

### DEVELOPMENT OF X-RAY POWDER DIFFRACTION FOR PROTEIN CRYSTALLOGRAPHY USING SYNCHROTRON RADIATION

K. Miura K. Kato K. Inoue T. Ueki

Japan Synchrotron Radiation Research Institute Life & Environment Research Division SPring-8, 1-1-1 Kouto, Mikazuki SAYO HYOGO 679-5198 JAPAN

We have developed to obtain clear X-ray powder diffraction of some protein micro crystals at public Structural Biology II beamline (BL40B2) at SPring-8. Submicron sized crystals were appeared with rapid microbatch system and collected with centrifugation in capillary. X-ray diffraction data were collected using a imaging plate detector (300 mm x 300 mm area) attached with a vacuumed beam path to remove excess scattering. Using all micro crystals (bovine Insulin, hen egg lysozyme, bovine pancreas trypsin), powder rings are clearly detected after 5 min to 60 min X-ray exposure with oscillation methods. The diffraction is determined up to 3 Å resolution. In addition, between lysozyme crystal and lysozyme crystal complex with N-acetyl chitotetraose (NAG4), there is significantly difference in intensity of some diffractions within 10° of 2θ angle. The structure refinement comparing with a single crystal X-ray diffraction data is now under going. This suggests that the rapid structure determination for drug design will be able to apply using micro crystals.

Figure: 1/4 part of X-ray powder diffraction image of lysozyme and NAG4 complex crystal, 30 min exposure, camera distance 420 mm, wavelength 1 Å



**Keywords: MACROMOLECULAR, POWDER DIFFRACTION, DRUG DESIGN**

### INFLUENCE OF MUTATIONS OF HIV-1 PROTEASE ON BINDING OF HYDROXYETHYLENAMINE AND ETHYLAMINE TYPE INHIBITORS

H. Petrokova J. Dohnalek T. Skalova J. Duskova J. Hasek

Institute of Macromolecular Chemistry AS CR Heyrovskeho Nam. 2 PRAGUE 162 06 CZECH REPUBLIC

HIV protease playing an essential role in the HIV life cycle is one of the most attractive targets for the design of drugs against AIDS. However, development of drug-resistant mutants of HIV protease in patients results in decreased effectiveness of protease inhibitor therapy. The mutant of HIV-1 protease under investigation (A71V, V82T and I84V) emerged in the patient in the course of the treatment with indinavir. We have determined crystal structures of HIV-1 proteases both wild type and mutant complexed with inhibitor series Boc-Phe-Ψ[(R/S)-CH(X)CH<sub>2</sub>NH]-Phe-Glu/Gln-Phe-NH<sub>2</sub> (where slashes denote alternatives and X= OH or H). Structural differences were analyzed in relation to the inhibitor efficiency. Two possible binding modes of the inhibitor isostere to the protease active site were found. The first with the isostere nitrogen binding between catalytic aspartates and the other one with nitrogen pointing towards one of the aspartates, forming thus different hydrogen bonding patterns. Beside this, neither the presence of hydroxy group of isostere nor the specific R/S configuration of the hydroxy group seem to be absolutely necessary for good binding of inhibitor to protease as it was assumed previously [1]. Thus the de novo design of the inhibitor remains a complex task of optimization of many related factors. The research was supported by the Grant Agency of the Czech Republic project no. 204/00/P091.

[1] Babine R. E., Bender S. L. (1997) Chem. Rev. 97, 1359

**Keywords: HIV-1 PROTEASE INHIBITORS DRUG DESIGN**

### DISCOVERY OF A SIALIC ACID BINDING SITE IN ONE OF THE LECTIN DOMAINS OF VIBRIO CHOLERAEE NEURAMINIDASE

I.M. Moustafa M.A. Taylor H. Connaris V. Zaitsev G.L. Taylor

St-Andrews University Biomolecular Sciences Centre North Haugh ST-ANDREWS FIFE KY16 9ST UK

Vibrio cholerae neuraminidase (VCNA) plays a significant role in binding and uptake of the virulence factor cholera toxin (CT) by susceptible cells: it cleaves sialic acid from higher order gangliosides to give GM1, the CT receptor; and increases the lipid fluidity of the surrounding environment facilitating the access of the bacterium to the target epithelial cells. The 2.4 Å crystal structure of VCNA showed the canonical neuraminidase β-propeller domain associated with two flanking lectin-like domains. Here we present the 1.9 Å of VCNA in complex with the transition-state analogue inhibitor, DANA, which will form the basis of structure-based drug design. The nature of ligands recognized by the lectin domain has remained a mystery until now. Surprisingly, in the 1.9 Å inhibitor complex, α-sialic acid has been found in a beautiful binding pocket in the N-terminal lectin domain. We could not think of any source of sialic acid either during crystallization or preparation of the enzyme. However, the mass spectrum of the DANA solution exhibited a trace of sialic acid. This result gives an answer to the question: how VCNA can bind to cell surfaces and remain active. The presence of sialic acid recognition site in this enzyme suggests that VCNA is able to target itself to the very receptor it seeks to remove. Perhaps VCNA should be renamed as haemagglutinin-neuraminidase (HN).

**Keywords: NEURAMINIDASE LECTIN SIALIC ACID**

### REAL AND VIRTUAL DISORDER IN COMPLEXES OF HIV PROTEASE

T. Skalova J. Hasek J. Dohnalek H. Petrokova

Institute of Macromolecular Chemistry AS CR Heyrovskeho Nam. 2 PRAHA 6 162 06 CZECH REPUBLIC

Mutant form of HIV-1 protease (A71V, V82T, I84V) in complex with new pseudo tetrapeptide inhibitor Boc-Phe-φ[CH<sub>2</sub>CH<sub>2</sub>NH]-Phe-Glu-Phe-NH<sub>2</sub> was studied using X-ray diffraction and molecular modeling. Diffraction was measured at the synchrotron facility ESRF in Grenoble at temperature 100K. Data were processed using software packages HKL and CCP4. Refinement was done in CNS using visualisation program O. The structure with resolution 2.2 Å was refined in space group P6<sub>1</sub> to R = 0.206 and Rfree = 0.252. The asymmetric unit contains approximately C<sub>2</sub> symmetric dimer of the protease. Similar as in many other HIV protease complexes, the asymmetric inhibitor was localised in the active site in two opposite orientations, each of them with occupation factor approximately 0.5. In addition, we observed alternative conformations of two protein residues T82 and V84 in each monomer that is in direct contact with inhibitor. The experimentally measured structure is an average structure where the inhibitor overlaps with its approximately C<sub>2</sub> symmetrically equivalent. The fact that the inhibitor is not symmetric can cause significant deviations from the expected C<sub>2</sub> symmetry of the protease in the parts where it is in contact with the inhibitor. As a result the asymmetric changes in the protease could be observable as alternative conformations. Molecular mechanics study of energies for all possible combinations of measured conformations was done to distinguish real and virtual alternative conformations.

The research was supported by the project A4050811/1998 of the Grant Agency AS CR and project 204/00/P091 of GA CR.

**Keywords: HIV-1 PROTEASE, PEPTIDOMIMETIC INHIBITOR, X-RAY CRYSTALLOGRAPHY**