

PS04.08.12 CRYSTALLOGRAPHIC INVESTIGATIONS OF ERYTHROCRUORIN FROM LUMBRICUS TERRESTRIS.

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Lumbricus hemoglobin is an extracellular respiratory protein complex located in the hemolymph of the common earthworm where it functions to transport oxygen and carbon dioxide. It is composed of four unique heme binding polypeptides (abcd chains) and three linker chains which are required for assembly of the entire molecule. We are investigating the crystal structure of the entire molecule and isolated subunits in order to learn the mechanism for the self-limited assembly of a cooperative complex from more than 200 polypeptide chains. We have recently crystallized the abcd assemblage in 2.2 M phosphate buffer pH 6.7. These crystals show symmetry of the space group C2221 and diffraction corresponding to at least 2.8 Å resolution with cell constants of $a=138.2$ $b=171.1$ and $c=201.2$ Å. We have also grown crystals in which the Calcium has been replaced with various Lanthanides. The modulation of diffraction intensities at different wavelengths due to anomalous scattering of these lanthanides will be used to solve the phases problem. The structure of the abcd complex will then be fitted into cryo-electron microscopy images of Lumbricus erythrocruorin in order to provide initial phases for the whole molecule crystal diffraction data.

PS04.08.13 X-RAY STRUCTURE OF EUKARYOTIC E3, LIPOAMIDE DEHYDROGENASE, FROM YEAST.

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α -Keto acid dehydrogenase complex is a family of well-organized multienzyme systems which are composed of three kinds of enzymes, E1, E2, and E3. Depending on the substrates, E1 and E2 are varied to construct the different architectures with 532 symmetry for pyruvate dehydrogenase complex and 432 symmetry for α -ketoglutarate dehydrogenase complex in eukaryotes. The third component E3 (lipoamide dehydrogenase) is, however, commonly used among them. To reveal the structure of E3 which binds to both complexes and to elucidate the reaction mechanism, the crystal structure of E3 from yeast was solved by the molecular replacement method with diffraction data collected up to 2.98(max. 2.49)Å resolution using synchrotron radiation. The initial phases were improved by non-crystallographic symmetry averaging and solvent flattening of electron density. The atomic coordinates of the molecular model constructed by computer graphics were refined with molecular dynamics. The final R-factor is 19.0% at 2.49Å resolution. Compared with prokaryotic Gram negative bacteria E3 (*A. vinelandii*) which binds only to complexes with 432 symmetry, large differences occur in the loop regions with insertion or deletion of amino acids. There are no differences in topology of the secondary structures. One β -sheet (246-276 residues) is changed the normal on the molecular surface. It is expected that E3s have different molecular surfaces between those incorporated into the cores with only 432 and with both 432 and 532 symmetries. A characteristic feature, found on an electrostatic molecular surface, may be concerned with such binding properties.

PS04.08.14 CRYSTAL STRUCTURE OF A NEW HEAT-LABILE ENTEROTOXIN, LT-IIb, THAT CAN ADP-RIBOSYLATE Gs-ALPHA.

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The LT-IIb crystal structure is the latest addition to structures determined in the cholera toxin (CT) family which encompasses a common theme of conserved structural similarities despite little sequence identity. The LT-IIb subunit organization is identical to CT and heat-labile enterotoxin (LT-I): a catalytic A subunit, capable of ADP-ribosylating Gs α , and a B pentamer which serves to bind to the ganglioside receptor located on the outside of the target cells. The sequence similarity of LT-IIb and LT-I is substantial when comparing their A subunits but was undetectable when comparing their B subunits. The crystal structure was determined by single isomorphous replacement (SIR) using a K₂PtCl₄ derivative. An initial 15-5 Å SIR map, without anomalous data, showed features of a 5-fold arrangement of long rods. These rods could be superimposed onto the 5 long α -helices found in the LT-I B pentamer and provided the first evidence of structural similarity between the B subunits of the two toxins. Solvent flattening combined with phase extension and 5-fold averaging improved the electron density dramatically. The LT-IIb structure is currently refined to 2.25 Å resolution with an R-factor of 19.1% with good geometry. The B-pentamer of LT-IIb shows the same 'OB-fold' as the pentamers of LT-I, CT, and other AB₅ toxin structures reported to date, such as pertussis toxin and shiga toxin. This constitutes a remarkable level of structural homology even in the absence of detectable sequence identity. The nature of the B pentamer pore, which is involved in binding the A subunit, among members of the cholera toxin family is very different except for a conserved ring of solvent accessible hydrophobic surface present in all members of the family. We speculate that this hydrophobic ring is critical for AB₅ assembly in the periplasm of the pathogens producing these toxins.

Viruses**MS04.09.01 STRUCTURAL STUDIES OF POLIOVIRUS ASSEMBLY AND CELL-ENTRY INTERMEDIATES.**

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The high resolution structures of polio and related picornaviruses have provided considerable insights into those properties that are relevant to the extracellular form of the virus. It is known, however, that the virus undergoes significant conformational rearrangements during assembly and cell entry. We have recently described the structure of the empty capsid assembly intermediate of poliovirus (1). This form of the virus lacks the viral RNA and has yet to undergo the maturation cleavage of the immature capsid protein precursor VP0 to yield VP4 and VP2. The structure demonstrates that the cleavage of VP0 is required for the correct formation of a network of interactions of VP4 and the amino terminal extension of VP1 on the inside surface of the virion. Interestingly, those portions of the network which form only after VP0 cleavage involve normally internal portions of the capsid protein which are externalized during conformational changes which are induced by binding to the poliovirus receptor. These results, together with analysis a wide variety of poliovirus variants with altered stability and/or receptor interactions, have