A2 16:15 - 16:30

X-ray Crystallographic Analysis of Alginate Lyases from *Spingomonas* sp. A1

Hye-Jin Yoon, Wataru Hashimoto, Kousaku Murata and Bunzo Mikami

Research Institute for Food Science, Kyoto University, Japan

Alginate which is produced by brown seaweeds and certain kinds of bacteria heteropolysaccharide comprised of β-D-mannuronate and the C5 epimer α-L-guluronate. *Sphingomonas* sp. A1 intracellularly produces 3 kinds of alginate lyases, A1-I, -II and -III. A1-I is suggested to be autocatalytically processed to form A1-II and -III. A1-II is specific to the polyguluronate block, while A1-III selectively depolymerize the polymannuronate block. A1-III is a potent drug of cystic fibrosis because it can destroy alginate-film formed by pathogenic bacterium, *Pseudomonas aeruginosa*, in human lungs of the patients.

In order to determine the structure of alginate lyases from *Sphingomonas* sp. A1, we have crystallized A1-I, -II and -III by hanging drop vapour-diffusion method. Small crystals of A1-I and -II were characterized by a synchrotron radiation source at SPring-8. It was found that the crystals of A1-I are monoclinic ($P2_1$) with unit cell dimension of a=120.9, b=86.05, c=82.73 Å and β=125.43° , containing two A1-I in an asymmetric unit. The crystals of A1-II are tetragonal ($P4_32_12$ or $P4_12_12$) with unit cell dimension of a=b=144.07 and c=296.38 Å, containing 16 molecules of A1-II in an asymmetric unit. The large crystals of A1-III obtained in the presence 49% ammonium sulfate are monoclinic and belong to the space group $C2$ with unit cell dimensions of a=49.18, b=93.08, c=82.10 Å and β=104.12° .

The crystal data of A1-III up to 1.71 Å resolution were collected by a Bruker multiwire detector with R_{sym} of 5.0%. The structure of A1-III was determined by the multiple isomorphous replacement method, and the model was refined at 1.78 Å resolution with a final R-factor of 18.0%. The final model of A1-III contained 351 amino acid residues, 299 water molecules and 2 sulfate ions. A1-III is composed of an (α_6/α_5)-barrel similar to (α_6/α_6)-barrel found in glucoamylase and a certain kind of cellulase. A1-III has a deep tunnel-like cleft formed by the α-barrel and loops. His192 which is located in the bottom of the cleft is suggested to be one of the catalytic residue important for the subtraction of a proton from C5 atom of mannuronate.