Preparation of large-volume crystals for structure analysis of human casein kinase-2 by neutron crystallography

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Casein kinase 2(CK2) is one of the ubiquitous Ser/Thr kinases and is involved in the cell cycle and the survival and proliferation of cells. CK2 is a heterotetrameric structure comprising two α- or α-subunits and two regulatory β-subunits, in which two β-subunits dimerize the two catalytic α- or α-subunits[1]. In order to understand the biological function of the alpha catalytic subunit of CK2 (CK2α), we aim to analyze the structure of CK2α including information of the hydrogen and hydrating water molecule by neutron crystallography.

The gene coding CK2α was inserted into pET24a and expressed in *E. coli* strain BL21DE3, in which the mobile region (330-335) and chemically reactive thiols (Cys147 and Cys220) were removed by amino acid mutation. The recombinant CK2α was successfully expressed in *E. coli* as a soluble protein and was purified by several steps of ion-exchange chromatography. A total of 150 mg protein was obtained from a 6 L culture, and was used for crystallization trials. The preparation of large crystals was performed using a macro seeding method specially developed for CK2α. After mixing the protein solution (40 mg/mL) with the same portion of 25 mM Tris-HCl buffer (pH 8.5) containing 0.8 M ammonium sulfate, 1 mM dithiothreitol and 5 % acetonitrile, seed crystals were added to each well. Then, the concentration of ammonium sulfate was raised to 1.2 M over a period of several days by replacing the reservoir solution. Finally, a large crystal with a volume of approximately 2 mm^3 was reproducibly obtained. From the X-ray diffraction study, we confirmed that the crystals obtained diffracted to approx. 1 Å resolution at 100K after soaking the crystal into the deuterated cryo protectant. The neutron diffraction data collection is planned to obtain a high resolution neutron structure of CK2α.

Reference

[1] K. Niefind, B. Guerra, I. Ermakowa, O. G. Issinger, EMBO J. 20, 5320-5331. (2001)