



Chien Ho

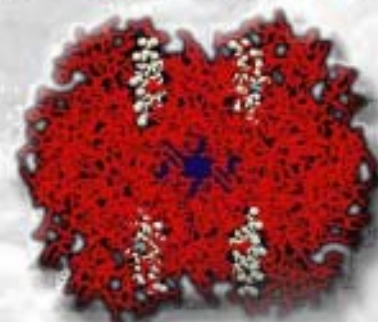
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Recent Multinuclear NMR Studies of Hemoglobin: New Insights into the Structure-Function Relationship in Hemoglobin in Solution at Atomic Resolution

Hemoglobin is one of the most studied proteins and has served as a model for understanding intra- and inter-molecular interactions and for gaining insights into signaling pathways in multimeric, allosteric proteins. The oxygenation process of human normal adult hemoglobin (Hb A) is cooperative and the oxygen-binding properties are modulated by interactions of specific amino acid residues with allosteric effectors. By comparing the crystal structures of Hb A in the deoxy and ligated states, Perutz and coworkers have described detailed tertiary and quaternary structures of Hb A and have proposed a structural basis for the cooperative oxygenation of Hb A— However, the detailed mechanism for the cooperative oxygenation of Hb A is not fully understood and remains controversial.

With the development of an expression plasmid to produce authentic Hb A in *Escherichia coli*, we can design and express any desired mutant hemoglobin. We can also produce mutant and normal hemoglobins isotopically labeled with ^2H , ^{15}N , and/or ^{13}C , enabling the application of multinuclear NMR techniques. These techniques, in combination with other biochemical and biophysical methods, can provide insights into the structure-function relationship of hemoglobin in solution. For example, multinuclear NMR has made possible the assignment of the side-chain resonances of all 38 histidines in Hb A, which account for the vast majority of the Bohr effect. These assignments led to the observation of a hydrogen bond between the distal histidine and oxygen in both subunits of HbO₂ A, which stabilizes the binding of oxygen. Other recent results include the finding that amino-acid substitutions at the $\alpha_1\beta_1$ subunit interface can perturb the oxygen affinity and cooperativity of hemoglobin, suggesting a pathway for communication between the $\alpha_1\beta_1$ and $\alpha_1\beta_2$ interfaces. Also, the quaternary structure of carbonmonoxy-Hb A can be determined in solution at near-physiological conditions of pH, ionic strength, and temperature by NMR measurements of ^{15}N - ^1H residual dipolar couplings in weakly oriented samples. The structure is found to be a dynamic intermediate between two previously solved crystal structures, known as the R and R2 states. Exchange broadening at the subunit interface points to a rapid equilibrium between different structures that presumably include the crystallographically observed states. Thus, multinuclear NMR has emerged as an important tool in protein engineering, which can elucidate the structure-function relationship of mutant Hbs and Hb A as well as provide a new look at Perutz's classic "switch" mechanism for the cooperative oxygenation of hemoglobin.



Department of Biological Sciences Seminar Announcement

Structural Biology and Functional Genomics
Lecture Series 2004

Fri, 18 Jun 2004

4.00pm - 5.00pm

LT20

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