PS04.02.13 COMPLEXES OF THE CATALYTIC DOMAIN OF AVIAN SARCOMA VIRUS INTEGRASE WITH DIVALENT CATIONS. Grzegorz Bujacz[1], Mariusz Jaskólski[2], Jerry Alexandratos[1], Alexander Wlodawer[1], George Merkel[3], Richard A. Katz[3], Anna Marie Skalka[3]. [1]Macromolecular Structure Laboratory, NCI-Frederick Cancer Research and Development Center, ABL-Basic Research Program, Frederick, MD 21702, [2]Center for Biocrystallographic Research, Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznan, Poland, [3]Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, PA 19111

Retroviral integrases require divalent cations, such as Mg^{2+} or Mn^{2+}, for their enzymatic activity. Details of the placement of metal ions in the active site can enhance our understanding of the catalytic mechanism of these enzymes and be helpful in designing integrase inhibitors - potentially potent antiviral drugs. Structures of the enzymatically-active catalytic domain (residues 52-207) of avian sarcoma virus integrase have been solved in the presence of divalent cations Mg^{2+} and Mn^{2+}, at resolutions of 1.7 - 2.0 Å. A single ion of either type interacts with the aspartate side chains of the D,D(35)E catalytic center and uses four water molecules to complete its octahedral coordination. Binding of the required metal ions does not lead to significant structural modifications in the active site of ASV IN. Only small adjustments take place in the active site residue side chains upon binding of the metal cofactor. However, the conformation of the catalytic aspartates in the active site of ASV IN (both in the absence or presence of metal) differs significantly from that reported for the analogous residues in HIV-1 IN in the absence of metal. The location of the metal ion is similar to that reported for metal complexes of RNases H from E. coli and HIV-1, which are structurally related to integrase. This suggests that the observed constellation of the acidic residues represents a pre-formed catalytically competent active site. Only a single divalent cation was observed even at extremely high concentrations of the metals. The unexpected differences between the active sites of ASV and HIV-1 IN remain unexplained, but because of ability to create of complex with metal we suggest that at the current stage of investigation, the ASV IN structures provides a good model for inhibitor design.

PS04.02.14 PROBING THE ROLE OF THE BURIED CHARGE, ASP235, IN CYTOCHROME C PEROXIDASE BY ENGINEERING IN COUNTER CHARGES OR H-BONDS. Yi Cao, David B. Goodin and Duncan E. McRee, Dept. of Molecular Biology, The Scripps Research Institute

Cytochrome c peroxidase (CCP) contains a buried charge, Asp 235, that is thought to control the reduction potential of cytochrome c peroxidase and coupling of the W191 free radical to the heme (D. Goodin & D. McRee, 1993, Biochemistry 32:3313). In this study, the role of this buried D235 is probed by introducing a counter-ion to it (with mutants M172K, M231H and F202K), or by introducing one more H-bond into the H175- D235-W191 H-bond network (with mutant F202Y). The X-ray crystal structures of these four mutant proteins show that: 1) in the M172K mutant, K172 surprisingly does not form a salt bridge with D235-Od1 as designed, but instead H-bonds are formed between these two groups with H2O as the bridge; 2) in the M231H mutant, H231 forms either a salt bridge or a H-bond with D235-Od2; 3) In the F202K mutant, K202 forms a salt bridge with D235-Od2, and 4) In the F202Y mutant, Y202 does form a H-bond with D235-Od1 as designed (with distance 2.5A between Y202- O and D235-Od1). For M231H and F202K, the H-bond D235(Od2)- W191 breaks and the indole ring of W191 is found to be reoriented in a similar way to that observed in the D235A and D235N mutants. No other major changes are observed in the protein structures of these mutants. This result reveals that the H-bond between the buried D235 and H175 in CCP is more stable than a potential salt bridge between D235 and K172. The result of the activity assay further shows that introducing a H-bond between D235 and Y202 in F202Y mutant (possibly weakening the D235- H175 H-bond) decreases the activity for cytochrome c oxidation and increases the activity for oxidation of some small molecule substrates.

PS04.02.15 X-RAY STRUCTURE DETERMINATION OF HUMAN CERULOPLASMIN AT 3.0 Å RESOLUTION. G L. Card, I. Zaitseva, V. N. Zaitsev, B. Bax, A. Ralph, P. F. Lindley, CCLRC, Daresbury Laboratory, Warrington, WA4 4AD, UK

Human Ceruloplasmin (hCP) is a copper containing glycoprotein with a molecular weight of approximately 132 kDa, corresponding to some 1046 amino acid residues and 7-8% by weight carbohydrate. The precise functions and chemistry of the protein have not been defined, but it has been associated with ferroxidase activity, amine oxidase activity, anti-oxidant activity and copper transport, and it may indeed be multi-functional. Sequence analysis of the protein indicates a domain structure involving internal triplication and an extraordinary homology with the A-type domains of blood clotting factor VIII.

Preparations of hCP exhibit heterogeneity, particularly with respect to the carbohydrate moieties, and the protein is very susceptible to proteolytic cleavage, aggregation and loss of copper. Numerous modifications have been made to the isolation and purification procedures to obtain reproducible crystal for X-ray analysis. Recently a S200/300 gel filtration stage has been introduced to remove aggregated and proteolytically cleaved fragments immediately prior to crystallisation. This has enabled the production of trigonal crystals, spacegroup $P3_221$ with a = 213.92 Å and c = 85.63 Å.

Using the SRS synchrotron source at Daresbury Laboratory, data has been collected for the native crystals and two heavy atom derivatives. Difference Patterson syntheses for both derivatives were readily interpretable in terms of single site binding. Protein phases were calculated using the program MLPHARE, and lead to an electron density map at 3.4 Å resolution which clearly showed the molecular boundary. Improvement of this map using DM, incorporating solvent flattening and histogram matching, led to an electron density map at 3.0 Å which is readily interpretable in terms of the molecular structure. Map interpretation was undertaken using the computer graphics O program, leading to over 90% of the protein being traced. Refinement of the model has been undertaken using a combination of RESTRAIN and XPLOR. Positional parameters have been refined for all atoms, but only side and main chain group isotropic thermal parameters for the individual residues, due to the resolution limit of 3.0 Å. The final model has some 1017 residues and a final R factor of 22.0% (start R = 34.6 %) and Free R = 28.6 %, (start Free R = 34.1%), for all reflections in the 12-3.0 Å range.