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Letter to the Editor: Backbone resonance assignments of human adult hemoglobin in the carbonmonoxy form

Jonathan A. Lukin[a,*,**], Georg Kontaxis[b,*,***], Virgil Simplaceanu[a,*], Yue Yuan[a,****], Ad Bax[b] & Chien Ho[a,*****]

[a]*Department of Biological Sciences, Carnegie Mellon University, Pittsburgh, PA 15213, U.S.A.;* [b]*Laboratory of Chemical Physics, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892, U.S.A.*

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Biological context

Hemoglobin is the oxygen carrier in the blood and is responsible for carrying oxygen from the lungs to the tissues. Human normal adult hemoglobin (Hb A) is a C_2-symmetric tetrameric protein of approximately 65 kD, consisting of two 141-residue α-chains and two 146-residue β-chains. Each globin chain contains a heme group ligating one ferrous iron, which can combine reversibly with gaseous molecules, including O_2, CO, and NO. In the ligated form, Hb A is a diamagnetic molecule. However, in the unligated from, i.e. deoxyhemoglobin, it is paramagnetic with four unpaired electrons in each iron atom. The oxygenation of Hb A is cooperative, and the oxygen affinity of Hb A depends on pH (the Bohr effect) and is also regulated by intracellular metabolites, e.g., 2,3-bisphosphoglycerate (2,3-BPG). Hb A is a classic example of a multimeric allosteric protein. Hb A undergoes a quaternary structural transition in going from the deoxy or T (tense) state to the ligated or R (relaxed) state (Perutz, 1970). The crystal structures of deoxy-Hb A and carbonmonoxy-Hb A (HbCO A) have been determined to very high resolution (refer to PDB ID codes 1A3N for the T structure of deoxy-Hb A, 1IRD for the R structure of HbCO A, and 1BBB for the R2 structure of HbCO A). A basic assumption in correlating protein structure and function is that the structure of a protein in the crystalline state is the same as that under physiological solution conditions. In spite of extensive studies on this protein molecule, the molecular basis for the cooperative oxygenation of Hb A is not fully understood and several aspects remain controversial. Oxygen-binding properties of single crystals of Hb A show that cooperativity and allosteric interactions are absent in the crystalline state (Eaton et al., 1999), in contrast to those properties observed in solution or inside red blood cells. For a review on the structure-function relationship of Hb A, see Ho and Lukin (2000). By measuring the ^{15}N-^{1}H residual dipolar couplings (RDCs) in weakly oriented (^{2}H,^{15}N)-labeled rHbCO A, we have found that the solution structure of HbCO A is a dynamic intermediate between R and R2 crystal structures (Lukin et al., 2003). Indeed, backbone RDCs are sensitive probes for monitoring changes in tertiary and quaternary structures as a function of ligation, and backbone hydrogen exchange rates can be used to probe the stability of hydrogen bonds (Englander and Englander, 1994). To allow a full exploitation of these parameters, we here report the backbone NMR assignments of both the α- and β-chains of HbCO A.

Methods and experiments

Chain-selectively (^{2}H,^{15}N)- and (^{2}H,^{13}C,^{15}N)-labeled HbCO A samples were prepared as described (Simplaceanu et al., 2000) using our hemoglobin expression system in *Escherichia coli.* In addition, several recombinant Hb A samples were produced using specific amino-acid labeling in order to aid our resonance

*Equal contribution to this work.
**Present address: Affinium Pharmaceuticals, Toronto, Canada.
***Present address: Department of Theoretical Chemistry and Molecular Structural Biology, University of Vienna, Vienna, Austria.
****Present address: Department of Chemistry, Simon Fraser University, British Columbia, Canada.
*****To whom correspondence should be addressed. E-mail: chienho@andrew.cmu.edu.

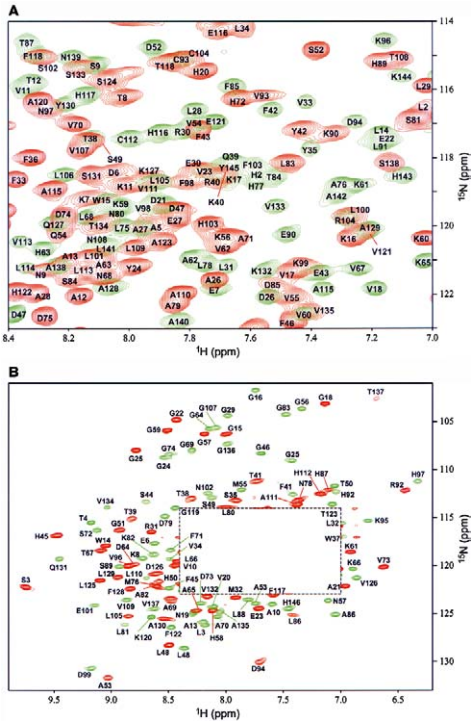

Figure 1. (A) Most crowded region of the superimposed 600 MHz (U-^{13}C/^{15}N/^{2}H) ^{1}H-^{15}N TROSY spectra of the α (red)- and β (green)-chains of rHbCO A. (B) Full spectrum of the amide resonances with the central crowded region shown in (A).

assignments. The Hb samples used for NMR measurements were generally 1 mM (tetramer) in 95% H_2O with 5% D_2O in 0.1 M sodium phosphate buffer at pH 7.0.

NMR experiments were carried out mostly at 29 °C, sometimes at 35 °C, on Bruker Avance DRX-500, DRX-600, and DRX-800 spectrometers, equipped with four or five RF channels, using triple resonance probes with triple-axis pulsed field gradients. Deuterium-decoupled, TROSY-based 3D triple resonance experiments HNCO, HNCA, HN(CO)CA, HN(CA)CO, HN(COCA)CB, and HN(CA)CB (Pervushin et al., 1998) with water flip-back were primarily used for backbone assignments. Additional information was obtained from 3D NOESY spectra at 500 and 600 MHz using mixing times of 100–300 ms. The NMR spectra were processed with NMRPipe (Delaglio et al., 1995) and analyzed with PIPP/CAPP (Garrett et al., 1991) as well as Fortran programs written in house. The assignment was also carried out using a new automated assignment program Monte (Hitchens et al., 2003).

Extent of assignment and data deposition

The secondary structure of both α- and β-chains is essentially α-helical, with connecting loops. As a result, spectral dispersion is relatively poor and required the use of chain-selectively labeled HbCO A samples (Figure 1). Complications due to solvent and conformational exchange added to the resolution problem, causing some of the resonances to be broad and weak. Nevertheless, we have assigned 89% of the α-chain and 94% of the β-chain of the backbone resonances for $^{1}H_N$, ^{15}N, $^{13}C_\alpha$ and $^{13}C'$, as well as determined the chemical shifts for many $^{13}C_\beta$ resonances. There are 9 residues plus 7 Pro residues (11%) in the α-chain and one residue plus 7 Pro residues (6%) in the β-chain for which the amide $^{1}H_N$ and ^{15}N resonances have not been assigned because they are not observed. A table of ^{1}H, ^{15}N and ^{13}C chemical shift assignments of HbCO A has been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under the accession number 5856.

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References

Delaglio, F., Grzesiek, S., Vuister, G., Zhu, G., Pfeifer, J. and Bax, A. (1995) *J. Biomol. NMR*, **6**, 277–293.

Eaton, W.A., Henry, E.R., Hofrichter, J.A. and Mozzarelli, A. (1999) *Nat. Struct. Biol.*, **6**, 351–358.

Englander, S.W. and Englander, J.J. (1994), *Meth. Enzymol.*, **232**, 26–42.

Garrett, D.S., Powers, R., Gronenborn, A.M. and Clore, G.M. (1991) *J. Magn. Reson.*, **95**, 214–220.

Hitchens, T.K., Lukin, J.A., Zhan, Y., McCallum, S.A. and Rule, G.S. (2003), *J. Biomol. NMR*, **25**, 1–9.

Ho, C. and Lukin, J.A. (2000) In *Embryonic Encyclopedia of Life Sciences*, Nature Publishing Group, London.

Lukin, J.A., Kontaxis, G., Simplaceanu, V., Yuan, Y., Bax, A. and Ho, C. (2003) *Proc. Natl. Acad. Sci.* USA, **100**, 517–520.

Perutz, M.F. (1970), *Nature*, **228**, 726–739.

Pervushin, K., Riek, R., Wider, G. and Wüthrich, K. (1998) *J. Am. Chem. Soc.*, **120**, 6394–6400.

Simplaceanu, V., Lukin, J.A., Fang, T.-Y., Zou, M., Ho, N.T. and Ho, C. (2000) *Biophys. J.*, **79**, 1146–1154.