Crystal structure Analysis of Macrophomate synthase by MAD method

Toyoyuki Ose(3853), Min Yao(3056), *Nobuhisa Watanabe(3812) and Isao Tanaka(3339)

Graduate School of Science, Hokkaido University

Macrophomate synthase (MPS) from the fungus *Macrophoma commelinae* IFO 9570 is a Mg(II)-dependent dimeric enzyme with molecular weight of 72kDa per dimer. MPS catalyzes an extraordinary complex five-step chemical transformation from 2-pyrone and oxalacetate to benzoate, involving decarboxylation, C-C bond formation, and dehydration. Based on the stereospecificity, it is proposed that involvement of a Diels-Alder reaction in which a double bond adds to a conjugated diene to form a six-memberd ring at the second step. Biosynthetic studies of Diels-Alder adducts provide not only further insight into the mechanism of above reaction, but also valuable information for the creation of artificial enzymes such as a catalytic antibody, which will be of great synthetic importance. It has not been discovered that Diels-Alder reaction catalysed by enzyme. In order to elucidate the catalitic mechanism of MPS at an atomic resolution, the structural information of MPS is necessary.

We had succeeded in getting crystals of MPS by hanging-drop vapor diffusion method. Several different shape crystals were grown up in different conditions. However, only poor diffraction spots (less than 4Å) were obtained using in-house X-ray source with Cu Kα. During one shift beam time at BL41XU, several crystals of different shapes were tried and we found the best crystal, which was grown to a size of approximately $0.01\times0.01\times0.15mm^3$ in a week at 20℃ (Fig), diffract to 1.8Å resolution with MAR-CCD camera. The indexing was carried out with MOSFLM in the CCP4 suite. It belongs to a hexagonal point group 622, with cell dimensions of $a=b=106.2$Å, $c=120.3$Å. There is a monomeric molecule in asymmetric unit corresponding to the V_M value of 2.75 $Å^3Da^{-1}$.

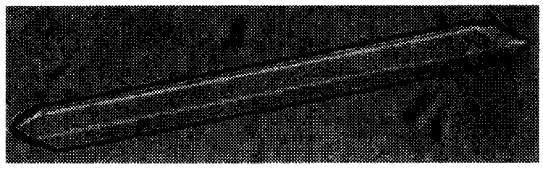

Fig. MPS crystal