Crystal strucutre analysis of 20S Proteasome from bovine liver

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A 20S proteasome forms a catalytic core of the 26S proteasome. The proteasome is a central enzyme of protein degradation in both the cytosol and nucleus. The enzyme is involved in many biological processes. Especially, for the mammalian cells, this enzyme is involved in the cellular immune response. Although X-ray crystal structures of 20S proteasome from the archaebacerium(1) and the yeast(2) has been reported, that from mammalian has not been reported.

The 20S proteasome from bovine liver was purified and crystallized(3) and we tried to collect the intensity data of native 20S proteasome. Out of about 60 crystals inspected by X-ray diffraction experiment, only one crystal was subjected to oscillation experiment under 100K and 0.708Å wavelength.

Total of 125 oscillation shots were obtained in this experiment. The camera length was 500mm, the exposure time was 120 sec, the oscillation angle was 0.75 degree and the number of oscillation was 40 ways. We tried to process these data in our laboratory, but we were able to use first 105 frames in scaling because of X-ray damage. The crystal diffracted X-ray up to 3.3Å resolution, but these images contained ice ring faintly. So we processed these data at 3.3Å resolution inside

ice ring. In the data set, total of 79,169 independent reflections with 68.6% completeness and 8.1% R-merge at 3.3 Å resolution were obtained from 192,072 observed reflections.

Then we merged this data with data that we have obtained total of 78,752 independent reflections with 81.3% completeness and 11.8% R-merge at 3.5 Å resolution. The crystals belong to orthorhombic and has cell dimensions of a = 122Å, b = 195Å, c = 320Å and the space group is P212121. Now we are analyzing the three dimensional structure by Molecular Replacement method using archaeonbacterium and yeast model.

REFERENCE

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