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Structural Basis for the Interaction between Casein Kinase 1 Delta and a Potent and Selective Inhibitor

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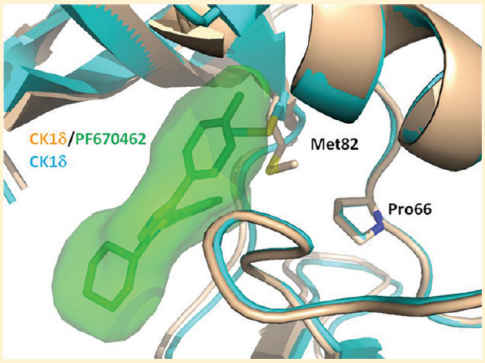
Supporting Information

ABSTRACT: Casein kinase 1 delta (CK1δ) and its closest homologue CK1ε are key regulators of diverse cellular growth and survival processes such as Wnt signaling, DNA repair, and circadian rhythms. We report three crystal structures of the kinase domain of human CK1δ, one apo and two complexed with a potent and selective CK1δ/ε inhibitor PF670462 in two different crystal forms. These structures provide a molecular basis for the strong and specific inhibitor interactions and suggest clues for further development of CK1δ/ε inhibitors.



INTRODUCTION

The casein kinase 1 (CK1) family of highly conserved serine/threonine protein kinases has six human isoforms α, γ1, γ2, γ3, δ, and ε that regulate diverse cellular processes including Wnt signaling, circadian rhythms, cellular signaling, membrane trafficking, cytoskeleton maintenance, DNA replication, DNA damage, and RNA metabolism.[1] CK1δ and CK1ε (CK1δ/ε), two closely related CK1 isoforms, have recently gained increased attention as potential drug targets. CK1δ/ε phosphorylate certain clock-related proteins as part of a complex arrangement of transcriptional/translational feedback loops that compromise the circadian oscillator in mammals.[2] In addition, CK1δ/ε are two key mediators of the Wnt signaling pathway, and several studies have highlighted their roles in cell survival and cancer progression. Kinome RNA interference screens showed that knockdown of CK1ε slowed cancer cell growth,[3] and CK1δ was also implicated in pancreatic cancer progression.[4] Moreover, both CK1δ/ε were suggested to be involved in psychostimulant-induced behaviors by acting on the Darpp-32-PP1 signaling pathway to regulate AMPA receptor phosphorylation in the nucleus accumbens.[5] Thus, inhibition of the kinase activity of CK1δ/ε is a promising strategy for multiple therapies in the treatment of abnormal circadian behavior, cancer, and drug use disorders.

Potent and selective small molecule inhibitors of CK1δ/ε have been recently reported.[6] Among them, PF670462 (**1**, Figure 1A) had an IC_{50} of 8−14 nM for CK1δ/ε and displayed good selectivity in two broad panel screens of kinases and was shown to alter CK1δ/ε dependent processes in circadian rhythms in both cultured cells and in animals.[7] Intriguingly, **1** only modestly inhibits cell proliferation despite being a potent inhibitor of Wnt signaling.[8] **1** also blocks amphetamine induced locomotion and its ability to increase phosphorylation of Darpp-32, decrease PP1 activity, and increase phosphorylation of the AMPA receptor.[5]

Detailed structural information of ligand recognition is important for further development of potent and selective CK1δ/ε inhibitors. However, no crystal structure of CK1ε has been reported and only two apo human CK1δ crystal structures have been published,[9] where the ATP binding site of one CK1δ protein is blocked by another CK1δ molecule; thus these two CK1δ structures are not ideal for structural studies with inhibitors. Herein, we report the high resolution structures of human apo CK1δ with a fully accessible active site as well as CK1δ in complex with **1** and describe the structural basis for the inhibitor's potency and selectivity over other kinases.

RESULTS AND DISCUSSION

The two previously published crystal structures of apo human CK1δ[9] were obtained with phosphorylated CK1δ (1−317). The residues C-terminal to Leu293 were disordered in both structures; thus we designed a new construct of CK1δ (1−294). After dephosphorylating CK1δ (1−294) with λ phosphatase, we obtained crystals of apo CK1δ in a crystal form ($P1$) that is different from those two previously published ($P2_12_12_1$ and $C222_1$). Our crystals diffracted to 2.3 Å with synchrotron radiation, and there are four crystallographically independent CK1δ molecules in the crystal structure. All four CK1δ molecules are quite similar to each other (with a backbone rmsd of 0.9 Å) as well as to those in previous structures (with a backbone rmsd of 1.0 Å), with the exception of the ATP binding site. The ATP binding sites of all four CK1δ molecules in this $P1$ crystal form are unoccupied and

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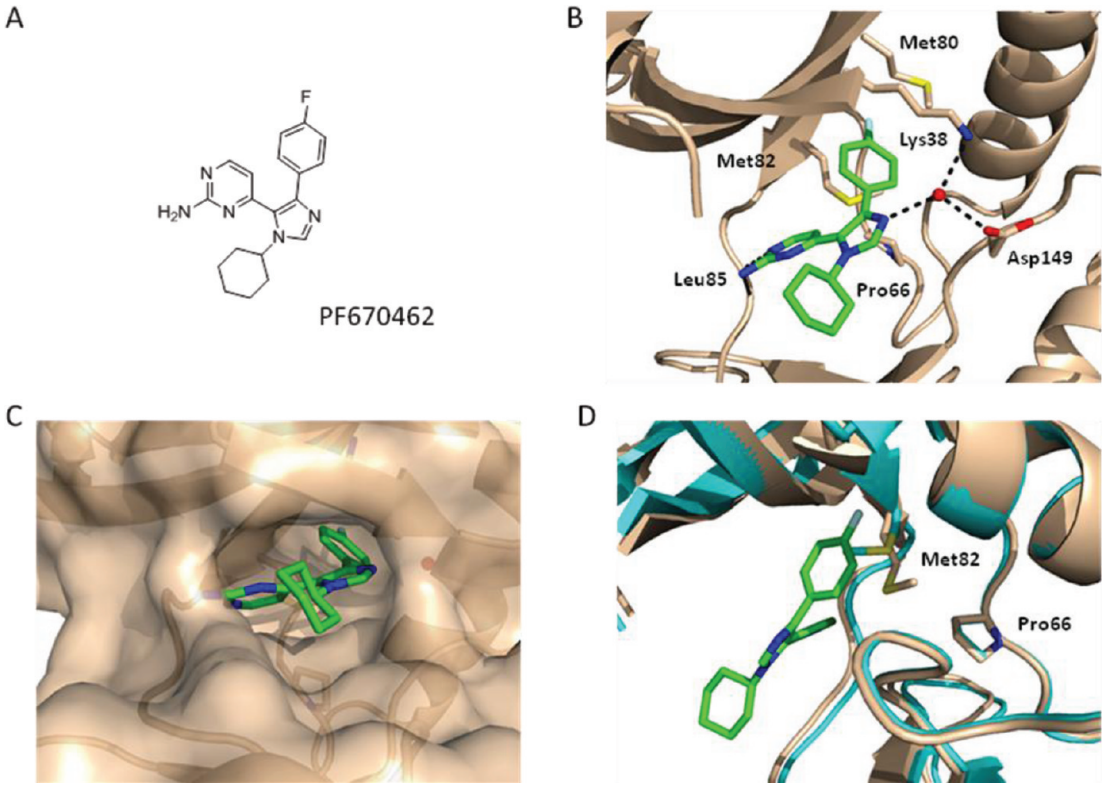

Figure 1. (A) Molecular structure of **1**. (B) The ATP binding site, showing interactions between **1** and CK1δ residues. For **1**, carbon atoms are shown in green, nitrogen in blue, and fluorine in light blue. (C) Surface representation of CK1δ with **1** bound. (D) Superposition of crystal structures of CK1δ/**1**, colored as in (B), and apo CK1δ, colored in cyan.

unhindered, unlike the previous crystal forms where the ATP binding site of one CK1δ molecule was blocked by the side chain of Arg16 from another CK1δ molecule. Soaking or cocrystallization with **1** resulted in a crystal structure of the CK1δ/**1** complex at 2.0 Å resolution. Furthermore, cocrystallization of CK1δ with **1** under a different crystallization condition yielded another crystal form ($P2_1$) that diffracted to 1.9 Å. The two CK1δ/**1** complexes observed in this crystal structure are highly similar (with a backbone rmsd of 0.8 Å) and they are also highly similar to those observed in the *P*1 crystal form (with a backbone rmsd of 0.8 Å).

The CK1δ/**1** complex structure (Figure 1B) reveals that **1** is anchored in the ATP binding site of CK1δ by interactions similar to those observed in other kinase/inhibitor complexes. The amino pyrimidine group of **1** forms two hydrogen bonds with the main chain NH and carbonyl oxygen of Leu85 from the interlobe linker (hinge) polypeptide of CK1δ. Unlike other kinase inhibitors, however, the fluorophenyl moiety of **1** is buried much more deeply in CK1δ, forming strong hydrophobic interactions with the side chains of Met80 and Met82. The imidazole nitrogen of **1** is hydrogen bonded through a water molecule to the side chain nitrogen of catalytic Lys38 and the side chain oxygen of Asp149 from the DFG motif. Surface representation of CK1δ illustrates the tight fit of **1** in the ATP binding pocket (Figure 1C).

1, the most potent CK1δ/ε inhibitor reported so far with an IC_{50} of 8−14 nM, was initially tested against 44 additional kinases and showed significant activity toward only two other kinases, epidermal growth factor receptor (EGFR) and p38 mitogen-activated protein kinase (p38), with IC_{50} values of 150 nM and 190 nM, respectively.[6a] It was later screened against another panel of 50 highly diverse kinases representing the major branches of the human kinome and found to strongly inhibit hepatocyte progenitor kinase-like/germinal center kinase-like kinase (HGK; also known as NIK).[6b] Intriguingly, **1** did not inhibit CK1γ2.[6b]

Structural superposition of the CK1δ/**1** complex and the apo CK1δ reveals one obvious difference: the side chain of the gatekeeper residue of CK1δ, Met82, is rotated by ∼180° around the Cα-Cβ bond toward Pro66 in the complex structure in order to avoid a steric clash with the fluorine atom of **1** and engages in strong hydrophobic interaction with the fluorine atom of **1** instead (Figure 1D). The side chain rotation of Met82 is only possible due to the small side chain of Pro66. Both Pro66 and Met82 are conserved in CK1ε and CK1α (Figure 2A), which explains why **1** is also a potent inhibitor for CK1ε and CK1α. Pro66 is also conserved in all three CK1γ isoforms (CK1γ1, CK1γ2, and CK1γ3), but Met82 is replaced in CK1γ by a leucine (Leu119 in CK1γ2). We hypothesize that the branched side chain of this leucine gatekeeper in CK1γ makes it much more difficult to rotate than the Met82 gatekeeper in CK1δ without clashing with other residues, resulting in much weaker inhibition of CK1γ by **1** (Figure 2B).

Structure based sequence comparison suggests that only a few kinases have residues with small side chains at the position equivalent to Pro66 in CK1δ.[10] For example, this position is occupied by an alanine (with an even smaller side chain than proline) in HGK, and Met82 of CK1δ is also highly conserved in HGK, resulting in strong inhibition of HGK by **1**. EGFR is another interesting example. Pro66 of CK1δ is replaced by a cysteine (Cys775; also with a small side chain) in EGFR, but the gatekeeper residue Met82 of CK1δ is replaced by a threonine (Thr790) in EGFR. Nonetheless, as observed in several cocrystal structures (e.g., 3POZ,[11] 3LZB,[12] 3BEL,[13] and

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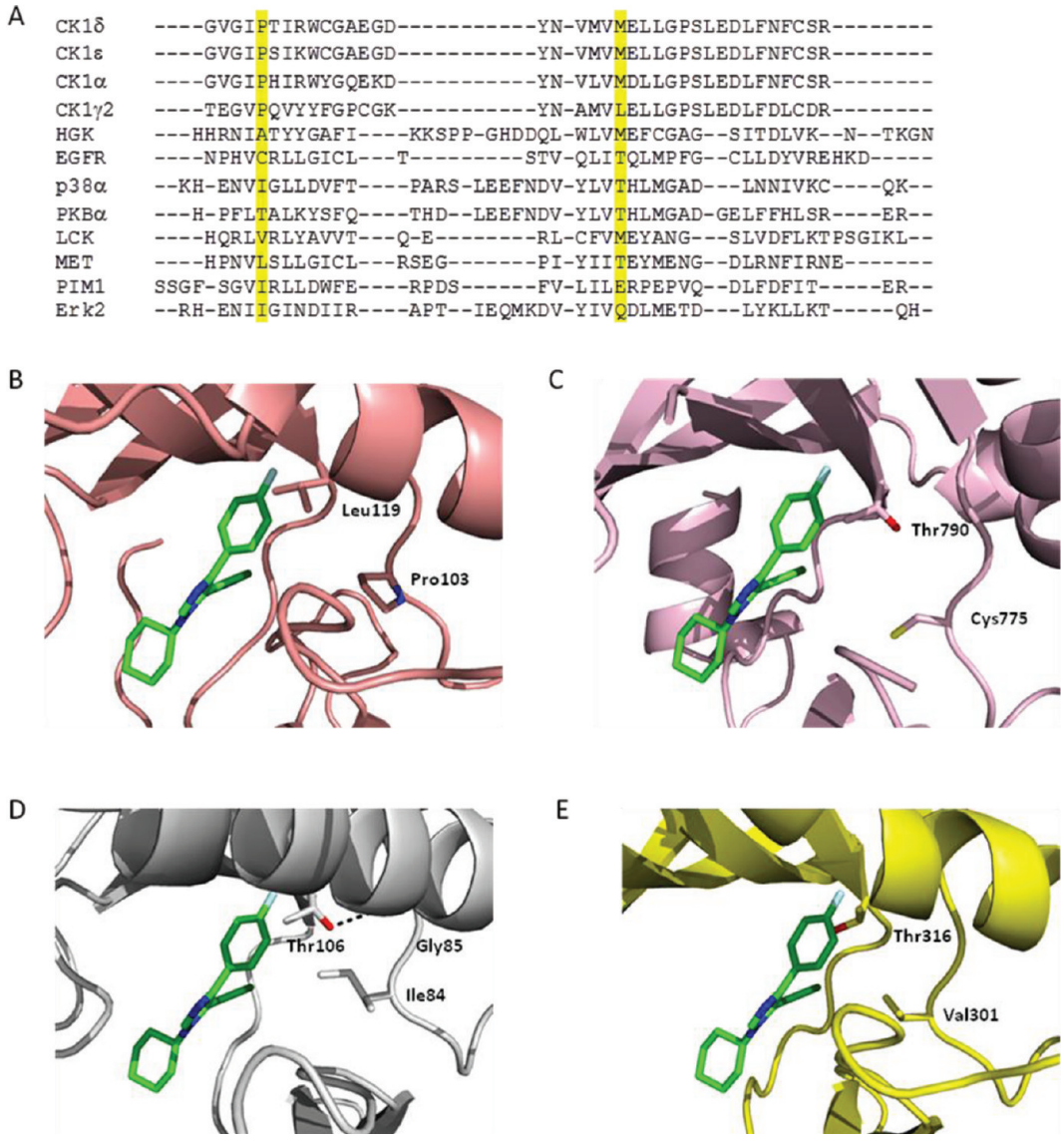

Figure 2. (A) Structure based sequence alignment of CK1δ, CK1ε, and other noted protein kinases. Key residues Pro66 and gatekeeper Met82, respectively, of CK1δ are highlighted, together with their equivalent residues in the other protein kinases. (B) **1** (in green) superimposed onto CK1γ2 (in pink; PDB entry 2C47). (C) **1** (in green) superimposed onto EGFR (in purple; PDB entry 3POZ). (D) **1** (in green) superimposed onto p38 (in gray; PDB entry 1YQJ). (E) **1** (in green) superimposed onto LCK (in yellow; PDB entry 2OFU).

1XKK[14]), Thr790 of EGFR adopts a conformation where its side chain, despite being branched, points toward Cys775, reminiscent of Met82 pointing toward Pro66 in CK1δ (Figure 2C). This side chain arrangement forms a pocket that nicely accommodates the fluorophenyl group, and we propose that this is why **1** inhibits EGFR. In contrast, Pro66 of CK1δ is replaced in most kinases by residues with larger side chains (for example, valine, threonine, leucine, and isoleucine in LCK, PKB, MET, and PIM, respectively), consistent with the weak or no inhibition of **1** against these kinases. However, like PIM, p38 also has an isoleucine (Ile84) at the position equivalent to Pro66 in CK1δ, and yet **1** still has good potency against p38. Interestingly, the gatekeeper residue of p38, Thr106, has been observed in most cocrystal structures[15] to adopt the same conformation as Thr790 of EGFR (Figure 2D), while a threonine gatekeeper in other kinases (such as LCK) does not adopt the same conformation (Figure 2E).[16] This conformation of Thr106 in p38 is possible because the residue immediately C-terminal to Ile84 is a Gly (Gly85) whose carbonyl oxygen is hydrogen bonded to the side chain hydroxyl of Thr106. The combination of Gly85 and Thr106 is unique to p38 when compared with other kinases. For example, Ile84 and Gly85 of p38 are conserved in extracellular-signal-regulated kinase 2 (ERK2) and serum-and glucocorticoid-induced protein kinase (SGK), but Thr106 of p38 is replaced by a glutamine and a leucine in ERK2 and SGK, respectively, resulting in no inhibition of **1** for ERK2 or SGK. Pro66 and Met82 of CK1δ are also highly conserved in some other kinases such as Misshapen NIK-related Kinase (MINK) and Traf2- and Nck-interacting kinase (TNIK) that have not been studied in the two reported kinase panels, and we predict that **1** would have significant activity against them as well. Our hypothesis that the interactions of the fluorophenyl moiety of **1** with residues Met82 and Pro66 of CK1δ account for the high selectivity of **1** over other kinases needs to be confirmed by either modifying **1** or mutating the related protein kinases.

Our CK1δ/**1** complex structures also suggest routes to further optimize CK1δ/ε inhibitors. As mentioned above, the

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imidazole nitrogen is hydrogen bonded through a water molecule to the side chain nitrogen of the catalytic Lys38 and the side chain oxygen of Asp149 from the DFG motif. Many highly potent kinase inhibitors, however, have direct hydrogen bond interaction with the side chains of these two residues, which are highly conserved among protein kinases. A bicyclic system containing the proper heteroatoms (such as a piperidinone fused to the imidazole of **1**) could form one hydrogen bond (through its carbonyl oxygen) with the side chain nitrogen of Lys38 and another hydrogen bond (through its nitrogen) with the side chain oxygen of Asp149, resulting in a potential improvement in potency.

In summary, the present structures provide insights into the molecular basis for the interactions of **1** with CK1δ/ε, which will be important for further optimization of CK1δ/ε inhibitors.

EXPERIMENTAL SECTION

Human CK1δ (1−294) with a cleavable N-terminal His_6 tag was expressed in *Escherichia coli* and purified using Co^{2+} chelating chromatography. The N-terminal His_6 tag was then cleaved and CK1δ was dephosphorylated by incubating with thrombin and λ phosphatase at 4 °C overnight. The untagged and dephosphorylated CK1δ was further purified by cation exchange chromatography. Crystals of CK1δ were obtained at room temperature in hanging drops with 100 mM citrate pH 5.0, 0.2 M Na_2SO_4, 15−20% PEG3350. These crystals belong to the space group *P*1 with unit cell parameters of a = 49.77, b = 85.16, c = 89.12 Å, α = 70.19, β = 73.86, γ = 86.15°. Paratone-N mineral oil was used as cryo protectant. Diffraction data for all crystals in this work were collected on beamline 21-ID-F at the Advanced Photon Source (APS) and processed and scaled with HKL 2000. The apo CK1δ structure was solved by molecular replacement with AMoRe using PDB entry code 1CKI as the template. **1** was purchased from CalBiochem, and crystals of CK1δ complexed with **1** were obtained initially by either incubating **1** with apo CK1δ crystals or cocrystallization of CK1δ with **1** under the same condition for which apo CK1δ crystals were obtained. Crystals of CK1δ/**1** were also obtained at room temperature in hanging drops with 100 mM citrate pH 5.0, 1.0 M LiCl, 22.5−35% PEG6000. These crystals belong to the spacegroup $P2_1$ with unit cell parameters of a = 49.00, b = 72.80, c = 88.99 Å, β = 103.21°. The two CK1δ/**1** complex structures were solved by molecular replacement with AMoRE using our apo structure as the template. Model building was carried out with QUANTA and refinement was done using CNS. Details on data processing and refinement statistics are given in the Supporting Information, Table 1.

ASSOCIATED CONTENT

Supporting Information

Details on data processing and refinement statistics. This material is available free of charge via the Internet at http://pubs.acs.org.

Accession Codes

Coordinates for the CK1δ, CK1δ&**1**[a], and CK1δ&**1**[b] structures have been deposited in the Protein Data Bank with access codes 3UYS, 3UYT, and 3UZP.

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ABBREVIATIONS

CK1, casein kinase 1; EGFR, epidermal growth factor receptor; p38, p38 mitogen-activated protein kinase; HGK, hepatocyte progenitor kinase-like/germinal center kinase-like kinase; LCK, lymphocyte-specific protein tyrosine kinase; PKB, protein kinase B; MET, Hepatocyte growth factor receptor; ERK2, extracellular-signal-regulated kinase 2; SGK, serum-and glucocorticoid-induced protein kinase; MINK, misshapen NIK-related kinase; TNIK, Traf2- and Nck-interacting kinase

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