Other

PS04.18.01 STRUCTURE OF THE R121D MUTANT N-TERMINAL LOBE OF HUMAN LACTOFERRIN. B.F. Anderson, W. Breyer, R.L. Kingston, H.R. Faber, C.L. Day and E.N. Baker, Department of Biochemistry, Massey University, Palmerston North, New Zealand.

The three dimensional structure of the iron free, R121D mutant N-terminal half molecule of human lactoferrin is currently being investigated by molecular replacement methods. Small colourless crystals were obtained by microdialysis from low-salt/ Tris/Isopropanol solutions and a data set complete to 3Å was collected on image plates with CuKα radiation. Although the diffraction pattern appears hexagonal the crystals are trigonal $P3_1$ with a=b=151.3Å, c=48.6Å, γ=120°,. The program AMORE, using the two domains of the recombinant N-terminal lobe of human lactoferrin, Lf_N, (Day et al., J. Mol. Biol., (1993). **232,** 1084-1100) independently, located two independent molecules in the assumed $P6_4$ asymmetric unit (correlation coefficients > 0.5). One molecule however seriously overlaps with its symmetry mates about the screw axis. A possible expanation of this is that there is merohedral twinning about a two fold axis parallel to the unique direction of a $P3_1$ crystal. Statistical tests on the observed data confirm that twinning is present with an approximate twin fraction of 0.4. Further rotation/translation searches with AMORE in space group $P3_1$ and using as search model one of the full molecules found previously gives four strong sites which can be divided into pairs related by a diad coincident with the 3_1 axis. Since this model gives a V_m of 4.4, our present efforts are directed toward locating the rest of the cell contents (probably one more molecule in the untwinned asymmetric unit) while continuing the search for less twinned crystals and higher resolution data.

The structure shows an 'open' conformation and as such appears very similar to that shown by the N lobe of apolactoferrin. Comparison with Lf_N shows that there has been a rotational movement of the N2 domain with respect to N1 of approximately 52°.

PS04.18.02 CRYSTAL STRUCTURE OF CALBINDIN D_{9k} BINDING MG^{2+}. Maria Andersson[1], L. Anders Svensson[1], Sara Linse[2]. 1Molecular Biophysics and [2]Physical Chemistry 2 at the University of Lund, Box 124, S-221 00 Lund, Sweden

Calbindin D_{9k} is thought to transport Ca^{2+}/Mg^{2+} in mammalians[1,2] and is present in bone and in the intestine. Calbindin D_{9k} has two EF-hand subunits, binding calcium cooperatively[3]. In this structure only one of two EF-hand loops binds Mg^{2+} and the other loop is devoid of metal ions. Significant structural differences exist between the $(Ca^{2+})_2$-calbindin structure[4] and the Mg^{2+}-calbindin structure. Due to these structural differences molecular replacement with $(Ca^{2+})_2$-calbindin as a model failed to solve the strucure and instead isomorphous replacement techniques had to be used. The final structure includes all 75 aminoacids, Mg^{2+}, 50 water molecules and is refined to 1.6 Å (87 % completeness). The final Rfactor is 19.6 %. The Mg^{2+}-calbindin structure further supports the idea of cooperativity. As Ca^{2+} levels increase intracellularly, Ca^{2+} goes into the empty site, inducing a conformational change which releases Mg^{2+}. Thereafter Ca^{2+} binds to the second site.

1Balmain N., *Clin. Orth. Rel. Research* **265**, 265-276, (1991)
2Hemmingsen C., Staun M., Olgaard K. *Miner Electrolyte Metab* **20**, 265-273, (1994)
3 Linse S., Brodin P., Drakenberg T., Thulin E., Sellers P., Elmdén K., Grundström T., Forsén S., *Biochemistry* **26**, 6723 - 6735, (1987)
4Szebenyi D. M. E., Obendorf S. K. and Moffat K., *Nature* **294**, 327-332 (1981)

PS04.18.03 MOLECULAR REPLACEMENT STUDIES OF A NUCLEASE INHIBITOR PROTEIN. C. Dennis', R. Pauptit*, A. Tucker*, R.James', C. Kleanthous', G. Moore' and M. Osbourne'. 'School of Biological Sciences, 'University of East Anglia, Norwich, UK; *Zeneca Pharmaceuticals, Alderley Park, Macclesfield,Cheshire, UK

Structural studies are being carried out on a group of homologous nuclease inhibitor proteins using an available NMR structure as a trail modelin molecular replacement. These inhibitor proteins bind tightly to the nuclease and neutralise their toxicity. The colicin E family are a group of bacteriocins which are secreted as part of a bacterial defence mechanism which are induced by DNA damaging agents, such as UV light and are secreted as part of a bacterial defence mechanism. Colicin E2, E7, E8 and E9 exert toxicity by a Dnase activity in their C terminal domain. This toxicity is neutralised inside the producing cell by the tight binding of a constitutively expressed 10kDa immunity protein. This immunity protein shows high specificity to its cognate colicin. The NMR structure of Immunity protein 9 (Im9) is mainly helical with large flexible loops. Crystallisation trials have been successful for Im7 and large orthorhombic crystals diffracting to 2.0Å have been obtained. The immunity proteins share 60% homology so molecular replacement studies of Im7 are currently underway using the NMR structure of Im9 as the trial model.

PS04.18.04 CRYSTAL STRUCTURE OF THE C DOMAIN OF SYNAPSIN IA FROM BOVINE. Lothar Esser1, Chyung-Ru Wang2, Cynthia Smagula1, Thomas Südhof1, and Johann Deisenhofer*1. [1]Howard Hughes Medical Institute Dallas, TX 75235; [2]Gwen Knapp Center for Lupus and Immunology Research, The University of Chicago, IL 60637

Synapsin Ia is a neuronal protein whose ability to bind to both synaptic vesicles and actin filaments depends on its phosphorylation state. Thus, a regulatory role in the process of synaptic transmission has been ascribed to it. Synapsin Ia is made up of five domains (A - E). Of these, the central hydrophobic domain C (a. a. 113-420) is the most conserved region among different species and contains binding sites for synaptic vesicle membranes and actin. Recombinant DNA technology was used to express a 36 kDa fragment of bovine Synapsin Ia comprising residues 110 through 420 (which we refer to in the following text as SynA). SynA was also expressed in form of a seleno-methionine variant. SynA crystallizes in the space group P3221 with two molecules per asymmetric unit. The cell dimensions of the seleno-methionine variant are a = b = 76.39 Å, c = 180.94 Å, $\alpha = \beta = 90°$, $\gamma = 120°$ at 150 K. A mercury derivative allowed phase determination by SIR to 2.8 Å resolution. However, phases were greatly improved and extended to 2.4 Å by MIR with optimized anomalous scattering techniques based on four data sets from two crystals. Data sets at selected wavelengths of a seleno-methionine SynA crystal and that of a mercury derivative of wild-type SynA, were collected at the beamline X4A in Brookhaven. The high quality of the electron density in ordered regions allowed an unambiguous assignment of the sequence. The current model consists of 286 amino acids (92%) and contains 18 seleno-methionine residues as well as 146 water molecules. The crystallographic R-factors for the current model are 28.9% (free) and 22.5% (work) for data in the resolution range between 6.0 Å and 2.4 Å. SynA can be classified as an alpha-beta structure. Further refinement and structure analysis is in progress.