M11.0B Metalloproteins, Electron Transport and EXAFS

Chair: J.M. Guss
Co-Chair: B.G.M. Hedman

Attendance: 117

This session was devoted to presentations in the area of metalloproteins, or electron transport or EXAFS, which were at the leading edge of current methodology. The topics covered ranged from the use of time-resolved methods in single crystal diffraction to the complementary roles played by crystallography and EXAFS in determining the structures of the metal centres in metalloproteins. Ilme Schlichting (Max Planck Institute, Dortmund, Germany) described single crystal X-ray structures of freeze trapped intermediates of P450cam coupled with single crystal microspectrophotometry. Highlights were a structure of the ternary complex between P450cam/camphor/dioxygen, formed by co-crystallisation with camphor, chemical reduction, and then oxygen diffusion, and a structure of a reaction intermediate, initiated from the ternary complex by X-ray radiolysis, which contained the species following dioxygen bond cleavage. S. Ramaswamy (Uppsala University, Sweden) described the single crystal structure of naphthalene dioxygenase, where the initial structure surprisingly contained a reaction intermediate; an indole covalently linked to dioxygen, which was bonded to the active site iron. Following refinement of the purification protocols for producing the enzyme, an “empty” active site was achieved, and product and other indole derivatives could be soaked into the crystals, leading to the conclusion that indole binds before dioxygen during the reaction. The relatively clear skies of Glasgow then provided a good view of the solar eclipse, which took centre stage for the next 30 minutes. Hans Freeman (University of Sydney, Australia) spoke about using polarised Fe K-edge X-ray absorption spectroscopy with oriented carbonmonoxy-myoglobin crystals to obtain accurate angular geometries between the ligand and metal centre. This gave a tilt angle of 8o for the Fe-C from the perpendicular of the porphyrin ring, and an 11o tilt for the Fe-CO. It was suggested that these measurements could be used to restrain metal-ligand angles in single crystal X-ray structures. Graham George (Stanford Synchrotron Radiation Lab, U.S.A.) highlighted the difficulties of interpreting X-ray crystal structures with reference to spectroscopic techniques. In the case of formaldehyde ferredoxin oxidoreductase, a tungsten containing enzyme, EXAFS suggested the presence of two W-oxo ligand interactions, whereas the crystal structure only had one built into the model, although re-interpretation of the electron density did suggest the presence of a second interaction. S. Samar Hasnain (Daresbury Laboratory, U.K.) demonstrated the complementarity of EXAFS and single crystal X-ray crystallography, using nitrite reductase, Cu,Zn superoxide dismutase (SOD) and rusticyanin as examples. EXAFS and single crystal structures shown that nitrite reductase was incapable of binding substrate or inhibitors, subsequent to an electron transfer between the two copper ion sites in the enzyme. Therefore, substrate nitrite must bind to the enzyme prior to electron transfer between the copper centres.

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