Multiple conformational states of the HPK1 kinase domain in complex with sunitinib reveal the structural changes accompanying HPK1 trans-regulation

Received for publication, January 14, 2019, and in revised form, April 17, 2019. Published, Papers in Press, April 24, 2019, DOI 10.1074/jbc.AC119.007466

© Eric Johnson, Michele McTigue, Rebecca A. Gallego, Ted W. Johnson, Sergei Timofeevski, Michael Maestre, Timothy S. Fisher, Robert Kania, Sansana Sawasdikosol, Steven Burakoff, and Ciarán N. Cronin

From the La Jolla Laboratories, Pfizer Worldwide Research and Development, San Diego, California 92121 and Department of Oncological Sciences, Icahn School of Medicine at Mount Sinai, New York, New York 10029

Edited by John M. Denu

Hematopoietic progenitor kinase 1 (HPK12 or MAP4K1) is a Ser/Thr kinase that operates via the c-Jun N-terminal kinase (JNK) and extracellular signal-regulated kinase (ERK) signaling pathways to dampen the T-cell response and antitumor immunity. Accordingly, selective HPK1 inhibition is considered a means to enhance antitumor immunity. Sunitinib, a multi-receptor tyrosine kinase (RTK) inhibitor approved for the management of gastrointestinal stromal tumors (GISTS), renal cell carcinoma (RCC), and pancreatic cancer, has been reported to inhibit HPK1 in vitro. In this report, we describe the crystal structures of the native HPK1 kinase domain in both nonphosphorylated and doubly phosphorylated states, in addition to a double phosphomimetic mutant (T165E,S171E), each complexed with sunitinib at 2.17–3.00-Å resolutions. The native nonphosphorylated cocrystal structure revealed an inactive dimer in which the activation loop of each monomer partially occupies the ATP- and substrate-binding sites of the partner monomer. In contrast, the structure of the protein with a doubly phosphorylated activation loop exhibited an active kinase conformation with a greatly reduced monomer–monomer interface. Conversely, the phosphomimetic mutant cocrystal structure disclosed an alternative arrangement in which the activation loops are in an extended domain-swapped configuration. These structural results indicate that HPK1 is a highly dynamic kinase that undergoes trans-regulation via dimer formation and extensive intramolecular and intermolecular remodeling of the activation segment.

Hematopoietic progenitor kinase 1 (HPK12 or MAP4K1) comprises an N-terminal kinase domain, an intermediate region, and a C-terminal Citron homology domain (see Fig. 1A). In T-cells, HPK1 has been implicated as a negative regulator of signal transduction via phosphorylation and activation of the T-cell receptor adaptor protein SLP-76. Likewise, in B-cells, B-cell receptor signaling is down-regulated through HPK1-mediated phosphorylation and subsequent ubiquitination of activated B-cell linker protein (BLNK), an SLP-76 paralog (1). Upon activation and expansion of T-cells, caspase-mediated cleavage of HPK1 separates the kinase domain, HPK1-N, from the C terminus, HPK1-C. This cleavage event reverses the role of HPK1 from an activator of NF-κB into an inhibitor of NF-κB and leads to sensitization toward activation-induced cell death (2–5). Several in vivo studies of Hpk1−/− mice have provided genetic evidence for HPK1 as a negative regulator of the immune response and established that HPK1 functions as a potent immune response inhibitory kinase downstream of T-cell receptor–generated activation signals (6–8). Thus, HPK1 kinase has emerged as a potential target for immunotherapy by small-molecule inhibitors (9, 10).

Sunitinib malate (SutentTM) is a multi-RTK inhibitor approved for the treatment of gastrointestinal stromal tumors, renal cell carcinoma, and pancreatic cancer. Although c-KIT, VEGFR, and platelet-derived growth factor receptor are its primary targets, sunitinib also binds to other kinases, including HPK1 (11, 12). Therefore, cocrystal structures of sunitinib bound to HPK1 are of interest as a starting point in the structure-based design of more potent and selective HPK1 inhibitors.

During our drug design campaign, we generated structures of the HPK1 kinase domain (KD) in complex with sunitinib and in a wide variety of conformations, including an inactive dimer (native nonphosphorylated kinase), an active dimer (native diphosphorylated kinase), and a three-dimensional (3D) domain-swapped dimer (phosphomimetic T165E,S171E mutant) in the inactive state. The diversity of conformational states observed, both in terms of the subunits and in distinct dimers, highlights the dynamic/flexible nature of the HPK1 protein.

The authors declare that they have no conflicts of interest with the contents of this article.

This article contains Figs. S1–S8 and Table S1.

The atomic coordinates and structure factors (codes 6NYF, 6NFZ, and 6NG0) have been deposited in the Protein Data Bank (http://wwwpdb.org/).

1 To whom correspondence should be addressed: La Jolla Laboratories, Pfizer Worldwide Research and Development, 10770 Science Center Dr. (CB2), San Diego, CA 92121. Tel.: 858-638-3765; Fax: 858-678-8281; E-mail: ciaran.cronin@pfIZER.com.

2 The abbreviations used are: HPK1, hematopoietic progenitor kinase 1; JNK, c-Jun N-terminal kinase; ERK, extracellular signal-regulated kinase; RTK, receptor tyrosine kinase; VEGFR, vascular endothelial growth factor receptor; KD, kinase domain; 3D, three-dimensional; ASU, asymmetric unit; PDB, Protein Data Bank; AL, activation loop; NCS, noncrystallographic symmetry; PISA, Protein Interfaces and Surface Area; GLK, germinal center kinase–like kinase; AS, activation segment; PAK, p21-activated kinase; SEC, size-exclusion chromatography; MALD, multiance static light scattering; SLK, STE20-like kinase; LOK, lymphocyte-oriented kinase; AMPPNP, 5′-adenylyl-β,γ-imidodiphosphate; r.m.s.d., root mean square deviation; TEV, tobacco etch virus; TCEP, tris(2-carboxyethyl)phosphine.
kinase and suggests a role for dimerization as a mechanism for its regulation.

Results

In vitro inhibition of HPK1 activity by sunitinib and enhanced IL-2 production in sunitinib-treated T-cells

It has been previously shown in vitro that sunitinib can bind to the kinase domain of HPK1 with high affinity, with a dissociation constant \( K_d \) of 16 nM (12). To characterize binding of sunitinib to HPK1 in a construct most relevant to the cellular environment, we produced recombinant WT full-length HPK1 and preactivated the protein by in vitro autophosphorylation. The inhibition constant \( K_i \) for sunitinib against activated WT full-length HPK1 was measured to be about 10 nM (Fig. S1). Incubating sunitinib with Jurkat T-cells results in enhanced IL-2 production when these T-cells are stimulated with anti-CD3 and anti-CD28 monoclonal antibodies (Fig. S2). Therefore, the development of small molecules capable of inhibiting HPK1 kinase activity with exquisite specificity would benefit greatly from crystal structures of the HPK1 kinase domain in complex with inhibitor.

Structure of the inactive trans-inhibited HPK1–sunitinib complex

Initial attempts to express and purify WT HPK1 kinase domain (1–307 and 10–307) resulted in very low yields. To overcome this, we investigated the purification of the 10–307 construct in the presence of sunitinib and obtained sufficient amounts of the nonphosphorylated protein (HPK1"0") for crystallization trials. Cocrystallization screening and crystal optimization (Fig. S3) generated a 2.17-Å-resolution structure of the HPK1"0"–sunitinib binary complex. The crystals belonged to the space group R32, and the HPK1"0"–sunitinib asymmetric unit (ASU) contained two molecules of the binary complex packed into a tight noncrystallographic dimer. Electron density for the protein backbone and side chains was of overall good to excellent quality. Structure refinement produced a well-validated atomic model with good refinement statistics of \( R_{free} = 0.219 \) and \( R_{work} = 0.192 \) (Table 1). The structure revealed the expected archetypal kinase fold: an N-terminal \( \beta \)-sheet–containing domain (N-lobe), a connecting hinge region, and a C-terminal \( \alpha \)-helical domain (C-lobe) (Fig. 1B). As anticipated from previous structures of sunitinib bound to c-KIT (PDB ID code 3GOE) (13) and VEGFR2 (PDB ID code 4AGD) (14), sunitinib binds in the ATP site.

Two HPK1 kinase domains are arranged in a homodimer with the activation loop (AL) of each protomer coming into the interdomain cleft of the cognate protomer and partially occupying the ATP- and protein substrate–binding sites. Notably, a portion of the inhibitor–binding pocket is formed from AL residues of the adjacent protomer; Ala-167 Cβ is positioned ~3 Å from the inhibitor F atom (Fig. 1C). In addition, the Arg-168 side chain from the noncrystallographic symmetry (NCS) molecule makes stabilizing interactions with its symmetry mate, including to the Oδ1 and Oδ2 atoms of Asp-155 in the DFG motif as well as the His-135 backbone O. This arrangement introduces considerable restraints on the DFG motif (residues 155–157), stabilizing it in the inactive orientation. Furthermore, the position of the NCS AL is incompatible with the \( \alpha \)C-helix (\( \alpha \)C) adopting an active, \( \alpha \)C-in configuration. More specifically, Thr-175 from the AL of the opposite protomer is inserted between the catalytic lysine (Lys-46) and the conserved \( \alpha \)C glutamate (Glu-62). These interactions stabilize the inactive \( \alpha \)C-out configuration and exclude formation of the canonical active kinase Lys-46–Glu-62 salt bridge. The dimer is further stabilized by a \( \beta \)-sheet interaction where residues 171–173 of the 2-fold NCS monomers form four backbone hydrogen bonds (Fig. 2A). The AL is anchored by the DFG and P + 1 motifs, and the position of the P + 1 loop is typical with loop residues making only intramolecular contacts.

The tight subunit packing and high number of intermolecular interactions involving the active-site pocket and key regulatory motifs suggest a biologically relevant role for the dimer. To explore this further and quantitatively evaluate the crystal packing interface, we performed analysis of the structure using the Protein Interfaces and Surface Area (PISA) module in the CCP4 program suite (15). The analysis predicted the dimer to be stable in solution and revealed involvement of 62 residues in the dimer interface and 2253 Å² of buried accessible surface area (Table S1 and Fig. S4). There is a significant \( \Delta G \) of ~22 kcal/mol for the dimer that includes 13 hydrogen bonds and 12 salt bridges in the interface.

Structure of the fully active diphosphorylated HPK1–sunitinib complex

Using the WT 1–307 construct purified in the presence of sunitinib, the cocystal structure of the diphosphorylated HPK1–sunitinib complex (HPK1"2P") was obtained at 3.0-Å resolution. The crystals also belong to the space group R32 with two molecules in the ASU. However, the two molecules did not pack into a tight NCS dimer like the HPK1"0" structure. The two molecules in the ASU suggested a monomeric kinase in a nonphysiological dimer resulting from crystal packing. In contrast to the NCS dimer, PISA analysis predicted a distinct crystallographic dimer to be the only assembly stable in solution. The relative orientation of the two subunits identified by PISA was similar to that observed in the inactive HPK1"0" dimer; in each case, the subunits are assembled in a roughly parallel or “head-to-head” arrangement where the active sites are oriented to position sunitinib’s terminal diethylamino group pointing away from the dimer interface and where the activation loops are arranged at the dimer interface in an overlapping antiparallel configuration (Fig. 3, A and B). Despite the similar arrangement of subunits in the dimer, the HPK1"2P" structure is much more open with vastly reduced interface residues and buried surface area, a \( \Delta G \) of only ~9.4 kcal/mol, few hydrogen bonds, and no salt bridges, indicating a significantly weaker interaction (Table S1 and Fig. S4). Compared with the +0P structure, there are significant differences in the AL conformation and in the specific interactions made by AL residues (Fig. 2B). However, the DFG and P + 1 anchor points for the AL remain intact in both structures. From a purely structural standpoint, the interconversion of the inactive closed HPK1"0" dimer to the active open HPK1"2P" dimer would require a relative subunit rotation of ~61° and a displacement of ~8 Å.
The most significant differences between the inactive HPK1 and production of the double phosphomimetic mutant (1–307/H11001 native complex in a domain-swapped dimer Structure of the phosphomimetic HPK1–sunitinib inhibitor HPK1 Thr-175 just preceding the P/H11001 of the AL starting at Asp-155 of the DFG and continuing to and /H11001 with the interface of the closed against side chains from the N-terminal domain the /H11001 Arg-168. lized by interactions with the sidechains of Arg-136 and ration. The phosphate oxygens of phospho-Ser-171 are stabili- mediated by interactions with the sidechains of Arg-136 and Arg-168.

Comparing the positions of Thr-165 and Ser-171 in the +OP and +2P states, it is clear why phosphorylation is incompatible with the interface of the closed +OP dimer. As noted above, in the +OP complex, Thr-165 of one protomer is tightly packed against side chains from the N-terminal domain β-sheet and αC of the other protomer. A large, negatively charged phosphate group cannot be accommodated in this small hydrophobic space, and thus phosphorylation of Thr-165 would necessitate a remodeling of the AL at residues 163–167. Similarly, phosphorylation of Ser-171 would be incompatible with the dimeric β-strand interactions observed at residues 171–174 of the HPK1 +OP dimer.

Structure of the phosphomimetic HPK1–sunitinib inhibitor complex in a domain-swapped dimer

Due to difficulties generating substantial amounts of the native +OP or +2P kinase domains, we investigated the production of the double phosphomimetic mutant (1–307 T165E, S171E, or HPK1 2PM). As validation, we compared enzyme kinetics and inhibition by sunitinib for HPK1 2PM and activated full-length WT HPK1. Although HPK1 2PM was found to be an order of magnitude less active than activated WT full-length HPK1, the apparent ATP $K_m$ values of HPK1 2PM and full-length WT HPK1 were found to be similar, 78 and 72 μM, respectively (Fig. S5). Sunitinib $K_i$ values were about 6 and 10 nm, respectively (Fig. S1). Notably, HPK1 2PM could be purified in relatively high yield and in the absence of inhibitor and was amenable to the production of high-diffraction-quality crystals with a wide variety of inhibitor chemotypes using a single crystallization condition. The cocrysalts obtained varied widely in their unit cell parameters and in the number of molecules in the ASU (2, 4, 8, and 12). Regardless of the crystallographic parameters, the structures invariably contained a dimer with a head-to-head subunit orientation and a 3D domain swap of the P + 1 loop. The features of HPK1 2PM that are characteristic of the active kinase fold include phosphorylation at Thr-165 and Ser-171, the αC-in position, the Lys-46–Glu-62 salt bridge and a DFG-in configuration. The phosphate oxygens of phospho-Ser-171 are stabilized by interactions with the sidechains of Arg-136 and Arg-168.

At the level of the subunit structure, the active HPK1 +2P and inactive HPK1 +OP kinase domains have a similar overall fold. The most significant differences between the +OP and +2P structures are an inward movement of αC and a rearrangement of the AL starting at Asp-155 of the DFG and continuing to Thr-175 just preceding the P + 1 loop. The features of HPK1 2PM that are characteristic of the active kinase fold include phosphorylation at Thr-165 and Ser-171, the αC-in position, the Lys-46–Glu-62 salt bridge and a DFG-in configuration. The phosphate oxygens of phospho-Ser-171 are stabilized by interactions with the sidechains of Arg-136 and Arg-168.

### Table 1

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$^a$ $R_{merge}$ = $\sum_{hkl} I(i)_h - I(i)_o$/$\sum_{hkl} I(o)_h$, where $I_h$ is the intensity of the $i$th observation, $I_o$ is the mean intensity of the reflection, and the summations extend over all unique reflections ($hkI$) and all equivalents ($i$), respectively.

$^b$ $R_{merge}$ is a measure of the quality of the data after averaging the multiple measurements, and $R_{merge} = \sqrt{\sum_{i} n_i (\langle I_{i\text{obs}} \rangle - I_{i\text{calc}})^2/\sum_{i} n_i (I_{i\text{obs}})^2}$, where $n_i$ is the multiplicity and other variables are as defined for $R_{merge}$.

$^c$ $CC_{1/2}$ is the Pearson correlation coefficient.

$^d$ $R_{merge} = \Sigma |F_{o} - F_{c}| / \Sigma F_{c}$, where $F_{o}$ and $F_{c}$ are observed and calculated structure factors, respectively, $R_{merge}$ was calculated from a randomly chosen 5% of reflections excluded from the refinement, and $R_{merge}$ was calculated from the remaining 95% of reflections.

**ACCELERATED COMMUNICATION: Structures of HPK1 kinase domain**
HPK1<sup>2PM</sup> complex also assembles into a parallel head-to-head dimer where the arrangement of subunits is strikingly similar to the active HPK1<sup>1P</sup>/H11001<sup>2P</sup> but with a much more extensive dimer interface (Fig. 3C). The most distinct feature of the HPK1<sup>2PM</sup> structure is the presence of an extended AS, protruding across the dimer interface such that the P + 1 and APE motifs adopt a 3D domain-swapped configuration (Fig. 4A). The structure of the AS consists of a pair of antiparallel amphipathic α-helices
(residues 159–171 and 180–189) connected by a well-ordered P + 1 loop. The residues of the P + 1 loop pack into an amphipathic pocket on the opposing HPK1 protomer and together with the APE motif define the domain swap (Fig. 3, B and C). In the domain-swapped dimer, the conserved APE glutamate, Glu-182, forms a bidentate ion pair with Arg-262 of the cognate protomer. This bidentate interaction is highly structurally conserved among kinases. The intermolecular pair is present in all reported domain-swapped kinases and is also observed as an intramolecular interaction in both HPK1 native structures described here.

The dimer interface contains 53 residues, corresponding to 1997 Å² of buried accessible surface area that includes four hydrogen bonds and nine salt bridges with a ΔG of −32.4 kcal/mol (Table S1 and Fig. S4), which is consistent with a physiologically relevant oligomer. In addition, the HPK1^2PM domain swap is very similar to published structures representing a diverse set of domain-swapped kinase domains ranging from less conserved members like CHK2 kinase to the closely related GLK (MAP4K3) (Fig. 4D).

Based on the observation of a DFG-out conformation, the αC-out position, and the absence of a Lys-46–Glu-62 salt bridge, the kinase subunits in the HPK1^2PM complex adopt the inactive fold. However, there is significant asymmetry within the HPK1^2PM ASU. For example, the AL appears much less ordered in chain A compared with chain B. Furthermore, the DFG conformation was intermediate for chain A but DFG-out for chain B. The position of αC was also ~3 Å further out for chain A compared with chain B.

**Sunitinib binding interactions**

The electron density maps clearly indicate sunitinib bound in each of the complexes. Superposition of the structures shows that the kinase hinge region of residues 91–101 agrees well in the three complexes and that the oxindole pyrrole of sunitinib makes backbone hydrogen-bonding interactions with Cys-94 and Glu-92 (Fig. S6). Although sunitinib binding is stabilized via interactions with the hinge backbone, the overall position of sunitinib is affected by differences in the position of other residues, primarily in the G-loop and AL. For example, the oxindole ring is translated ~0.9 Å farther into the binding pocket toward gatekeeper residue Met-91 in the +0P complex as compared with the +2P complex (Fig. S6C). The tertiary amine tail of sunitinib extends away from the kinase hinge into solvent, and its electron density was weak and ambiguous, indicating that the amine tail may occupy more than one preferred conformation.

**Discussion**

The crystal structures of the HPK1 KD described herein provide insight into the extraordinary structural plasticity of the HPK1 KD and its propensity for dimerization. The observation of the HPK1 KD in active and inactive kinase conformations as well as in both domain-swapped and nonswapped dimers offers a tantalizing clue for dimerization as a regulatory mechanism.

From a structural perspective, the inactive HPK1^1OP dimer appears inhibited in *trans*; the intermolecular exchange of the AL stabilizes the kinase in an inactive state. The extent of the interactions at the dimer interface and the involvement of key regulatory motifs and residues argue in favor of a biologically relevant dimer. Perhaps paradoxically, this AL exchange positions the autophosphorylation residue, Thr-165, between the catalytic lysine (Lys-46) and the conserved αC glutamate (Glu-62) at the active site of the adjacent protomer (Fig. S7). Superposition of the structures of HPK1^1OP and the insulin receptor kinase in complex with IRS2 KRLB peptide and ATP (PDB code 3BU5) positions the terminal phosphate of ATP within ~8 Å of HPK1’s Thr-165 side-chain Oγ1 atom. Therefore, it seems plausible to speculate that conformational dynamics could allow for formation of the Asp-Lys-Thr catalytic triad and the autophosphorylation of Thr-165. Such a conformational change could be initiated by the trans-phosphorylation of Ser-171 by PRKD1, which would be consistent with the observation that trans-phosphorylation by PRKD1 and subsequent autophosphorylation of Thr-165 appear to be prerequisite for full activa-
tion of HPK1 (22). Biochemical and crystallographic studies of dimeric p21-activated kinase 2 (PAK2) and the homologous PAK1 (PDB ID code 1YHV) indicate that dimerization positions the AL of one protomer in the active site of the companion protomer, which is required for the trans-autophosphorylation reaction (23, 24).

The observation of the HPK1/H11001 dimer is intriguing but possibly nonphysiological. Indeed, the ∆G of −9.4 kcal/mol predicted by PISA falls below the ∆G of >15–20 kcal/mol established as a range for reliable (1–2% errors) representation of complexes in crystals (25). Unfortunately, our efforts to determine the oligomeric state of the HPK1/H11001 and HPK1/H11002 in solution using size-exclusion chromatography with inline multiangle static light scattering (SEC-MALS) were inconclusive. In each case, the MALS analysis of the single peak eluting from the gel-filtration column predicted a mass in between those expected for the monomer and dimer (data not shown). In contrast, two distinct peaks were observed during SEC-MALS of the HPK12PM protein that corresponded well with the expected monomer/dimer masses (data not shown). These latter observations agree with the findings of Wu et al. (17). Considering the inconclusive SEC-MALS and PISA prediction for HPK1/H11001, conclusions regarding the physiological relevance of the dimer require additional experimental evidence.

HPK1 now adds to the growing list of domain-swapped kinase structures that includes both active and inactive kinase folds from a diverse set of kinase family members, including CHK2 (PDB code 2CN5) (26), SLK (PDB code 2J51) (27), LOK/STK10 (PDB code 2J7T) (27), DAPK3 (PDB code 2J90) (27), OSR1 (PDB code 3DAK) (28), and GLK/MAP4K3 (PDB code 5J5T) (16). Moreover, Wu et al. (17) also reported the structure of a T165E,S171E double phosphomimetic mutant of HPK1 in complex with AMPPNP (PDB ID code 6CQF). Despite different crystallization conditions, different crystal parameters, and different ligands bound, the HPK12PM structure compares closely with the structure of Wu et al. (17). Superposition of the structures yields r.m.s.d. values of 1.564 Å for 482 C atoms and 0.429 Å for 202 C atoms for the dimer and monomer, respectively (Fig. S8).

The precise physiological role of the domain-swapped kinase remains unclear. However, the observation of kinases from diverse subfamilies assembled as domain-swapped dimers argues in favor of a physiologically relevant phenomenon. For example, structural studies of SLK, LOK, and DAPK3, each assembled as domain-swapped dimers, led the authors to propose a mechanism for kinase autophosphorylation of nonconsensus sites (27). It has also been suggested that dimerization of HPK1 may provide an additional layer of regulatory control over substrate selectivity or cis/trans-phosphorylation activity (29).

Inherent conformational plasticity is essential for the interconversion between active and inactive kinase states. In addition, biophysical studies have suggested that catalysis and conformational dynamics are linked, whereas NMR spectroscopy and molecular dynamics simulations have connected domain swapping to conformational flexibility in other protein molecules (30). Therefore, domain swapping is a likely consequence of the intrinsic conformational flexibility of HPK1 and other kinase domains. Additional structural and biochemical studies are needed to elucidate the functional roles of dimerization and domain swapping for HPK1 and other protein kinases.

Figure 4. HPK1–sunitinib and comparable domain-swapped dimers from the PDB. A, the HPK12PM KD in a domain-swapped dimer. Chain A is shown as a green cartoon with transparent surface, and chain B is shown as a cyan cartoon. Inhibitor and WYMAPE residues are drawn as sticks. The active-site surface is colored yellow in subunit A. B, zoomed-in view of HPK12PM domain swap. Selected chain A side chains (green) surrounding YWMAPE residues from the P + 1 and APE motifs of chain B (cyan) are shown. C, equivalent view of HPK1/H11001 chain A (dark green) to illustrate the duplication of interactions in domain-swapped and nonswapped dimers. D, crystal structures of selected kinase domains from the PDB that assemble as domain-swapped dimers.
Experimental procedures

Production of recombinant HPK1

HPK1 (GenBank™ NM_001042600.2) insect cell expression constructs were synthesized at GenScript and included a TEV-cleavable N-terminal poly-His purification tag. Recombinant baculoviruses were prepared by using the Bac-to-Bac method (Invitrogen) and were used to infect ∼21 insect cells. Recombinant HPK1 kinase domain was purified by double immobilized metal affinity chromatography following cell lysis in 50 mM Tris-HCl, pH 8.0, 250 mM NaCl, 0.25 mM TCEP, 10 μM leupeptin (Sigma), 10 μM E-64 (Sigma), and one EDTA-free protease inhibitor tablet (Roche Applied Science)/75 ml of buffer with tag removal between immobilized metal affinity chromatography steps followed by gel filtration over a Phenomenex 30 × 2.1-cm BioSep-s3000 size-exclusion column in 25 mM Tris-HCl, pH 7.5, 250 mM NaCl, 0.25 mM TCEP. Sunitinib was added to the nonmutant kinase domains for stabilization and concentrated to ∼20 mg/ml. The HPK1 (1–307) T165E,S171E double mutant was prepared similarly but using 25 mM HEPES-NaOH, pH 7.5, 250 mM NaCl, 0.25 mM TCEP. Full-length HPK1 purification included 2 mM MgATP during the TEV cleavage dialysis step to allow for autophosphorylation.

Crystallization and crystallography

The sitting-drop vapor-diffusion method was used for crystallization. HPK1+OP crystals grew from 300 nl of protein (16 mg/ml) plus 300 nl of reservoir solution (4.19 mM NaCl, 0.1 mM HEPES, pH 6.5) at 21 °C. HPK1+2P crystals grew from 100 nl of protein (22 mg/ml) plus 100 nl of reservoir solution (0.2 mM ammonium fluoride, 20% PEG 3350) at 13 °C. HPK1+OP crystals grew from 200 nl of protein (15 mg/ml) plus 300 nl of reservoir solution (0.1 mM Tris, pH 8, 20% 1,6-hexanediol, 10 mM magnesium sulfate, 24 mM barium acetate) at 13 °C. Crystals were cryoprotected in reservoir solution plus 20% glycerol and flash frozen in liquid N2.

X-ray diffraction data were collected at the Advanced Photon Source beamline 17-ID and processed with XDS (31) and POINTLESS, AIMLESS, or XSCALE via autoPROC from Global Phasing (32). Structure solution was by molecular replacement using Phaser (33). Model building was done in Coot (34) and refinement used REFMAC (35), PHENIX (36), or BUSTER (37). Structure validation was performed with MolProbity (38).

References


Acknowledgments—We thank Nicole Grable for insect cell culture and Oleg Brodsky for assistance with SEC-MALS analysis.
swapped activation loop and C-terminal extension. Protein Sci. 26, 152–162 CrossRef Medline