PS02.07.15 MOLECULAR FEATURES OF OXYGEN AVIDITY DISCERNED FROM STRUCTURES OF *ASCARIS* HEMOGLOBIN DOMAIN I AND SEVERAL MUTANTS. F. Scott Mathews[a], Louise M. Cunane[a], Jian Yang[a], Andrew P. Kloek[b] and Daniel E. Goldberg[b,c]. [a]Department of Biochemistry and Molecular Biophysics, [b]Department of Molecular Microbiology and [c]Department of Medicine, Washington University School of Medicine, St. Louis, MO 63110 USA.

The structures of the N-terminal domain of *Ascaris* hemoglobin and of several mutants have been determined at 2.2 Å resolution. This perienteric hemoglobin from a parasitic nematode has an exceptionally high affinity for oxygen. It is an octameric protein containing two similar heme-binding domains per subunit. The recombinant monomeric N-terminal heme-binding domain (D1) retains full oxygen avidity. Its structure reveals a characteristic globin fold. A strong hydrogen bond between tyrosine B10 and the ligand distal oxygen, combined with a weak hydrogen bond between glutamine E7 and the proximal oxygen, grip the ligand in the binding pocket. A third hydrogen bond between these two amino acids appears to stabilize the structure. Mutation of B10 Y→L increases the oxygen dissociation rate of D1 about 500 fold. An initial difference Fourier indicated negative density at the site of tyrosine B10, consistent with substitution by leucine; refinement of the model indicated little additional change in structure, confirming the importance of the strong hydrogen bond of the tyrosine hydroxyl to dioxygen. Mutation of E7 Q→L increases the oxygen off-rate 5-fold. Analysis of this mutant shows a slight reduction of the E7 side chain volume and the elimination of the hydrogen bonds both to the oxygen and the tyrosine. In addition, there appears to be a slight reorientation of the heme group toward the mutated side chain and a 1.3Å movement of the FG loop toward the exposed edge of the heme group. Mutation of E7 Q→N increases the oxygen off-rate 50-fold. The mutated E7 Asn is too far from the oxygen to form a hydrogen bond, but a hydrogen bond to the B10 Tyr side chain is still maintained. There appears to be no movement of the heme group, in contrast to the E7 Q→L mutant, but the FG loop has moved further toward the heme, 1.6 Å, and the side chain of an Asp in this loop now appears to form a hydrogen bond to a heme propionate. This latter interaction may prevent the movement of the heme group to protect the oxygen-binding pocket, seen in the E7 Q→L mutant, leading to a large increase in the oxygen dissociation rate.

PS02.07.16 HOW THE ATOMIC STRUCTURE OF A CRYSTAL CAN BE SEEN WITHOUT A HIGH RESOLUTION MICROSCOPE. V. L. Indenbom.

Other

PS02.09.01 DIRDIF-96: PATTERSON METHODS, DIRECT METHODS ON DIFFERENCE STRUCTURES, AND COMPLETION OF THE STRUCTURE BY AUTOMATIC RECYCLING. Paul T. Beurskens, Randy Israel, Gezina Beurskens, Rene' de Gelder, W.P. Bosman and J.M.M. Smits, Crystallography Laboratory, University of Nijmegen, The Netherlands

Direct methods become more and more powerful every year .... but some structures cannot be solved easily, because of poor data sets?, low resolution data?, pseudo-symmetry?, heavy atoms?, or just bad luck?

The heavy-atom interpretation techniques in DIRDIF are fully automated and possible symmetry problems are solved by the special application of direct methods to difference structure factors.

Vector search methods offer an ideal possibility to use your chemical knowledge. The expected geometry of a molecular fragment may be obtained from structural publications (your own research, the Cambridge Data Base) or by Molecular Modelling. The intramolecular vectors are rotated in Patterson space, and acceptable orientations are positioned, all without user intervention: the programs are more powerful than the unexperienced user!

Finally, the heavy-atom structure, or the resulting structural fragment from the vector search techniques, is expanded to the full structure: again fast and fully automatic, using improved R_2 recycling criteria, and new strategies for rejecting Fourier peaks by checking expected peak heights and molecular geometries. In most cases the structure is solved.

But if the heavy atoms are not so heavy, or if the Patterson allows homometric solutions, or if the molecular fragment is not unique or not completely correct or very small, a large(?) number of tentative structural models may be obtained. User controlled recycling is then possible, but automatic multisolution-recycling is on its way: the program selects the most probable model (using various FOM's), tries to expand it to the complete structure, and if unsuccesful, selects the next probable model.

PS02.09.02 IMPROVEMENTS ON THE USE OF IMAGE SEEKING FUNCTIONS. Javier Borge, Santiago García-Granda, Departamento de Química Física y Analítica, Facultad de Química, Universidad de Oviedo, C/ Julián Clavería, 8, 33006 Oviedo, Spain

Rotation and translation function procedures only reach correct results when powerful mathematical techniques are used to measure the goodness of fit between observed and calculated Patterson maps. Usually, Image Seeking Functions (ISF) [1] solve satisfactorily this problem.

Several tests [2-3] based on computer generated random numbers showed how the behavior of the ISF depends largely on certain relations between statistical descriptors (such as mean and standard deviation) of the Patterson maps.

Real data tests, now performed, confirm the previous results from computer simulation. Useful transformations of observed and calculated data will be suggested to improve the results when applying ISF.

[1] M. J. Buerger. *Vector Space and its application in crystal-structure investigation.* 1st ed. John Wiley & Sons, Inc. New York. **1959**
[2] C. E. Nordman. L.-Y. R. Hsu in *Computational Crystallography.* (Ed.: D. Sayre). Oxford: Clarendon Press. **1982**. pp. 141 -149.
[3] S. García-Granda. J. Borge. A. Gutiérrez-Rodríguez. *Anales de Química. Int. Ed.* Submitted for publication.