and binds rhodopsin, the β and γ subunits, and cGMP-PDE. Diffraction quality crystals of the heterotrimeric complex are grown in microseeded hanging drops containing 10 mg/ml protein, 10% PEG-8000, 50 mM Tris, pH 8.0, 50 mM NaCl, 10% glycerol, and 0.1% β-mercaptoethanol. The space group is C2 with unit cell dimensions of a=133.4, b=91.4, c=83.2, β=120.1°. Progress to date will be reported.

PS04.10.22 REFINED CRYSTAL STRUCTURES OF TNF-ALPHA AND TNF MUTANT R31D. C. Reed*, Z.-Q. Fu*, J. Wu*, Y.-N. Xue, M.-J. Chen and I.T. Weber*. *Department of Pharmacology, Department of Microbiology and Immunology, Jefferson Cancer Center, Thomas Jefferson University, Philadelphia PA 19107, USA.

Crystal structures have been determined of recombinant human tumor necrosis factor-α (TNF-α) and its R31D mutant that preferentially binds to TNF receptor R1 with five times greater affinity than to receptor R2. Crystals of the wild type TNF were of space group $P4_12_12$ and had unit cell dimensions of a=b=94.7 and c=117.4 Å. Refinement of the structure gave an R-factor of 22.3% at 2.5 Å resolution. The crystals of TNF R31D mutant diffracted to 2.3Å resolution, and were of identical space group to the wild type with unit cell dimensions of a=b=95.4 and c=116.2 Å, and the structure was refined to an R-factor of 21.8%. Almost continuous electron density was observed throughout both structures, although the first five residues of the N-termini appear to be disordered. Comparison of the structures of the wild type and mutant TNF showed that the two trimers were similar with an rms deviation of 0.77 Å for main chain atoms, however, the subunits within each trimer were more variable with rms deviations of over 1.05 Å for pairwise comparison of main chain atoms. Model complexes of TNF with receptors R1 and R2 have been used to predict TNF-receptor interactions. The Arg 31 of wild type TNF is predicted to form an ionic interaction with an identical glutamic acid in both receptors R1 and R2. In the TNF R31D mutant, modeling suggested that this interaction is replaced by interaction with a histidine in R1, but there is no equivalent interaction in R2, consistent with the observed greater affinity of the R31D mutant for receptor R1 compared to R2.

PS04.10.23 REFINEMENT OF NATIVE AND MUTANT VEROTOXIN B-SUBUNIT STRUCTURES. Allan M. Sharp, Penelope E. Stein, Amechand Boodhoo, and Randy J. Read. Departments of Biochemistry, and Medical Microbiology and Immunology, University of Alberta, Edmonton, Alberta, Canada, T6G 2H7

The ability of enterohemorrhagic *E. coli* strains to cause hemorrhagic colitis and hemolytic uremic syndrome is a result of the production of a shiga-like toxin or verotoxin. This is an AB_5 type toxin, with a catalytic A-subunit attached to a pentamer of B-subunits that bind the cell surface glycolipid globotriaosylceramide (Gb-3). The solution of the wild-type verotoxin B-subunit pentamer, at 2.2 Å resolution, revealed a predominantly β-sheet structure, built around a central helix-lined pore [1]. Based on the distribution of conserved surface residues, the deep clefts at the interfaces between the subunits were proposed to be the sugar-binding sites. Mutation of a phenylalanine to an alanine residue in the cleft region (Mutant F30A) did eliminate the majority of glycoside binding [2]. Difference Fourier analysis of crystals of F30A at 2.0 Å resolution suggested that there were no major structural differences in the protein away from the mutation site. Further refinement of the native and mutant structures with XPLOR and TNT has reduced their R-factors to 0.195 and has allowed detailed analyses of the features of the structures, their distortion from ideal pentamers by crystal contacts, changes at their putative binding sites, and changes in occupancy at two crystallographic zinc binding sites.

A more recent 2.8 Å crystal structure of the verotoxin B-subunit complexed with Gb3 has shown that there are in fact three different classes of binding sites on the pentamer surface, one of them closely corresponding with the original prediction, and another contacting phenylalanine 30 [3]. The effects of the mutation may aid in elucidating the relative importance of the different sites.

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PS04.10.24 ATOMIC STRUCTURE OF POTD-THE PRIMARY RECEPTOR OF SPERMIDINE/PUTRESCINE TRANSPORT SYSTEM IN E.COLI. D.G.Vassylyev[1], S. Sugiyama[2], M. Matsushima[3], K. Kashiwagi[4], K. Igarashi[4], K. Morikawa[1], [1]Biomolecular Engineering Research Institute, 6-2-3, Furuedai, Suita, Osaka, 565, Japan, [2]Kyowa Hakko Kogyo Co. Ltd., Pharmaceutical Research Laboratories, 1188 Shimotogari, Nagaizumi-cho,Sunto-gun,Shizuoka,411,Japan, [3]Rational Drug Desighn Laboratories, 4-1-1 Misato, Matsukawa, Fukushima 960-12, Japan, [4]Faculty of Pharmaceutical Sciences, Chiba Univ., 1-33 Yayoi-cho, Inage-ku, Chiba 263, Japan

The crystal structure of PotD (a periplasmic binding protein which is the primary receptor of polyamine transport system in E.coli) in complex with spermidine has been solved at 2.5Å resolution. The PotD protein (325 amino acids) consists of two domains with a deep cleft (20Å long, 5Å wide, 14Å deep) in the interface between them. This cleft was found to be a binding site of spermidine in the complex. The three positively charged nitrogens of spermidine are recognized by four acidic side chains of PotD in the cleft while five aromatic residues anchor the spermidine methylene backbone by van der Waals interactions. The overall fold of PotD is similar to other periplasmic binding proteins despite the fact of low sequence similarity.

Crystals of the complex belong to the space group $P2_1$(a=145.3Å, b=69.1Å, c=72.5Å, β=107.6, Z=8). The structure was solved by MIR method in combination with solvent flattening and 4-fold N.C.S. averaging and refined at 2.5Å resolution to a final R-factor of 0.199 (R-free = 0.280).

PS04.10.25 CRYSTALLOGRAPHIC ANALYSIS OF THE MAP KINASE P38. Zhulun Wang[1], Jiahuai Han[2] & Elizabeth J. Goldsmith[1]. Department of Biochemistry, UT Southwestern Medical Center at Dallas, Dallas, TX 75235[1]. Department of Immunology, The Scripps Research Institute, La Jolla, CA 92037[2]

The mitogen-activated protein (MAP) kinase cascades are major signaling pathways that transmit extracellular information from the cell surface into the intracellular responses. Various extracellular stimuli, such as, growth factors, heat, UV-irradiation, inflammatory cytokines and hyperosmolarity, activate MAP kineses by dual phosphorylation on threonine(T) and tyrosine(Y). Three distinct MAP kineses signal transduction pathways have been defined so far in mammalian cells based on their differential activation selectivity and substrate specificity. As a new member of the MAP kinase family, p38 has been identified in a stress-activated signal transduction pathway. The three dimensional structure of p38 and comparison of p38 to ERK2 will help to elucidate the mechanisms of specificity determination in this family.

His6-tagged recombinant murine p38(45kDa) protein has been expressed, purlfied and crystallized by vapor diffusion method