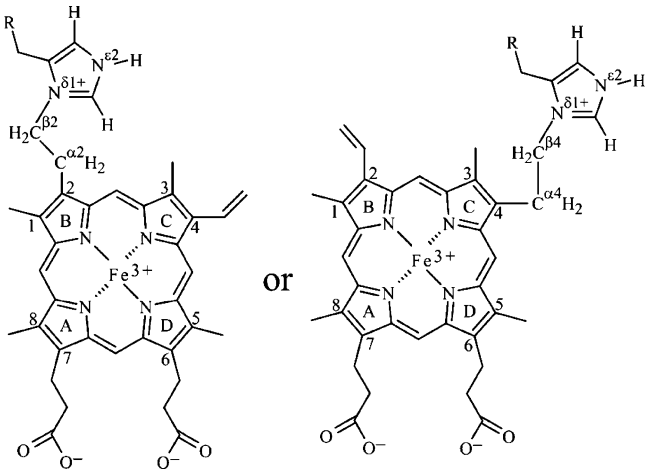
Scheme 1. Possible Chemical Structures of the Heme−Histidine Bond



Supporting Information Available: One-dimensional spectra of free heme and heme−histidine complex. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Ponka, P. *Am. J. Med. Sci.* **1999**, *318*, 241−256.
(2) Rodgers, K. R. *Curr. Opin. Chem. Biol.* **1999**, *3*, 158−167.
(3) Schulz, H.; Hennecke, H.; Thöny-Meyer, L. *Science* **1998**, *281*, 1197−1200.
(4) Thöny-Meyer, L. *Biochemistry* **2003**, *42*, 13099−13105.
(5) Enggist, E.; Thöny-Meyer, L.; Güntert, P.; Pervushin, K. *Structure* **2002**, *10*, 1551−1557.
(6) Barbush, M.; Dixon, D. W. *Biochem. Biophys. Res. Commun.* **1985**, *129*, 70−75.
(7) Wüthrich, K.; Shulman, R. G.; Wyluda, B. J.; Caughey, W. S. *Proc. Natl. Acad. Sci. U.S.A.* **1969**, *62*, 636−643.
(8) Lamar, G. N.; Viscio, D. B.; Smith, K. M.; Caughey, W. S.; Smith, M. L. *J. Am. Chem. Soc.* **1978**, *100*, 8085−8091.
(9) Yu, C.; Unger, S. W.; Lamar, G. N. *J. Magn. Reson.* **1986**, *67*, 346−350.
(10) Shulman, R. G.; Glarum, S. H.; Karplus, M. *J. Mol. Biol.* **1971**, *57*, 93.
(11) Vu, B. C.; Vuletich, D. A.; Kuriakose, S. A.; Falzone, C. J.; Lecomte, J. T. J. *J. Biol. Inorg. Chem.* **2004**, *9*, 183−194.
(12) Riek, R.; Pervushin, K.; Fernandez, C.; Kainosho, M.; Wüthrich, K. *J. Am. Chem. Soc.* **2001**, *123*, 658−664.
(13) Sudmeier, J. L.; Ash, E. L.; Gunther, U. L.; Luo, X. L.; Bullock, P. A.; Bachovchin, W. W. *J. Magn. Reson. Ser. B* **1996**, *113*, 236−247.
(14) Ernst, R. R.; Bodenhausen, G.; Wokaun, A. *Principles of Nuclear Magnetic Resonance in One and Two Dimensions*; Oxford University Press: Oxford, 1990.
(15) Stevens, J. M.; Daltrop, O.; Higham, C. W.; Ferguson, S. J. *J. Biol. Chem.* **2003**, *278*, 20500−20506.
(16) Loundon, G. M. *Organic Chemistry*. Oxford University Press: New York, 2002.
(17) Vu, B. C.; Jones, A. D.; Lecomte, J. T. J. *J. Am. Chem. Soc.* **2002**, *124*, 8544−8545.

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