X-ray structure analysis of drug-resistant HIV-1 protease, and protease inhibitor complexes, and rHCAI and substate/ inhibitor complexes

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HIV is the causative agent of AIDS, and inhibitors of HIV-protease are being deveoped as potential drugs against AIDS. A complication in the drug-development process is the emergence of drug-resistance by the pathogen. To understand this process at a molecular level, and also to help design better and more effective inhibitors of HIV-1 protease, we have collected X-ray diffraction data on the BL41XU beam-line with kind support of Dr. Nobuo Kamiya. We have collected data on the three protein crystals: 1. drug-resistant single-site mutant HIV1-PR, 2. HIV1-PR complexed to a locally designed and synthesised inhibitor, and 3. Saporin.

The diffraction data was collected by the oscillation-Weissenberg method using an oscillation angle of 6 - 10 degrees. The data has been processed using software packages AUTO and DENZO (partly at SPring8 and partly at BARC), and the results are summarised in the following Table:

Crystal Size	Number of frames	Resolution	Rm
1. 0.05 * 0.05 * 0.2mm	19	2.2	10.0%
2. "	14	2.5	10.2%
3. 0.03 * 0.03 * 0.01m	24	5.0	15.0%

Sample : 1. Mutant HIV1-PR 2. Inhibitor complex 3. Saporin

Efforts were made to collect data under cryo-conditions, but it was found that the mosaicity increased very much suggesting that the cryo-conditions were not optimised. The processed frames were therefore all collected at room-temperature. The Rm values are probably high because the crystals were too small and/or suffered radiation damage.

The structure of the inhibitor complex has now been solved. We see difference density for the inhibitor molecule in the active-site. Electron density interpretation and refinement are currently under progress.

Few frames of saporin crystals could be processed and scaled. The space group has been obtained as I4 with a=91.5, b=91.5 and c=150.9A. Since the crystals were so small, even this crystal data has been a new result not obtainable through our inhouse RAXIS IIC diffractometer.

High-resolution data on crystals of HIV1-PR-inhibitor complex are needed to know more accurately the detailed interaction between the inhibitor and the protease to be able to suggest chemical modifications to the inhibitor to acheive lower Ki values.