The Multiwavelength Anomalous Dispersion Experiment at 0.90 Å Producing Atomic Resolution Experimental Electron Density for 36 kDa Enzyme

A. Joachimiak,[1] E. Howard,[2] T. Petrova,[2,3] B. Chevrier,[2] R. Sanishvili,[1] A. Mitschler,[2] A. Podjarny[2]

[1] *Structural Biology Center, Argonne National Laboratory, Argonne, IL, U.S.A.*

[2] *UPR de Biologie et Genomique Structurale, IGBMC, Illkirch, Alsace, France*

[3] *Institute of Mathematical Problems of Biology of the Academy of Sciences of Russia, Pushchino, Moscow Region, Russia*

MAD has emerged as the most powerful method for *de novo* protein structure determination. This method has also the potential of providing experimental phases free of model bias and may contribute valuable information about the protein structure, the solvent molecules, unusual chemistry and the multiple conformations. Crystals of a SeMet derivative of human aldose reductase (hAR) have been obtained that are isomorphous to the native monoclinic crystals (P21, a=49.324 Å, b=66.956 Å, c=47.378 Å, α=90.000, β=92.262, γ=90.000) that diffract x-rays to subatomic resolution (0.62 Å) using synchrotron radiation. Crystals contain one monomer of 36 kDa in asymmetric unit and six potential Se sites. MAD experiments at atomic resolution have been performed on the crystals of SeMet labelled hAR in the ternary complex with coenzyme $NADP^+$ and two different inhibitors. Diffraction data were collected at the 19-ID beamline of the Structural Biology Center at the Advanced Photon Source for three wavelengths (peak, inflexion and high energy remote). The data collection was subject to the constraints of detector size, a fixed wavelength around the Se absorption edge (0.98 Å), and crystal decay and therefore the resolution was limited to 0.90 Å. Data were shown to be of high quality up to 1.1 Å resolution.

MAD phasing was performed using the SOLVE. The Se atoms were located and refined, giving rise to experimental phases with figure of merit > 0.9 up to 1.1 Å resolution, and extending to 0.90 Å. The phases obtained from the MAD experiment were compared with those calculated from the model of hAR refined to 0.66 Å. The MAD map was found to be highly informative. Analysis of the MAD maps showed peaks corresponding to every ordered protein atom. The experimental electron density maps show features such as multiple conformations for side chains, networks of H-bonds and water molecules, and unexpected peaks of density near amino acid residues. These fine stereochemical details are particularly clear for the well-ordered active site region. The hAR structure was refined using the amplitudes corresponding to the remote wavelength (averaged F+ and F-). The final map corresponds to: 314 aldose residues, the coenzyme $NADP^+$, the inhibitor IDD 594 and 663 H_2O molecules (see Fig. 1).

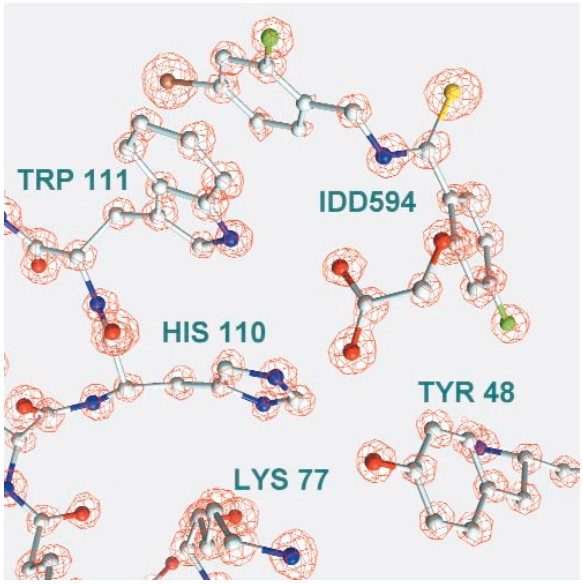

FIG. 1. Experimental MAD map of the active site of aldose reductase at 0.9 Å resolution. Note that every atom is resolved and that the peak heights correspond to the atomic species.

Acknowledgments

This work was supported by the Centre National de la Recherche Scientifique (CNRS), by the collaborative project CNRS-NSF, by the Institut National de la Santé et de la Recherche Médicale and the Hôpital Universitaire de Strasbourg (H.U.S), and by the U.S. Department of Energy, Office of Health and Environmental Research, under contract W-31-109-Eng-38.