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Structural insight into human CK2α in complex with the potent inhibitor ellagic acid

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ABSTRACT

We determined the 2.35-Å crystal structure of a human CK2 catalytic subunit (referred to as CK2α complexed with the ATP-competitive, potent CK2 inhibitor ellagic acid. The inhibitor binds to CK2α with a novel binding mode, including water-mediated hydrogen bonds. This structural information may support discovery of potent CK2 inhibitors.

Protein kinase CK2 (former name: casein kinase 2) is a highly conserved serine/threonine protein kinase, and >300 protein substrates of CK2 have been identified.[1] The CK2 holoenzyme comprises two catalytic and two regulatory subunits.[2] CK2α as catalytic subunit is constitutively active with or without the regulatory subunit referred to as CK2β.[3]

CK2 has important roles in the growth, proliferation, and survival of cells. It is expressed in a wide variety of tumors.[4] Downregulation of CK2 in tumor cell lines leads to cell death because CK2 suppresses apoptosis in tumor cells.[5] We recently reported that CK2 was a target protein for glomerulonephritis (GN) therapy, supported by experiments showing that administration of antisense oligodeoxynucleotide against CK2 or low-molecular-weight CK2-specific inhibitors effectively prevented progression of renal disease in a rat model of GN.[6]

Several planar chemical compounds have been identified as ATP-competitive and selective CK2 inhibitors, such as ellagic acid,[7] emodin,[8] apigenin,[9] TBB,[10] IQA,[11] and DRB[12] (Fig. 1). Ellagic acid (4,4′,5,5′,6,6′-hexahydroxydiphenic acid 2,6,2′,6′-dilactone), a naturally occurring tannic-acid derivative, was first identified as a novel potent CK2 inhibitor using a virtual screening approach (K_i = 0.02 μM).[7] Ellagic acid selectively inhibits the catalytic activity of CK2 (IC_{50} = 0.04 μM) compared with other kinases such as PKA (IC_{50} = 2 μM) or PKC (IC_{50} = 8 μM).[7] Trials to produce potent and selective CK2 inhibitors are in progress.[13,14]

For stimulating drug discovery, the structures of human CK2α complexed with three ATP site-directed inhibitors, including emodin[8] (Protein Data Bank (PDB) code: 3BQC and 3C13), DRB[15] (PDB code: 2RKP), and AMPPNP (Fig. 2; 5′-adenylyl-beta, gamma-imidodiphosphate)[16,17] (PDB code: 1PJK and 2PVR) have been reported. Among them, emodin and DRB bind to CK2α with unique binding modes in the ATP-binding site. As well as emodin and DRB as natural compounds, ellagic acid was expected to bind to CK2α with another novel binding mode. This would contribute to the development of CK2 inhibitors.

The crystal structure at 2.35 Å shows that ellagic acid binds to CK2α in the ATP-binding region (Fig. 3),[18] although the relatively broad electron density map corresponding to the inhibitor suggests mere disorder of the inhibitor molecule. This binding mode is different to the one analyzed using computational methods by Cozza et al.[7] Ellagic acid has no immediate interaction with the hinge region (Glu114 to Asn118) by which the N-terminal lobe and C-terminal lobe are connected. This is despite the fact that interaction of ATP, GTP and many ATP-competitive inhibitors in the hinge region is a dominant factor in binding to CK2α, and that docking simulation showed that ellagic acid made immediate interaction with the hinge region.

In this complex, four water molecules (W1, W2, W3, W4) independently act as a bridge between CK2α and ellagic acid. The latter interacts with the hinge region, and this is mediated by W1 and

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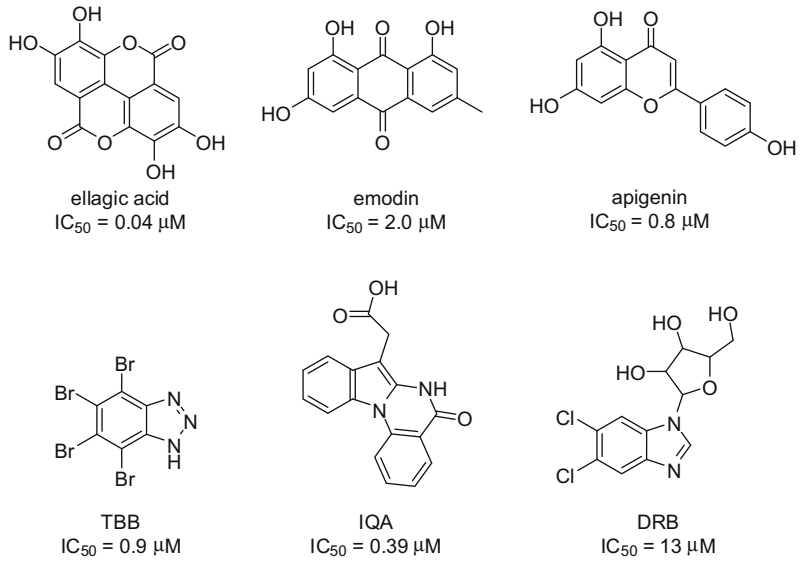

Figure 1. Chemical structures and inhibitory activities of CK2 inhibitors.

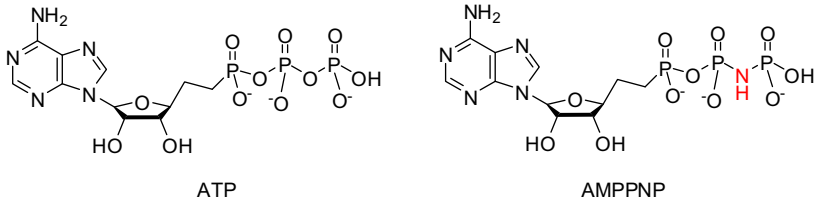

Figure 2. Chemical structures of ATP and AMPPNP as an ATP analogue.

W2 (Fig. 4). This water-mediated interaction makes prediction of the binding mode difficult because several kinase inhibitors form immediate hydrogen bonds at the hinge region. W1 forms hydrogen bonds with one of the hydroxyl groups of ellagic acid (2.5 Å), the carboxyl oxygen atom in the backbone of Glu114 (3.3 Å) and the nitrogen atom in the backbone of Val116 (3.3 Å). Superimposition of AMPPNP onto the ellagic acid–CK2α complex reveals that the position of W1 in the ellagic acid–CK2α complex fits the N1 atom in the purine moiety of AMPPNP, in which the N atom forms an immediate hydrogen bond with the nitrogen atom in the backbone of Val116 in the hinge region (Fig. 5). The remained water W1 in the ellagic-CK2α complex reminds the fact that a potent inhibitor binds to adenosine deaminase leaving the water molecule at the binding site of the N1 atom in substrate adenosine.[19] This positional compatibility suggests that unique inhibitors possessing this water-mediated interaction may be produced via structure-based drug discovery or virtual screening. Resulting compounds are likely to have selectivity to CK2α over other kinases.

W2 and W1 line ellagic acid and CK2α (Fig. 4), forming hydrogen bonds with a hydroxyl group of ellagic acid (2.8 Å) and with the nitrogen atom of the side chain of Asn118 (3.3 Å). W2 lies at the boundary between the ATP-binding pocket and the solvent-accessible region.

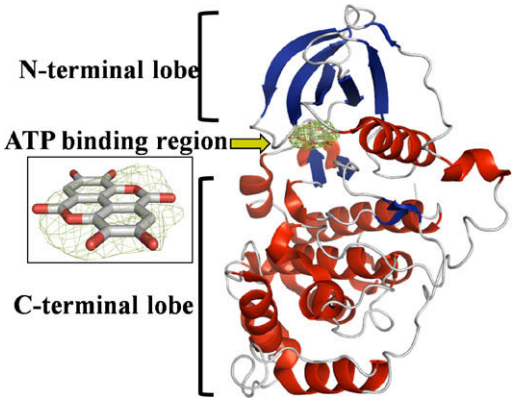

Figure 3. Overall structure of the catalytic subunit of human CK2 in complex with ellagic acid. The N-terminal lobe, which is rich in β-strands and ends at Asn118, is implicated in nucleotide binding. The C-terminal lobe is mainly α-helical and serves as a docking site for substrates. Ellagic acid binds in the ATP-binding region near the hinge region, which unites both lobes.

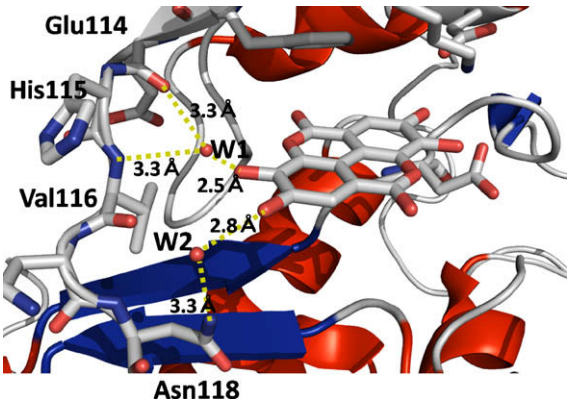

Figure 4. Interaction between ellagic acid and human CK2α at the hinge region. Ellagic acid binds to CK2α through the water molecules W1 and W2.

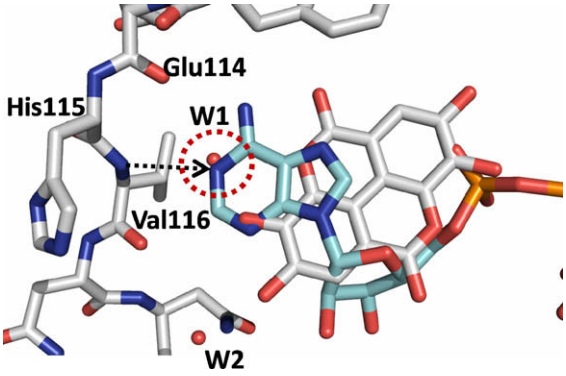

Figure 5. Superimposition of AMPPNP from 2PVR structure in PDB onto the ellagic acid–CK2α complex. N1 in the purine frame of AMPPNP fits the W1 water molecule.

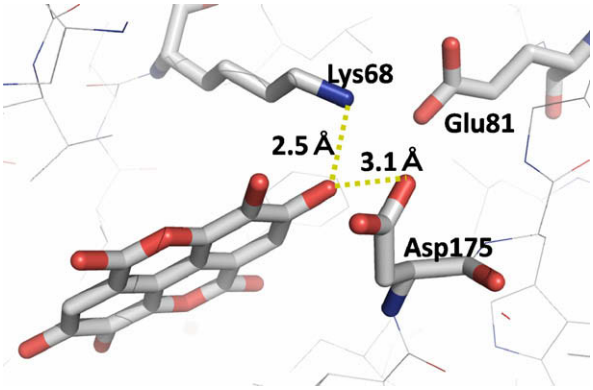

Figure 7. Ellagic acid interacts with Lys68 and Asp175.

The carbonyl oxygen atom of Arg47 in the glycine-rich loop (a highly conserved region among protein kinases with high flexibility) interacts with ellagic acid, and this interaction is mediated by W3 (Fig. 6). W3 forms two hydrogen bonds with the carbonyl oxygen of Arg47 (2.8 Å) and a carbonyl oxygen of ellagic acid (2.6 Å). This interaction allows the glycine-rich loop to restrict conformational flexibility as judged by a density map corresponding to the loop (Fig. 6).

W4 tethers ellagic acid to Ck2α at His160. Compared with other human CK2α structures including emodin-,[8] DRB-,[15] and AMPPNP-[16,17] complexes, the side chain of His160 is remarkably close to ellagic acid; this interaction (hydrogen bond or OH–π interaction) is mediated by W4. This observation is supported by density maps corresponding to His160 (Fig. 6). This induced fit may help to stabilize this complex structure.

Other than these water-mediated interactions, immediate hydrogen bonds and van der Waals interactions are observed in binding between ellagic acid and CK2α. Ellagic acid forms a hydrogen bond with an active residue Lys68 (2.5 Å; Fig. 7). Lys68 is essential for enzyme activity, and binds to the α- and β-phosphate of ATP. Ellagic acid interacts with Asp175 (3.1 Å), which is also essential for enzyme activity (Fig. 7). The latter unique interaction is not observed in the predicted complex by computational analysis.[7] Protonated ellagic acid may form a hydrogen bond or π–π interaction with Asp175, even though X-ray analysis did not reveal if ellagic acid is protonated.

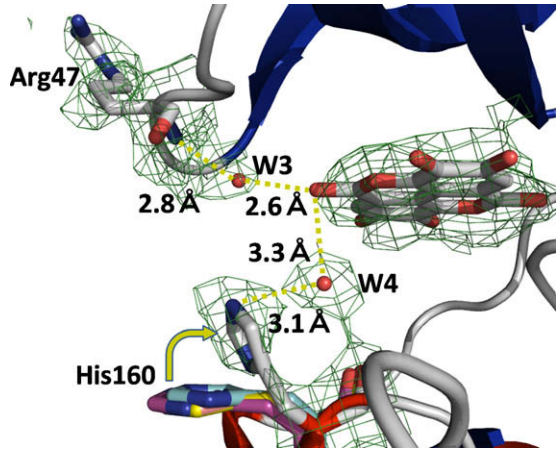

Figure 6. Interactions between ellagic acid and Arg47 (mediated by a water molecule, W3) and between ellagic acid and His160 (mediated by a water molecule, W4). Electron density maps are colored in green. The other colors of His160 are extracted from the other CK2α–inhibitor complexes (PDB code: 1PJK, 2PVR, 2RKP, 3BQC, 3C13).

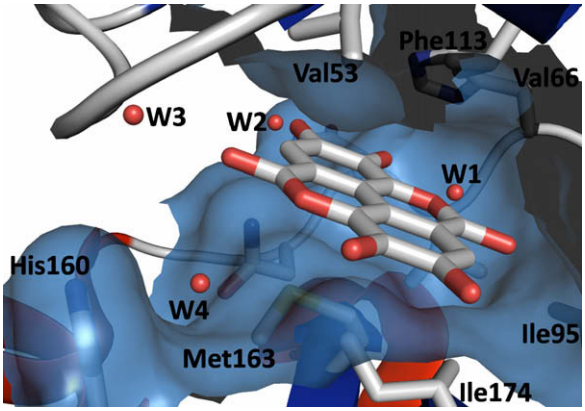

Figure 8. Hydrophobic residues in the ATP-binding region involving Val53, Val66, Ile95, Phe113, His160, Met163 and Ile174 surround ellagic acid.

The condensed planar structure comprising four flat rings in ellagic acid would effectively gain enthalpy from the van der Waals contacts with many hydrophobic residues, including Val53, Val66, Ile95, Phe113, His160, Met163 and Ile174, in the ATP-binding region of CK2α (Fig. 8). The smaller ATP-binding site of CK2α due to bulky residues should allow high selectivity for other kinases such as PKA and PKC (Table. 1). The rigid conformation of ellagic acid possibly confers a small entropic penalty in binding to CK2α.

In conclusion, we determined the crystal structure of human CK2α complexed with ellagic acid, providing structural insight for potential drug discovery. First, ellagic acid forms several water-mediated or immediate hydrogen bonds with the participation of W1, W2, W3, W4, Glu114, Val116, Asn118, Arg47, His160 and Lys68. These hydrogen bonds allow ellagic acid to bind to CK2α strongly and selectively. Ellagic acid has a specific interaction with His160 compared with the other human CK2–inhibitor complexes. Second, the highly flat and hydrophobic character of ellagic acid is advantageous in the van der Waals interactions at the smaller ATP-binding site comprising many hydrophobic residues, including Val53, Val66, Ile95, Phe113, His160, Met163 and Ile174. These structural insights would be highly beneficial for developing novel and potent CK2 inhibitors.

Table 1
Residues of PKA and PKC corresponding to bulky residues in the ATP-binding site of CK2α

CK2	Val66	Met163	Ile174
PKA	Ala70	Leu173	Thr183
PKC	Ala407	Leu511	Ala521

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18. The C-terminal truncated form of human CK2α was cloned into the pGEX6P-1 expression vector (GE Health care) and expressed in *Escherichia coli* as a GST-fused protein at the N-terminus. E. coli strain HMS174 (DE3) cells carrying pGEX/CK2α were cultured in LB medium supplemented with ampicillin at 310 K. Expression was induced by addition of 0.5 mM IPTG when the absorbance 600 nm reached 0.5, and cultivation was continued for 21 h at 298 K. The cells were harvested, resuspended in the buffer, and sonicated. After removing the cellular debris by centrifugation, the supernatant was purified by the affinity chromatography with a GSTrap HP column and anion-exchange chromatography with a MonoQ column (GE-Healthcare). The purified protein was concentrated to 10 mg/ml. An excess amount of powdered ellagic acid was suspended in the protein solution. The centrifuged supernatant was used for crystallization as a protein solution. Crystallization trials were performed using a crystal screening kit Crystal Screen HT (Hampton Research) by the sitting-drop vapour-diffusion method. Well formed crystals of the CK2α-ellagic acid complex were obtained under the optimized condition including 25% ethylene glycol as a precipitant. After dipping into Paratone-N oil (Hampton Research), the crystals were frozen using a nitrogen gas stream at 100 K. A diffraction data set was collected on BL6A beam line at the Photon Factory. The data were processed with the program *HKL-2000*[20]. Molecular-replacement calculations were carried out with the program *Molrep*[21] in the CCP4 suite.[22] All refinements and electron-density map calculations were performed using the programs *DS modelling* and *CNX* (Accelrys). Final refinement was converged to $R = 22.19\%$, $R_{free} = 27.41\%$. The PDB accession code is 2ZJW.
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