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X-Ray diffraction experiments for drug target protein of human casein kinase II

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1 Introduction

Casein kinase II (CKII) exhibits broad phosphorylation reaction on various important regulatory proteins such as survival factors in eukaryotic cells. Since the relationship of CKII over-expression to carcinogenesis and cancer metastasis has been reported, CKII is considered to be one of the drug target proteins. Here, we aimed to obtain the structural information with location of hydration water molecules, and to elucidate the catalytic reaction of CKII for development of effective inhibitors.

2 Experiment

All crystals were prepared in vapor diffusion hanging drops by a micro seeding method. A reservoir solution containing 0.1 M Tris–HCl (pH 8.5), 1.2 M ammonium sulfate, 5% acetonitrile, and 2 mM DTT was mixed with the protein solution, and equilibrated at 293K before seeding. The obtained crystals were soaked into a cryo protectant solution, composed of 0.1 M Tris–HCl (pH 8.5), 35% PEG MME 5000, 0.2 M ammonium sulfate and 10% (w/v) glycerol, and were mounted with a cryoloop and flash-cooled in a nitrogen-gas stream at 100 K. The collected data were indexed, integrated and scaled using DENZO and SCALEPACK programs. The structural refinement was performed by the program PHENIX.

3 Results and Discussion

We have found the hydrogen bond network going through the protein molecule from catalytic residue of Asp156 to bulk solvent at the opposite side by neutron crystallography [1]. Asp156 is well-known to enhance the nucleophilicity of the substrate OH group to the γ-phospho group of ATP by elimination of the proton. Interestingly, the network originating at Asp156 involves the ion pair of His148 and Asp214 inside the protein molecule. According to the neutron structure analysis, the hydrogen atom in the water molecule bound to Asp214 displayed delocalization in a distance of 0.2 Å probably because of quantum effect. Additionally, we confirmed the catalytic activity of CKII and mutant enzymes. The results indicated that the mutations on His148 to Ala, Ser, and Asn significantly affect to the catalytic activity. Table 1 lists the data collection and refinement statistics. The analysis revealed water molecules occupying in the space of side chain in the all mutants, and can keep hydrogen bonding [1]. An example in H148A is shown in Fig. 1. These results demonstrate that the ion pair of His148 and Asp214 plays a key role in catalytic reaction at the active site, and the long and potential hydrogen bonding network may assist the general base catalyst to extract a proton with link to the bulk solvent via intermediates of the ion pair. These findings will lead us to know the catalytic mechanism more deeply for drug design.

Table 1: Diffraction data and refinement statistics

Protein	WT1*1	H148A*1	H148S*1	H148N*1	WT2
Space Group*2	$P2_1$	$P2_1$	$P2_1$	$P2_1$	$P2_1$
Temperature	100	100	100	100	100
Resolution	1.05	1.20	1.50	1.65	1.70
No. of ref.*3	137,800	92,189	49,697	37,338	34,389
Redundancy	6.4	4.5	4.7	3.3	3.3
R-merge	0.042	0.058	0.036	0.039	0.043
(outer shell)	0.591	0.563	0.540	0.336	0.342
Completeness	0.945	0.955	0.999	0.998	0.999
(outer shell)	0.906	0.927	0.999	0.997	0.998
Refinement					
R-factor	0.160	0.152	0.194	0.197	0.184
Free-R	0.180	0.181	0.230	0.239	0.222
PDBID	5ZN1	5ZN2	5ZN3	5ZN4	5ZN5

*1; two mutations of C147A and C220A are included.
*2; unit cell parameters for all derivatives are similar to that of WT1 (a=58.6, b=45.5, c=63.2 Å, β=111.8˚).
*3; number of unique reflections.

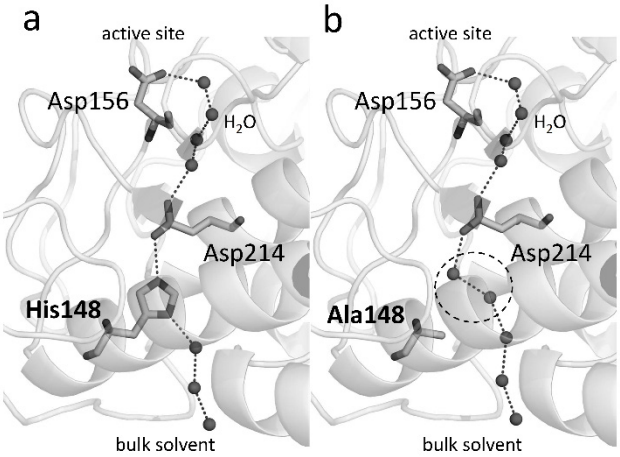

Fig. 1: Refined X-ray crystal structures of WT1 (a) and H148A mutant (b). The two additional water molecules are observed in the space near Ala148 in (b).

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References

[1] C. Shibazaki *et al.*, *J. Mol. Biol.* **430,** 5094-5104 (2018).

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