s7.m6.p5.la **A Gd-complex to obtain heavy atom derivatives for SAD and MAD experiments. Results with tetragonal hen egg-white lysozyme.** E. Girard, L. Chantalat, J. Vicat and R. Kahn. *Institut de Biologie Structurale J.P. Ebel CEA/CNRS 41, rue Jules Horowitz, 38027 Grenoble cedex 01,France*
Keywords: Gd-complex, MAD, SAD.

Since obtaining good isomorphous derivatives is often the bottleneck of the multiple isomorphous replacement method, phasing methods taking advantage of anomalous dispersion are now widely used for *de novo* structure determination in macromolecular crystallography. In that context, the single-wavelength anomalous dispersion (SAD) phasing method is particularly interesting if only because it can be applied to data collected using conventional X-ray sources. SAD phasing is more likely to succeed when using anomalous scatterers that exhibit high f'' values. For CuK_α radiation, lanthanides, especially samarium (f'' = 13.3 electrons) and gadolinium (f'' = 12.0 electrons) are of particular interest. Surprisingly, no SAD experiment using these elements has been reported up to now.

A neutral gadolinium complex is presented as a good candidate to obtain heavy-atom derivatives and solve macromolecular structures using anomalous dispersion. Tetragonal crystals of a gadolinium derivative of hen egg-white lysozyme have been obtained by co-crystallization using different concentrations of this complex. Diffraction data from three derivative crystals (prepared with 100 mM, 50 mM and 10 mM of Gd-complex) have been collected up to a resolution of 1.7 Å, using the CuK_α radiation from a rotating anode. From the gadolinium anomalous signal, two strong binding sites of the gadolinium complex to the protein have been located in both 100 mM (with site occupancies of 0.83 and 0.75) and 50 mM derivatives (site occupancies of 0.63 and 0.49). A single site is occupied (0.28) in the 10 mM derivative. Phasing using the anomalous signal at a single wavelength (SAD method) lead to an electron density map of high quality. The structure of the 100 mM derivative has been refined with final residual values, R and R_{free}, of 17.8 % and 20.8 % respectively. Two molecules of the gadolinium complex are close together with a distance of 6 Å between gadolinium atoms. Both molecules are located close to tryptophan residues. Four chloride ions have been found. The SAD electron density map, only enhanced by solvent flattening, highly correlated to the final map calculated from the refined model (0.775 correlation for the 100 mM derivative), allows easy model building.

s8a.m1.p52.la **Site Directed Mutagenesis of Nitrite Reductase. The Crystallographic Studies of Recombinant Nitrite Reductase and Mutations.** M.J. Ellis¤ #, F.E. Dodd¤, R.R. Eady*, G. Sawers*, M. Prudencio*, S.S. Hasnain¤ and B.E. Smith*. ¤*CCLRC Daresbury Laboratory, Warrington, WA4 4AD, UK.* # *School of Applied Sciences, De Montfort University, Leicester, LE1 9BH, UK.* **Unit of Nitrogen Fixation, John Innes Centre, Colney, Norwich, NR4 7UH, UK.*
Keywords: enzyme catalysis, protein engineering.

Nitrite reductase is the key enzyme of the denitrification process and catalyses the first committed step that leads to the gaseous products NO, N2O or N2 resulting in a significant loss of fixed nitrogen from the atmosphere.

Denitrifying bacteria use two entirely different enzymes in this process, one based on haem cd1 and the other on type 1 and type 2 copper centres. The copper nitrite reductases are subdivided into spectroscopic blue and green groups which have azurins and pseudoazurins as their respective native redox partners.

Site directed mutagenesis has been used to examine the roles of the type 1 and type 2 copper ligands and to test a proposed proton transfer route from the surface of the protein to the type 2 copper site. The structure of recombinant nitrite reductase was examined using X-Ray fluorescence, EXAFS and crystallographic techniques at the Synchrotron Radiation Source, Daresbury Laboratory. The crystal structure of recombinant protein was shown to have very little metal content and was determined to a resolution of 1.2Å. The crystal structure of some mutants of nitrite reductase have been solved including a mutation of the methionine 144 to alanine to a resolution of 2.25Å, aspartic acid 92 to asparagine to a resolution of 1.9Å, and cystein 130 alanine to a resolution of 1.35Å.

All structures have been compared to the structure of the wildtype native protein with respect to the overall shape of the protein, the type 1 and type 2 copper sites (colour and catalytic centres respectively) and the proton channel.