Crystallographic analysis of proteins related to drug-design, V.

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We did not have any experiences with Synchrotron Radiation (SR) before utilizing hatch-A of Hyogo-beamline (BL24). So first, we have collected a test data of bovine pancreatic trypsin crystals. The main purpose is searching the conditions and parameters about data collection experiments.

Crystallization:

Bovine pancreatic trypsin was purchased from Sigma. Crystallization was performed by sitting drop vapor diffusion method at 18°C. It took a few days to appear the crystals from droplets.

30%(w/v)PEG8000
0.1M Tris-HCl pH8.5
0.2M AmS
2mM $CaCl_2$

Data collection:

We used several trypsin crystals to optimize camera_length (from the crystal to the detector distance) , exposure time and collimator size for the oscillation data collection. A single crystal of trypsin (0.07x0.05x0.05mm) was used for data collection at -173°C with Rigaku Raxis-IV and cryo_equipment. Data collection conditions are below.

camera_length = 300mm
oscillation angle = 1.5°
exposure time = 300sec/frame
number of frames = 85

Crystallographic data:

We have also collected a data set of trypsin in our laboratory to compare with the SR-data. The crystal was obtained from the same crystallization condition.

SR-data

space group:$P3_121$
crystal constants:
a=54.8 Å b=54.8 Å c=108.3 Å
$\alpha=\beta=90°$ $\gamma=120°$
resolution : 1.8 Å
Rmerge:6.22%
Crystallographic R (no waters):27.4%

In-house-data

space group:$P3_121$
crystal constants:
a=54.7 Å b=54.7 Å c=108.4 Å
$\alpha=\beta=90°$ $\gamma=120°$
resolution : 1.8 Å
Rmerge:6.34%
Crystallographic R (no waters):25.3%

Conclusion:

The resolution of this SR-data was limited by long camera_length to avoid high background. There is no significant difference in the crystallographic data between the SR-data and the In-house-ata but we have an advantage in the electron density map (2fo-fc) of the SR-data.