Crystal structure analysis of α- and β-amylases from *Bacillus* species

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Three samples, *Bacillus licheniformis* α-amylase (BLA), phospholipase D (PLD) and lysozyme, were checked at the machine time on December 8-9, 1997. Data were collected at room temperature except for the cryo-experiment. The common experimental conditions are as follows;

X-ray wave-length	1.00A.
IP size	800 x 400 mm
Crystal to IP distance	560mm
Coupling constant	1.0 deg./mm
No. of osc.	1

1-1. Data collection of BLA at 100K

Data collection at 100K was examined in order to study the dynamic reaction mechanism of BLA and its substrate. Crystal data of BLA are: P212121, a=86.92, b=117.80, c=119.36A, Z=8. Crystals were soaked in 28% ethylene glycol solution before the data collection. Experimental conditions are;

Ring current	15.2 to 13.9 mA
No. of IP	10
Rotation range	6.0 deg.
Exposure time	6.0 s.
Osc. speed	1.0 deg./s.

Only two-thirds of the independent region was covered. At that time we must stop the data collection because of the diffuseness of reflection spots. A total of 53,538 reflections between 30 and 3.0A resolution were obtained, of which 14,228 reflections were independent. An Rmerge was 0.163 (0.36 for the 3.1-3.0A shell). Completeness of the data was 0.563 (0.562 for the 3.1-3.0A shell). This shows that the condition of the cryo-protectant must be still more refined.

1-2. Xe derivative of BLA

The Xe derivative was prepared under the Xe pressure of 10Kg/cm^2. Experimental conditions are;

Ring current	16 to 14 mA
No. of IP	16
Rotation range	6.0 deg.
Exposure time	3.0 s.
Osc. speed	2 deg./s.

The full oscillation range was covered before the crystal deterioration. However the cell parameter, b, was significantly changed from 118.33 to 112.37A. Therefore we stopped the merging of the data.

2. Data collection of PLD

The intensity data set of two crystal forms could be successfully collected.

PLD type II: P212121, a=62.78, b=85.34, c=100.54A. A total of 62,625 reflections between 30 to 2.5A resolution; independent reflections 17,113; completeness 0.88 (0.79 for the 2.59-2.5A shell); Rmerge 0.045 (0.127); crystal size 0.3 x 0.3 x 1.0 mm.

PLD type IV: P212121, a=60.77, b=87.61, c=91.96A. Crystal size 0.06 x 0.06 x 0.1 mm.

No. of IP	10
Rotation range	5.0 deg.
Exposure time	2.5 s.
Osc. speed	2.0 deg./s.

A total of 40,815 reflections between 30 to 3A resolution; independent reflections 10,018; completeness 0.97 (0.96 for the 3.11-3A shell); Rmerge 0.088 (0.194). An excellent data set was obtained in very short exposure time such as 1s per 1deg. oscillation using the small crystal at this station.