

## X-ray crystallography of calcium-dependent inhibitory factor

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Calcium binding proteins play important roles in intracellular signal transduction. In general, these molecules act as activation factors, but the calcium-dependent inhibitory factor (CIF) from *Physarum polycephalum* inhibits myosin light chain kinase activity in the presence of  $\text{Ca}^{2+}$ . To elucidate this unusual mechanism based on the molecular structure, the crystallographic analysis of CIF is proceeded.

The CIF crystals belong to a trigonal space group  $P3_121$ (or  $P3_21$ ), with  $a=b=64.4\text{ \AA}$  and  $c=207.2\text{ \AA}$  and its asymmetric unit contains one molecule with  $M_r=38000$ . We had already collected diffraction data up to  $3.0\text{ \AA}$  resolution. But, in spite of several trials for getting higher resolution data, which include cocrystallization with chemical additives, cryocrystallography, mutation of the cysteine residue into serine and raising the concentration of precipitant, there had been no more improvement. Therefore, we tried to collect the diffraction data at the station BL41XU under some cryoprotectant conditions and obtained the following results.

- 1) Some crystals diffracted up to about  $2.5\text{ \AA}$  at  $100\text{K}$ . The diffraction data was processed by the AUTO system to give a data set containing 16829 unique reflections to  $2.5\text{ \AA}$  with an overall  $R_{\text{merge}}$  factor of 9.90%.
- 2) For these crystals, glycerol was more suitable as a cryoprotectant than sucrose.
- 3) When the concentrations of both ammonium sulfate (precipitant) and glycerol were raised, the crystals did not give the diffraction at a higher resolution.
- 4) Higher resolution data were collected by transferring the crystals sequentially into 5, 10, 15, 20, 25 and 30% glycerol. The resolution was also improved by transferring them directly into 30% glycerol, but the diffraction was slightly inferior.
- 5) Using about 30% glycerol as a cryoprotectant, the best data were obtained by 30min soaking and the crystals were damaged by 5min or 6hr soaking.

Now, we are determining the tertiary structure of CIF by MIR method.