Poster Presentation

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Weak interaction of an inhibitor in the 20S proteasome

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Proteasomes are the multicatalytic protein complexes with huge molecular weight. It is well known that the ubiquitin proteasome system plays an important role in regulated proteolysis. The 26S proteasome is composed of two 19S regulating components and a 20S proteasome. The 20S proteasome forms barrel shape and is composed of four rings, α- and β-rings; each ring contains highly homological seven α-subunits (1~7) and seven β-subunits (1~7), N-terminal tails of α-ring subunit form a gate to facilitate substrate entry. Each of 1, 2 and 5 β−subunit has different enzyme activity; 1 is caspase-, 2 tryptic- and 5 chymotryptic- like activity, respectively. We found a new protease inhibitor compound-A, which interacts weakly against proteasome. In order to elucidate the binding mode of the compound-A to proteasome, structure determination of the 20S proteosme complexed with compound-A was carried out. Isolated and concentrated 20S proteasome was co-crystallized with compound-A. Sitting drop vapor diffusion method was applied using 0.1M MES-NaOH (pH 6.5), 35% MPD, 50 mM magnesium acetate as a reservoir solution and 10 mg/ml proteasome and 20 mM compound-A as a sample solution. Crystals are isomorphous with the previous report [1]. Diffraction images were recorded at 100 K by using ADSC Quantum 210r CCD detector at NW12A of Photon Factory, Tsukuba, Japan. Initial phases were determined by molecular replacement method using the structure of the yeast 20S proteasome [1RYP] as a starting model and the structure model with a ligand was refined by using Refmac5 in CCP4 program package with an R value of 16.5% at 2.85 Å. The electron densities have been observed at the active site as mentioned above in the β-ring. Binding site of compound-A is closed to Tyr170 and Thr1 of the β5 subunit. The compound-A binds weakly to their residues by hydrogen bondings. It is quite different from the binding mode of the known potent proteasome inhibitor bortezomib [2].

[1] *M. Groll et al., Nature, 1997, 386, 463-471.,* ***[2]*** *M. Groll et al., Structure, 2006, 14, 451-456.*

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