03-Crystallography of Biological Macromolecules

The neuraminidase of strain A/Tokyo belongs to the N2 subtype and the subunit contains 469 amino acids. The product of the Fronase from N2 A/Tokyo virus neuraminidase, residue 83 to residue 469, was crystallized by vapor diffusion in hanging drops. Diffraction data at 2.63 Å was collected. Space group is C2221 and cell dimensions are a = 120.36 Å, b = 139.60 Å, c = 140.75 Å. The structure was determined by molecular replacement, using one of N1 subtype neuraminidase structure as the starting model. After XPLOR refinement, the crystallographic R factor is 29%.

03.12 – Other Macromolecular Structures

PS-03.12.01 CRYSTALLOGRAPHIC REFINEMENT OF TRICHOSANTHIN AT 1.1Å RESOLUTION. By Xing-qi Ma, Leijin, Hai Yun Gong, Da Cheng Wang, Institute of Biophysics, Academia Sinica, Beijing, China.

Trichosanthin (TCS) is a member of a larger group of proteins called ribosome-inactivating proteins (RIP). These proteins all function to catalytically inactivate eukaryotic 60S ribosomal subunits leading to rapid shutdown of protein synthesis.

Interest in RIP is growing due to several recent discoveries. The antiviral activity of the RIPs has focused attention on their use as potential anti-HIV agents and the abortifacient activity of Tian Hua Fen, a popular Chinese medicine widely used in China prepared from the root tuber of Trichosanthes kirilowii, has been shown to be due to Trichosanthin which is identified as an RIP as recently.

TCS crystalizes in the space group P2_12_12_1 with one protein molecule (247 amino-acid residues) in the asymmetric unit. The cell constants are a = 38.23 b = 76.53 c = 79.12. The diffraction data employed in the initial stage of the study were collected from the area detector at 1.1Å resolution, from which an initial TCS structural model was built up. Recently we have obtained a set of data at 1.1Å resolution using synchrotron radiation from KEK of Japan. The reflection with F>1.5σ(F) are 62% of possible total reflections. The overall merging R factor was 5.6%. The earlier refinement provided starting parameters for the work here. The TCS model was further refined at this very high resolution by the reciprocal space refinement with energy restraint (EREF), and the "foreign" solvents were excluded. The conventional R-value is now 0.25 for the 59604 reflections with F>1.5σ(F) and 0.11Å resolution. The precision of the model is much improved over the earlier refinement. In the best determined regions of the TCS molecule, H atoms are visible in the difference map. Structural heterogeneity is observed for a significant fraction of the amino-acid residues in the protein. Most common are flexible side chains on the protein surface. Discrete disorder extends into the ordered solvent regions of the crystal as well. Now the crystallographic refinement and the model rebuilding are still in progress.

PS-03.11.17 STRUCTURAL DETERMINATION OF THE NEURAMINIDASE OF INFLUENZA VIRUS A N2 SUBTYPE. By Liou Zhou and Ming Luo, Center for Macromolecular Crystallography, Department of Microbiology, University of Alabama at Birmingham, U.S.A.

In general the influenza viruses are spherical, enveloped particles with two types of surface glycoproteins spike, haemagglutinin and neuraminidase. Neuraminidase may facilitate mobility of the virus and from the site infection, and thus may be an important factor in the spread of the infection. It is a tetramer of M.W. 240,000 reducing to 200,000 when sublimated from the Fronase. The sequences of several strains of N1, N3, N7, N8, N9 and B subtype are known. Influenza A virus is the classic pandemic virus which infects human and affects persons in all area of the world.