Neutron and subatomic X-ray studies of perdeuterated Aldose Reductase

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Human Aldose Reductase (AR), an enzyme in the polyol pathway belonging to the aldo-ketoreductase family, is implied in diabetic complications. Its ternary complexes (AR-coenzyme NADPH-selected inhibitor) provide a good model to study the inhibition and enzymatic mechanisms. Indeed, X-ray electron density maps solved at very high resolution of AR complexes with different inhibitors (IDD-594, 0.66 Å; IDD-552; IDD-393; Fidarestat, 0.90 Å) show within the active site crucial protonation states. In some cases,different protonation states appear simultaneously, each with a partial hydrogen atom occupation.

Therefore, we have started neutron diffraction experiments. First trials based on H2O/D2O exchange, using crystals of 0.1 mm3, showed neutron diffraction up to only ~4.5 Å. New crystallisation trials, with fully deuterated protein (EMBL,Grenoble) complexed with the inhibitor IDD-594, succeeded. The quality of these crystals was tested by X-Ray diffraction. Data collection at the SBC-APS achieved a resolution of 0.8 Å at 15K (refined mosaicity 0.2°) and the structure was refined using SHELX. Neutron Laue diffraction measured on LADI (ILL,Grenoble) achieved a resolution of 2.2 Å at room temperature, despite a rather small crystal volume of only 0.15 mm3. Growth of larger crystals is under way.

After refinement with CNS, the resulting neutron density maps showed clearly the deuterium atoms in the active site region. In particular, the interaction of Tyr48 with Lys77, important for the catalytic reaction, is confirmed by a proton (deuterium) channel between these two residues.

This work is supported by the U.S. Department of Energy, Office of Biological and Environmental Research under contract No. W-31-109-ENG-38, the Centre National de la Recherche Scientifique (CNRS), by collaborative projects CNRS-DFG and ECOS-SUD, by the Institut National de la Santé et de la Recherche Médicale and the Hôpital Universitaire de Strasbourg (H.U.S), the ILL, the EMBL, and by the Institute for Diabetes Discovery, Inc. through a contract with the CNRS.