X-Ray Crystallographic Studies on Tobacco Necrosis Virus, Peroxidase, and α-Amylase Inhibitor

Keiichi Fukuyama (0003453)*, Yutaka Oda (0003512), Hiroyuki Itakura (0003508), and Shinya Asada (0003510)

Department of Biology, Graduate School of Science, Osaka University, Toyonaka 560-0043, Osaka

We proposed to measure high resolution X-ray diffraction data of tobacco necrosis virus, peroxidase, and α-amylase inhibitor, in order (1) to understand their functions based on the more detailed structures, and (2) to evaluate the diffraction data collected with the new facilities and synchrotron radiation of SPring-8. Since the virus crystals were not allowed to bring in the experimental hall of SPring-8, we tried to measure the diffraction intensities for the last two crystals.

Peroxidase is a heme-containing enzyme that catalyzes the oxidation of a variety of organic and inorganic compounds and ions using hydrogen peroxide. We discussed the catalytic mechanism of peroxidase on the basis of the crystal structure of *Arthromyces ramosus* peroxidase (ARP) [1-3]. Although we prepared ARP crystals for higher resolution measurement, they started dissolving in the experimental hall and did not give high resolution diffraction spots. We did not expect that the temperature in the hall was set to 27°C. ARP crystals are rather sensitive to temperature, and without keeping the crystals at 20°C for several hours we could not collect its high resolution data at all.

α-Amylase inhibitor (AI) that we crystallized is a protein isolated from wheat kernel that has inhibitory activity on α-amylase, the enzyme responsible for cleavage of α(1-4) glucoside bonds present in glycogen and starch. This inhibitor is composed of 124 amino acid residues and forms a tetramer. The structure was solved by MIRAS coupled with the molecular averaging and refined on the basis of the native data at 2.06 Å resolution measured with conventional X-ray [4].

AI crystal was flash cooled to 100K. Its diffraction data were recorded on the imaging plates (IPs) with Weissenberg mode with coupling constant of 1.0°/mm using the camera at BL41XU. Data were processed and scaled up to 1.9 Å resolution. Summary of the data collection is shown in Table 1. In conclusion, the present measurements are helpful for the next more successful data collection.

Table 1. Summary of the data collection

Wavelength	0.708 Å
Crystal to IP distance	560 mm
Oscillation angle	4°
No. of IPs	25
No. unique reflections	30,913
Completeness	96.2 %
R_{merge}	5.0 %

References

1) N. Kunishima *et al.*, *J. Mol. Biol.* **235**, 331-344 (1994).
2) K. Fukuyama *et al. J. Biol. Chem.* **270**, 21884-21892 (1995).
3) H. Itakura *et al. FEBS Lett.* **412**, 107-110 (1997).
4) Y. Oda *et al. Biochemistry* **36**, 13503-13511 (1997).