to this interface and estimation of disulfide-bond strain were evaluated by energy minimization of wild-type and disulfide bonded αHL subunits. The resulting structures were essentially identical and the disulfide bond adopts favorable stereochemistry. We will present data on the formation, crystallization and crystallographic analysis of heteroheptameric transmembrane channels.

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PS04.03b.17 CLONING AND PURIFICATION STUDIES OF A CLASS OF MEMBRANE PROTEINS THAT ARE RESPONSIBLE FOR PILIN ASSEMBLY AND TOXIN SECRETION. Marie Zhang[1], Jeff Pepe[2], Steve Lory[2], Wim W. J. Hol[1], [1]Howard Hughes Medical Institute, Department of Biological Structure and Biochemistry, Biomolecular Structure Center [2]Department of Microbiology, University of Washington, Seattle, WA 98195

Pseudomonas aeruginosa is an opportunistic pathogen of humans and is a major cause of morbidity and mortality in patients with cystic fibrosis, severe trauma and AIDS. Among the various virulence factors produced by *Pseudomonas aeruginosa*, two of them approved to be the most damaging to the host. One of two is the formation of pilin, which have been shown to be important during colonization of mucosal surfaces by mediation the attachment of the bacterium to epithelial cells. The other one is the secretion of toxic material, such as exotoxin A, alkaline phosphatase and phospholipase C, etc., that could affect host cell's normal cellular activity.

A class of membrane proteins (PilB, PilC and PilD) have to shown to be responsible for the formation of pilin as well as the formation of a set of the secretion machinery (XcpT, U, V, and W) that is responsible for the toxin secretion. Among this class of protein, PilD have shown to be essential for pilin assembly. PilD is a peptidase which will cleave off the leader peptide on pilin and Xcp family of proteins, then subsequently methylation the N-terminal amino acid. It belongs to a new class of peptidase and a new class of methyl transferase.

PilD has been cloned and expressed both in *E. coli* and P. *aeruginosa.* Purification and crystallization are currently underway. The structure of PilD should provide us with many useful information of this new class of dual functional enzyme. It will certainly aid the designing of drugs that could target this harmful bacteria.

Nucleic Acids

MS04.04.01 DNA STRUCTURE AND GENE REGULATION: THE IMPORTANCE OF BEING FLEXIBLE. Zippora Shakked, Department of Structural Biology, Weizmann Institute of Science, Rehovot 76100, Israel.

To elucidate the role of DNA structure in gene-regulatory interactions, we have investigated the crystal structures of various DNA targets in their free state and compared them to their complexes with the cognate proteins. Our results and comparisons with other re A gulatory systems demonstrate that certain sites of the DNA are more flexible than others and can undergo significant structural changes at low energy cost. Pyrimidine-purine base-pair doublets belong to this category. Such sites can be exploited to produce a specific interface by permitting the required DNA deformation either locally as in the *trp* operator and the CAP regulatory element, or globally by inducing a conformational transition from one helical form to another as in the TATA-box-containing DNA. These findings may explain the abundance of such sites in DNA sequences that are involved in transcriptional control.

MS04.04.02 FORMATION OF TRIPLE HELICES IN THE CRYSTAL STRUCTURE OF d(GGCCAATTGG). Alain Dautant[1], Bernard Gallois[1], Gilles Précigoux[1], Dominique Vlieghe[2], Luc Van Meervelt[2] & Olga Kennard[3], [1]Unité de Biophysique structurale, CNRS, Université de Bordeaux, 33405 Talence, France, [2]Department of Chemistry, Katholieke Universiteit Leuven, Celestijnenlaan 200F, B-3001 Heverlee, Belgium, [3]Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK

Although X-ray crystal structures of DNA triplets are already known, no high resolution structure could be established for triple helices. We present a way of obtaining high-resolution data for short triple helices, based on special choices of oligonucleotide lengths and sequences for crystallization. This crystal engineering technique is based on the previously determined structure of d(GCGAATTCG), where single triplets arose from interaction between double helices with overhanging bases at the 5'-ends of both strands(1,2). The crystal structure of d(GGCCAATTGG) was established to a resolution of 2 Å(3). This sequence forms a canonical B-DNA double helical octamer structure with at both 5'-sides two single stranded overhangs of two guanine nucleotides. These overhangs do interact with symmetrical equivalent structures to form short triple helices containing two successive (C-G)*G triplets. Surprisingly, two different kinds of triplexes are observed. The first one is formed by parallel interaction of the third strand with the second G-strand, and displays Hoogsteen-like hydrogen bond patterns. The second triplex is formed by antiparallel interaction between the third and the second strand, using reverse Hoogsteen-like hydrogen patterns to form triplexes. Both triplexes give us detailed information of parallel and antiparallel triplex formation. It is also a clear example of the asymmetric behavior of inherent symmetric sequences.

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