PS04.18.13 3D-STRUCTURE OF HUMAN TRANSTHYRETIN LEU-55-PRO MUTANT. Paula Sebastiao[1], Zbigniew Dauter[2], Maria Joao Saraiva[2] and Ana Margarida Damas[2], Embl, c/o Desy, Notkestrasse 85, 22607 Hamburg, Germany[1], Instituto de Ciencias Biomedicas de Abel Salazar, Largo Prof. Abel Salazar, 4000 Porto, Portugal[2]

The aim of the present study is the elucidation of the molecular mechanism(s) involved in amyloidogenesis by the structural characterization of transthyretin (TTR) variants.

The 3D-structure of variant human transthyretin Leu-55-Pro implicated as the causative agent in early-onset Familial Amyloid Polyneuropathy (FAP) is presented and the molecular mechanism involved in its extreme pathogenecity is suggested. FAP is an autosomal dominant disease characterised by the systemic deposition of amyloid with a particular involvement of the peripheral nerves. Amyloid FAP fibril formation is due to TTR aggregation in an insoluble protease resistant fibril with an antiparallel ß-pleated sheet structure which leads to neurotoxicity and organ dysfunction.

Transthyretin (TTR) also known as thyroxine-binding prealbumin is a tetrameric human plasma protein (MW 54980) composed of identical 127 residue subunits, each having a ß-sheet structure. Each monomer subunit presents two four-stranded ß-sheets DAGH (inner-sheet) and CBEF (outersheet).

The molecular characterization of this amyloidogenic TTR variant showed that the Leu-55-Pro mutation disrupts ß-strand D involved in the inner ß-sheet DAGH. The electrostatic surface potential in this TTR variant differs from that in the wild-type protein. Pathological effects may arise as this enhances the oligomerization of the protein and/or interaction of transthyretin with other amyloid components namely glycosaminoglycans.

PS04.18.14 INHIBITOR-COMPLEXED STRUCTURES OF AN HIV-1 PROTEASE MUTANT DISPLAYING CROSS RESISTANCE TO ALL CLINICAL TRIAL CANDIDATES Youwei Yan, Ying Li, Hilary B. Schock, and Zhongguo Chen, Merck Research Laboratories, West Point, PA 19486

Crystal structures of a mutant HIV-1 protease (M46I/L63P/V82T/I84V) complexed with either indinavir (MK-639, Merck) [Chen et al., (1995) *J. Biol Chem.* 269. 26344-26348]. retonavir (ABT-538, Abbott), or VX-478 (Vertex) are resolved at 2.0Å, 2.2Å, and 1.8Å resolution, respectively, with R factors of 17~18%. The comparison is of interest because this mutant protease has been shown to display cross resistance to a panel of protease inhibitors that are either in or being considered for clinical trials [Condra et al., (1995) *Nature 374*, 569-571]. When superpositioned against the structures of the wild- type enzyme complexed with indinavir and VX-478 [Chen et al. (1994) *J. Biol. Chem, 270*, 21433-21436; Kim at al., (1995) *J. Am. Chem. Soc.* 117,1181-1182], no gross difference is found in the tertiary structure of the enzyme. The C_α tracings of the native structures are essentially identical, and the four substituted sidechains are clearly revealed in the electron density maps. In the inhibitor-bound form, the V82T substitution introduces an unfavorable hydrophilic moiety for binding at the S1 but not the S1', site of the indinavir and VX-478 complexes (at neither sites of the ABT-538 complex). The I84V substitution generates a cavity that should lead to a decrease in van der Waals contacts with the inhibitor in all three complexes. These changes are consistent with the observed increase in the K_i value for the inhibitors as a result of the mutations. Residues 46 and 63 are not located in the binding site and have no interactions with these inhibitors. Thus the role of the M46I and L63P substitutions in drug resistance is not obvious from our crystallographic data; the conformational perturbations induced by them are minor.

PS04.18.15 ELECTROPHILE-NUCLEOPHILE INTERACTION IN PROTEIN STRUCTURES. Pinak Chakrabarti and Debnath Pal, Division of Physical Chemistry, National Chemical Laboratory, Pune 411008, India.

Although the various nonbonded interactions in protein structures have been well identified, their use to explain protein folding, substrate recognition and catalysis has not been an unqualified success. We have been investigating the existence of other strong directional interactions in proteins. When in close proximity in small organic molecules, an electrophile and a nucleophile have been found to orient themselves in a very specific way so as to affect the overall conformation of the molecule.[1] Our analysis of proteins structures, as stored in the Brookhaven Protein Data Bank, has identified a similar interaction where a thiolate anion (S) (metal-bound cysteine) acts as a nucleophile and a peptide carbonyl group (C) an elcetrophile; in the majority of the cases the two atoms belong to the same residue. The S...C distance is shorter than the sum of the respective van der Waals radii; there is a direct correlation between this length and the angular deviation of the S-C vector from the normal to the carbonyl plane. Other systematics have also been identified. This interaction changes the normal distribution of the conformation of the cysteine side-chain. Besides providing stability to metalloproteins such an interaction is likely to modulate the redox potential of the metal center.

1) Bürgi, H.B., Dunitz, J.D. and Shefter, E. (1973) *J. Am. Chem. Soc.* **95**, 5065-5067.

PS04.18.16 ACTIVATION OF PERTUSSIS TOXIN BY ATP: STRUCTURE AND BIOLOGY. Bart Hazes and Randy J. Read, University of Alberta, Dept. of Medical Microbiology & Immunology, 1-41 Med. Sci. Bldg. Edmonton, AB T6G 2H7 Canada; Stephen A. Cockle, Connaught Centre for Biotechnology Research, 1755 Steeles Ave, West Willowdale, ON M2R 3T4 Canada.

Pertussis toxin is a virulence factor of *Bordetella pertussis*, the etiologic agent of whooping cough. ATP activates this toxin by destabilizing the quaternary structure of the holotoxin. The molecular basis for this effect has now been revealed by the pertussis toxin-ATP complex structure which we will present. In short, ATP binds at the heart of the hexameric toxin where it destabilizes the C-terminus of the toxic subunit through unfavorable steric and electrostatic interactions with the triphosphate moiety.

A more general consequence of our work is that the structural results contribute to our understanding of how pertussis toxin and other important protein toxins may enter the cytosol of eukaryotic cells. Several lines of evidence now converge on a mechanism in which these toxins undergo retrograde transport from the cell surface via the Golgi to the ER. In the ER the toxic subunit then dissociates from the holotoxin prior to membrane translocation. Proteolytic cleavage and disulfide bond reduction seem to be the preferred mechanisms for dissociation of the toxic subunit. However, for pertussis toxin we now propose that ATP, which is first encountered in the ER, triggers the dissociation of the holotoxin.

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