

KP218 An approach for producing a potent CK2 α inhibitor using X-ray and calorimetry analyses.

Yusuke Sekiguchi^a, Harumi Fukada^b, Tetsuko Nakaniwa^a, Takayoshi Kinoshita^a, Shinya Nakamura^c, Isao Nakanishi^{c,d}, Kazuo Kitaura^d, Hiroaki Ohno^d, Yamato Suzuki^d, Akira Hirasawa^d, Gozoh Tsujimoto^d, Toshiji Tada^a

^aGraduate School of Sciences, ^bGraduate School of Life and Environmental Sciences, Osaka Prefecture University, Osaka 599-8570, Japan, ^cDepartment of Pharmaceutical Sciences, Kinki University, Osaka 577-8502, Japan, ^dGraduate School of Pharmaceutical Sciences, Kyoto University, Kyoto 606-8501, Japan
sekiguchi07@b.s.osakafu-u.ac.jp

Protein kinase CK2 (former name: casein kinase 2) is a highly pleiotropic serine/threonine protein kinase, composed of two catalytic (α , or α') and two regulatory subunits (β). They forms an $\alpha_2\beta_2$, $\alpha\alpha'\beta_2$, or $\alpha'\beta_2$ heterotetrameric structure. Catalytic subunit CK2 α (Fig.1) is constitutively active either with or without the regulatory subunit CK2 β , with more than 300 protein substrates already recognized. CK2 plays important roles in cell growth, proliferation, and survival, while it is highly expressed in a wide variety of tumors. Furthermore, CK2 is a target protein for glomerulo nephritis (GN) therapy, because an administration of either anitiseense oligodeoxynucleotide against CK2 or low molecular weight CK2 α -specific inhibitors effectively prevents the progression of renal pathology in the rat GN models.

To design a novel and potent CK2 α inhibitor, we determined X-ray crystal structures of CK2 α -inhibitor complexes, and measured enzyme kinetic parameters for the respective inhibitors. Supporting with computational analysis, these data show the contributions of individual residues in ligand-binding, and confer useful information for structure-based drug design.

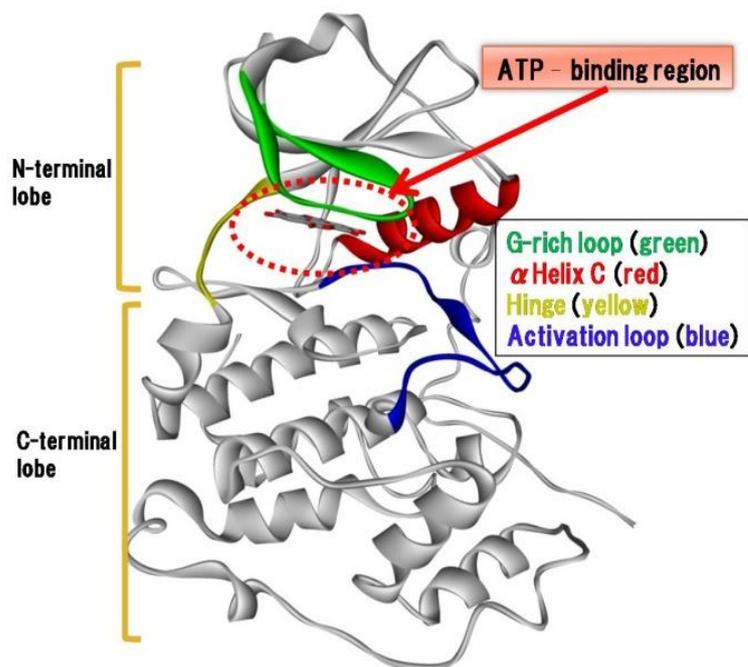


Fig.1 Overall structure of human CK2 α

METHODS

1. X-ray crystallography

We constructed an expression plasmid of human CK2 α and transformed it to the *E. coli* strain HMS174 (DE3). Harvest cells were thawed, sonicated and centrifugated, and then the extracted supernatant was purified by affinity and anion-exchange chromatographies. Crystals of CK2 α complexed with the individual inhibitors including ellagic acid, apigenin, cc4791 and cc4820 (Fig. 2) were obtained by the sitting-drop vapour-diffusion method, and diffraction data sets were collected at Photon Factory and at our laboratory. The structures of the inhibitor-CK2 α complexes were solved by the molecular displacement method.

2. Isothermal Titration Calorimetry (ITC)

ITC is a thermodynamic technique for monitoring any chemical reaction. When substances bind, heat is either generated or absorbed. Measurement of this heat allows accurate determination of binding constants (K_B), reaction stoichiometry (n), enthalpy (ΔH) and entropy (ΔS).

Five inhibitor solutions (Fig.2) are titrated into the CK2 α solution at 293K using VP-ITC (Microcal) until reaching to thermodynamic equilibrium.

3. Computational analysis

To investigate which amino acid residue is critical to ligand binding, the interaction in the individual amino acid residue was calculated in each complex using MOE. First, complex structure assigned MMFF94x forcefield parameter was optimized with energy minimization. During geometry optimization, all atoms except for hydrogen atoms were tethered to their crystallographic position. Then the residue-based interaction energy was estimated from MM/GBVI approach to consider solvation effect.

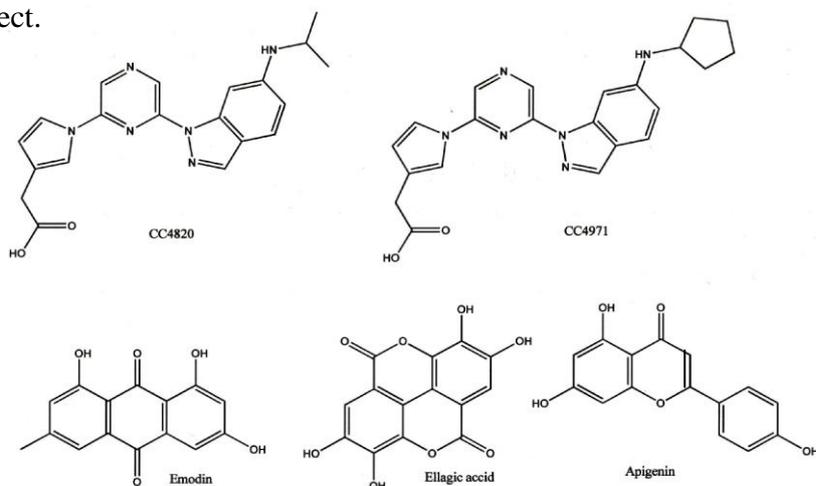


Fig. 2 Structures of CK2 α inhibitors