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Structure analysis of drug target protein CK2 by high resolution X-ray and neutron crystallography

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1 Introduction

Casein kinase 2 (CK2) is one of the ubiquitous Ser/Thr kinases and has a heterotetrameric structure comprising two α-subunits and two regulatory β-subunits. In order to understand the molecular interaction between the alpha catalytic subunit of CK2α and the inhibitors, we aim to analyze the structure of CK2α including information of chemical bond length, alternative conformations and anisotropic thermal motion by high resolution X-ray, and structure of hydrogen atoms and hydrating water molecules by neutron crystallography.

2 Experiment

The gene coding CK2α was inserted into a vector and expressed in *Escherichia coli*, in which chemically reactive thiols and the C-terminal region were removed by amino acid mutation. Usually, 25 mg protein was obtained from one liter culture. The complex with inhibitor emodin or CX-4945 was prepared by mixing before crystallization. Large crystals were prepared using a macroseeding method. Seed crystals produced in the initial crystallization drop were transferred to other equilibrated crystallization drops which is mixture of precipitant solution containing 0.1 M Tris-HCl (pH 8.5), 1.2 M ammonium sulfate, 5% acetonitrile and 2 mM DTT, and 20 mg/mL protein solution. Crystals of CK2α was reproducibly obtained with a volume of approximately 2 mm^3. The large crystals of ligand free and emodin complex of CK2α were soaked into the deuterated cryoprotectant solution, and diffracted to 1.9 and 2.0 Å resolution at 100 K in the neutron experiment, respectively. For joint refinement with neutron and X-ray diffraction data, X-ray diffraction data were collected with the same crystals used for neutron data collection. In addition, high resolution X-ray diffraction data were collected using smaller crystals soaked by hydrated solution at PF beamline (See Table 1).

3 Results and Discussion

As a result of joint refinement, we have clearly observed omit maps of hydrogen atoms in water molecules located at active site and dissociable hydrogen atoms in two of three hydroxy groups in emodin (See Fig. 1). When compared structure of the conserved H_2O19 between ligand free and emodin complex, positions of hydrogen atoms are significantly different based on neutron maps while the position of oxygen atom in H_2O19 is almost the same according to electron density maps. In addition, any significant omit maps of hydrogen atoms of the hydroxy group interacting to the H_2O19 in emodin is not observed, indicating that the hydroxy group is dissociated and has negatively charged. These information could aid in the drug design to develop anticancer agents.

Table 1: Statistics of collected diffraction data

Sample	Ligand free	Emodin complex	CX-4945 complex
Beam line	BL-5A	BL-5A	BL-5A
Resolution, Å	1.05	1.10	1.30
Space group	$P2_1$	$P2_1$	$P2_1$
Cell constants a, b, c, Å	58.6, 45.5, 63.5	58.6, 45.4, 63.6	58.2, 45.4, 62.4
Outer Shell, Å	1.09-1.05	1.14-1.10	1.35-1.30
Reflections	880377	636397	477145
Unique reflections	137800 [13178]	126644 [12574]	74581 [7278]
Completeness, %	94.5[90.6]	99.9[99.9]	99.2[98.1]
R-merge, %	4.2[59.1]	3.4[36.2]	3.9[35.7]
I/σI	58.4[2.8]	61.0[4.0]	55.9[3.5]
Redundancy	6.4[3.6]	5.0[3.4]	6.4[3.5]

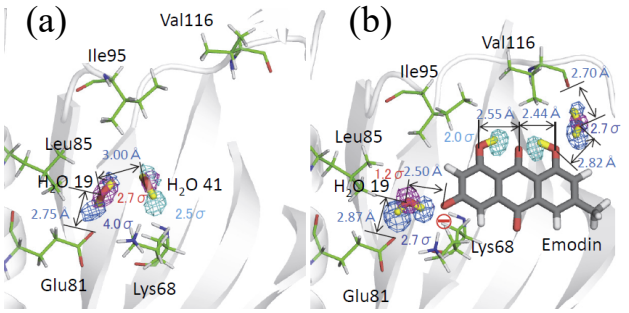

Fig. 1: Active site structures obtained by joint neutron and X-ray refinement. Ligand free CK2α (a) and CK2α emodin complex (b) were shown with maps. Neutron *Fo*-*Fc* omit maps of hydrogen atoms are colored in blue or cyan. X-ray 2*Fo*-*Fc* maps of oxygen atoms in water molecules are colored in red.

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