

PS04.07.06 CRYSTAL STRUCTURE OF THE HUMAN ADENOVIRUS PROTEASE WITH ITS 11 AMINO-ACID COFACTOR AT 1.6Å RESOLUTION. Jianzhong Ding, William J. McGrath, Walter F. Mangel, and Robert M. Sweet, Biology Department, Brookhaven National Laboratory, Upton, New York 11973

The human adenovirus serotype 2 protease (AVP) is essential for the production of infectious virus. Its function is to cleave multiple copies of 6 different virion proteins 2500 times to render a virus particle infectious. It is unique in that it requires two cofactors for maximal activity. One cofactor is pVIc, the 11 amino-acid peptide from the C-terminus of adenovirus precursor protein pVI. The second cofactor is viral DNA. The cofactors stimulate the catalytic rate constant 350-fold with pVIc, and 6000-fold with pVIc plus the viral DNA. Comparison of the structure of AVP with the cysteine protease papain and the chymotrypsin-like cysteine proteases indicates that AVP has a new fold. Thus, AVP represents a new subclass of cysteine protease.

We have extended the resolution of our previously reported X-ray structure of AVP with its pVIc cofactor from 2.6Å to 1.6Å through the use of a flash-frozen crystal. We used both programs X-PLOR and SHELXL to produce a final model at 1.6-Å resolution with $R_{\text{working}} = 13.3\%$, and $R_{\text{free}} = 17\%$.

In a related study, we solved the structures of this complex where the cofactor peptide pVIc was replaced by two mutant peptides in which the cysteine residue was replaced by either serine or alanine. Both mutant peptides are capable of serving as cofactors, although with altered affinities for the protease. The high-resolution structure has become the basis for structure-based rational drug design, and the structures with the mutants of pVIc have provided insights into the structural basis of cofactor activity.

This work was supported by National Institute of Allergy and Infectious Diseases Grant AI26049, by the Office of Health and Environmental Research of the United States Department of Energy and by the National Science Foundation.

PS04.07.07 STRUCTURE DETERMINATION OF YEAST COFILIN. A. A. Fedorov*, P. Lappalainen**, E.V. Fedorov*, D.G. Drubin**, S.C. Almo* **Department of Biochemistry, Albert Einstein College of Medicine, Bronx, NY 10461; **Department of Molecular and Cell Biology University of California 94720.

Cofilin is a widely distributed actin-associated protein that binds both F- and G-actin, and severs actin filaments in a pH dependent, calcium independent manner. These activities can be inhibited by phosphoinositides. Two crystal forms of yeast cofilin have been obtained: monoclinic (space group C2, with two molecules in asymmetric unit) and orthorhombic (space group P2₁2₁2₁, one molecule in asymmetric unit). The crystal structure of the orthorhombic form has been determined by multiple isomorphous replacement and anomalous scattering methods and refinement of the structure at 2.3Å resolution is in progress. Cofilin is built around a central five-stranded mixed β-sheet which is sandwiched between a pair of α-helices on each face. A comparison to the actin-severing proteins severin, gelsolin and villin, which display the same overall topology, will be presented. The structure of cofilin provides a basis for understanding its interactions with actin and phosphoinositides.

PS04.07.08 PROGRESS TOWARDS THE CRYSTAL STRUCTURE OF FFH, THE BACTERIAL HOMOLOG OF SRP54. Douglas Freymann, Robert Keenan, Robert Stroud & Peter Walter, Dept. of Biochemistry, University of California, San Francisco, CA 94143-0448.

Ffh is the bacterial homolog of the signal sequence recognition protein SRP54 - we have obtained diffraction quality crystals of both the intact Ffh and its amino-terminal domain. The signal recognition particle, SRP, in eukaryotes comprises six proteins and one RNA, and functions to target nascent secretory and membrane proteins to the translocation apparatus of the endoplasmic reticulum. In prokaryotes a ribonucleoprotein with similar function comprises at least one protein, Ffh, and the 4.5S RNA. SRP54 and Ffh have been shown to mediate recognition of the signal sequence of the nascent polypeptide. They are multi-domain proteins. The amino-terminal 'NG' domain is a GTPase whose activity is modulated by interaction with the C-terminal methionine-rich 'M' domain (which interacts directly with the signal peptide and with the RNA component of the particle) and by interaction with the membrane associated SRP receptor. We seek to understand the structural basis of signal sequence recognition and the function of GTP binding and hydrolysis in targeting. Progress towards the structure determination of Ffh will be reported.