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Orientation of Deoxyhemoglobin at High Magnetic Fields: Structural Insights from RDCs in Solution

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Recent NMR structural studies using $^{15}N-^{1}H$ residual dipolar coupling (RDC) measurements have shown that the solution structure of human normal adult carbonmonoxyhemoglobin (HbCO A) is distinctly different from the classical crystal structures (R and R2) for HbCO A; in fact, it is a dynamic ensemble between the R and R2 structures.[1] Supporting the facile interconversion of these structures, recent X-ray crystallographic results indicate that a number of R-type structures for HbCO A can be obtained under different crystallization conditions.[2−5] There are also several published crystal structures for deoxyhemoglobin A (deoxy-Hb A),[6−8] but no solution structure for this protein has been reported. The functional properties of Hb A in single crystals and in solution are very different.[9−11] Thus, to correlate the structure, dynamics, and function of Hb A, we need to determine its structures in solution in both ligated and unligated (deoxy) forms. We present data on the deoxy form in the following paragraphs.

Recent developments, including transverse relaxation-optimized spectroscopy (TROSY) and RDC collection methods, made it possible to use NMR for the study of larger proteins and protein complexes in solution. There are, however, several technical challenges that need to be overcome before one can apply these techniques for the characterization of deoxy-Hb A in solution. Among them is the problem of spectral assignment, hampered by the broadening and shifting of some resonances by the paramagnetism of the heme groups in deoxy-Hb A. Another challenge lies in difficulties encountered when using typical alignment media (such as bicelles, stretched gels, etc.) as the entire alignment system must be in a gastight, oxygen-free environment. However, the paramagnetism of deoxy-Hb A may actually provide a solution to this latter challenge by allowing us to align the molecule at high magnetic fields for the RDC measurement without the aid of external alignment media.[12,13] Single molecule field-induced alignment depends on the anisotropy of the magnetic susceptibility tensor. Although a study on deoxymyoglobin has been reported recently,[14] there is thus far no knowledge of the actual magnetic susceptibility tensor of the hemes in hemoglobin in the deoxy state, making the possibility of field-induced alignment somewhat unpredictable.

To demonstrate alignment, one-bond $^{1}H-^{15}N$ spin−spin couplings ($^{1}J_{NH} + {}^{1}D_{NH}$) have been measured at four different magnetic fields (11.7, 14.1, 19.8 and 21.1 T) on Bruker DRX-500, Bruker DRX-600, Varian Inova-800, and Varian Inova-900 spectrometers, equipped with triple resonance ($^{1}H,^{13}C,^{15}N$) cryogenic or conventional probes and pulsed-field gradients. The spin−spin couplings were measured using interleaved heteronuclear single quantum coherence (HSQC) and temperature-compensated TROSY pulse sequences.[15] The chain-specific ($^{15}N,^{2}H$)-labeled recombinant Hb A samples were prepared as described previously.[16]

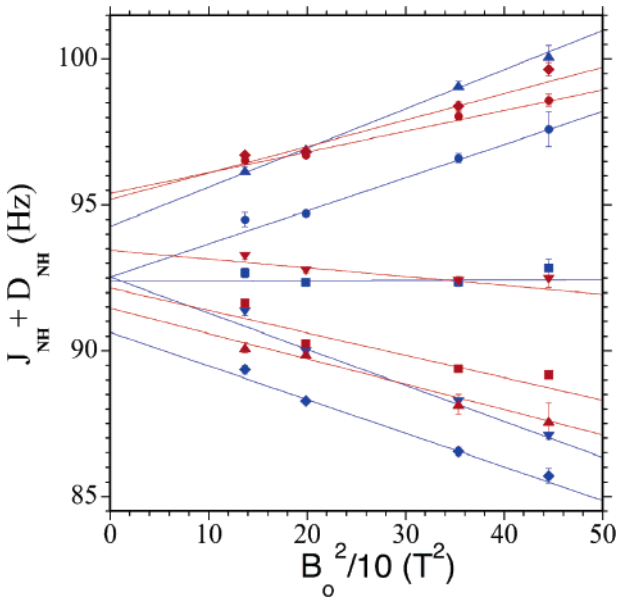

Figure 1. Magnetic field dependence of observed ($^{1}J_{NH} + {}^{1}D_{NH}$) couplings of chain-specific ($^{15}N,^{2}H$)-labeled recombinant deoxy-Hb A. As an illustration, we selected five amino acid residues each from the α(blue)- and β-(red)-chains of Hb A: αT39 (blue ●) and βG24 (red ●), αH45 (blue ◆) and βG25 (red ◆), αF46 (blue ▼) and βR30 (red ▼), αS81 (blue ■) and βQ39 (red ■), and αV96 (blue ▲) and βF42 (red ▲). ($^{1}J_{NH} + {}^{1}D_{NH}$) values in Hz are plotted versus the square of the magnetic field (B_0) in T^2 (Tesla^2). The solid lines represent the linear fits of the data to the equation: $^{1}J_{NH} = {}^{1}J_{NH}$ (iso) + cB_0^2, where c is the slope. The intercept to the Y-axis $^{1}J_{NH}$ (iso) is the true isotropic $^{1}J_{NH}$ value, which is used for the determination of RDCs.

When data at several field strengths are available, the residual dipolar couplings can be determined from the slope of the field dependence of the spin−spin coupling constants if an alignment is present. Magnetic field alignment of deoxy-Hb A in solution induced by the anisotropic magnetic susceptibility of the high-spin Fe^{2+} hemes is indeed observed (Figure 1), thus opening the possibility of using RDC data for structural studies on deoxy-Hb, especially at the highest magnetic fields available today.

Recently, we have assigned about 80% of the backbone resonances of both the α- and β-chains of deoxy-Hb A using TROSY-based triple-resonance NMR experiments on chain-specifically ($^{2}H,^{15}N,^{13}C$)-labeled samples.[17] This allows us to calculate an alignment tensor and compare the observed $^{15}N-^{1}H$ RDCs to values calculated using a recent, high-resolution crystal structure of deoxy-Hb A (1.91 Å; pdb ID: 1XXT).[8] The comparison of experimental and back-calculated RDCs shows a reasonably good agreement between data sets (Figure 2). However, the deviations are a little larger than the experimental errors suggested by the field-dependent plots (Figure 1). Also, the errors are in many cases larger

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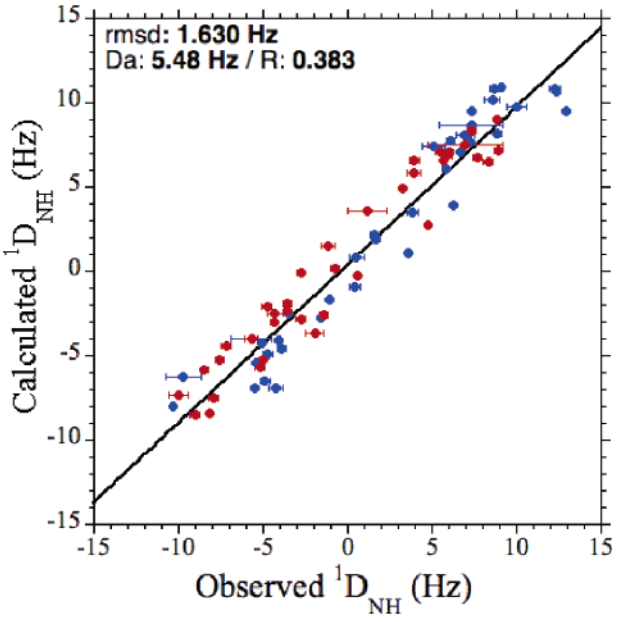

Figure 2. Correlation between the observed RDCs and calculated ones, based on the crystal structures of deoxy-Hb A (pdb ID: 1XXT). Well-resolved amino acid residues (38 and 37 in number) from the α- and β-chains are used, and their RDCs are distinguished by the colors blue and red, respectively. The line indicates the weighted least-squares fit of the data, and the root-mean-square deviation (rmsd) is 1.63 Hz.

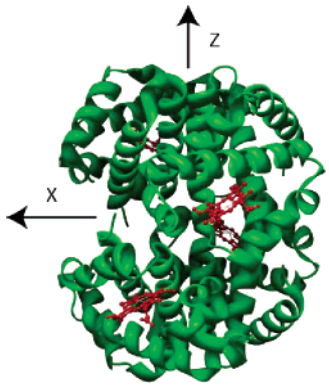

Figure 3. Structure of deoxy-Hb A with axes of the magnetic susceptibility tensor superimposed. The crystal structure 1XXT was used in the calculation, and RDC data were applied, assuming exact symmetry relationships of the chains: $\alpha_1-\alpha_2$ and $\beta_1-\beta_2$. The program REDCAT[21] was used to determine the susceptibility tensor axes, and the modeling program CHIMERA[22] was used to add hydrogens and produce the figure.

than those expected from "structural noise" (about 10% of the maximum coupling, i.e., ∼1.3 Hz).[18] These facts suggest that there may be some differences between the solution- and crystal structures of deoxy-Hb A.

The principal alignment frame, as determined from the calculations, is shown superimposed on the 1XXT structure[8] in Figure 3. The axes are in the directions appropriate for a susceptibility tensor dominated by the combined effects of individual heme susceptibility tensors. The magnitude of the anisotropy is consistent with individual anisotropies of susceptibilities approximately equal to that of the $S = 1/2$ state of cyanometmyoglobin.[19]

The RDC data presented above will be used to refine a structure for the solution form of deoxy-Hb A. This normally requires additional RDC data.[20] As a step in this direction, Pf1 phage has been used successfully to align deoxy-Hb A, and its RDCs have been measured in this medium (results not shown). Due to the viscosity of the Pf1 medium, however, it is difficult to prepare the deoxy-Hb A sample, and the measurements are further hampered by increased line widths. Another option involves additional isotopic labeling of deoxy-Hb A for field-induced alignment measurements.

Our present results demonstrate clearly the feasibility of achieving spontaneous preferential orientation of deoxy-Hb A in solution at high magnetic fields and provide a first step for a detailed structural determination of deoxy-Hb A in solution, a state which better approximates the physiological conditions.

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References

(1) Lukin, J. A.; Kontaxis, G.; Simplaceanu, V.; Yuan, Y.; Bax, A.; Ho, C. *Proc. Natl. Acad. Sci. U.S.A* **2003**, *100*, 517−520.
(2) Silva, M. M.; Rogers, P. H.; Arnone, A. *J. Biol. Chem.* **1992**, *276*, 17248−172.
(3) Mueser, T. C.; Rogers, P. H.; Arnone, A. *Biochemistry* **2000**, *39*, 15353−15364.
(4) Safo, M. K.; Abraham, D. J. *Protein Sci.* **2001**, *10*, 1091−1099.
(5) Safo, M. K.; Abraham, D. J. *Biochemistry* **2005**, *44*, 8347−8359.
(6) Fermi, G.; Perutz, M. F.; Shaanan, B.; Fourme, R. *J. Mol. Biol.* **1984**, *175*, 159−174.
(7) Tame, J. R.; Vallone, B. *Acta. Crystallogr., Sect. D: Biol. Crystallogr.* **2000**, *56 (Pt 7)*, 805−811.
(8) Kavanaugh, J. S.; Rogers, P. H.; Arnone, A. *Biochemistry* **2005**, *44*, 6101−6121.
(9) Eaton, W. A.; Henry, E. R.; Hofrichter, J.; Mozzarelli, A. *Nat. Struct. Biol.* **1999**, *6*, 351−358.
(10) Lukin, J. A.; Ho, C. *Chem. Rev.* **2004**, *104*, 1219−1230.
(11) Barrick, D.; Lukin, J. A.; Simplaceanu, V.; Ho, C. *Methods Enzymol.* **2004**, *379*, 28−54.
(12) Bertini, I.; Luchinat, C.; Parigi, G.; Pierattelli, R. *ChemBioChem* ***2005***, *6*, *1536−1549*.
(13) Prestegard, J. H.; Bougault, C. M.; Kishore, A. I. *Chem. Rev.* **2004**, *104*, *3519−3540*.
(14) Bertini, I.; Luchinat, C.; Turano, P.; Battaini G.; Casella, L. *Chem. Eur. J.* **2003**, 9, 2316−2322
(15) Bax, A.; Kontaxis, G.; Tjandra, N. *Methods Enzymol.* **2001**, *339*, 127−174.
(16) Simplaceanu, V.; Lukin, J. A.; Fang, T.-Y.; Zou, M.; Ho, N. T.; Ho, C. *Biophys. J.* **2000**, *79*, 1146−1154.
(17) Sahu, S. C.; Simplaceanu, V.; Ho, N. T.; Giovannelli, J. L.; Ho, C. *J. Biomol. NMR* **2005**. In press.
(18) Zweckstetter, M.; Bax, A. *J. Biomol. NMR* **2002**, *23*, 127−137.
(19) Tolman, J. R.; Flanagan, J. M.; Kennedy, M. A.; Prestegard, J. H. *Nat. Struct. Biol.* **1997**, *4*, 292−297.
(20) Seidel, R. D., III; Amor, J. C.; Kahn, R. A.; Prestegard, J. H. *J. Biol. Chem.* **2004**, *279*, 48307−48318.
(21) Valafar, H.; Prestegard, J. H. *J. Magn. Reson.* **2004**, *167*, 228−241.
(22) Pettersen, E. F.; Goddard, T. D.; Huang, C. C.; Couch, G. S.; Greenblatt, D. M.; Meng, E. C.; Ferrin, T. E. *J. Comput. Chem.* **2004**, *25*, 1605−1612.

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