Structural Basis of Constitutive Activity and a Unique Nucleotide Binding Mode of Human Pim-1 Kinase*

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Pim-1 kinase is a member of a distinct class of serine/threonine kinases consisting of Pim-1, Pim-2, and Pim-3. Pim kinases are highly homologous to one another and share a unique conserved hinge region sequence, ER-PXPX, with its two proline residues separated by a non-conserved residue, but they (Pim kinases) have <30% sequence identity with other kinases. Pim-1 has been implicated in both cytokine-induced signal transduction and the development of lymphoid malignancies. We have determined the crystal structures of apo Pim-1 kinase and its AMP-PNP (5′-adenylate-β,γ-imidodiphosphate) complex to 2.1-Å resolutions. The structures reveal the following. 1) The kinase adopts a constitutively active conformation, and extensive hydrophobic and hydrogen bond interactions between the activation loop and the catalytic loop might be the structural basis for maintaining such a conformation. 2) The hinge region has a novel architecture and hydrogen-bonding pattern, which not only expand the ATP pocket but also serve to establish unambiguously the alignment of the Pim-1 hinge region with that of other kinases. 3) The binding mode of AMP-PNP to Pim-1 kinase is unique and does not involve a critical hinge region hydrogen bond interaction. Analysis of the reported Pim-1 kinase-domain structures leads to a hypothesis as to how Pim kinase activity might be regulated in vivo.

The proto-oncogene Pim-1 is a serine/threonine kinase (1, 2) and was first identified as a common integration site for the Moloney murine leukemia virus, which may induce T cell lymphomas in mice (3). Overexpression of Pim-1 in mice makes these mice more susceptible to lymphomagenesis, likely due to Pim-1 cooperation with c-Myc (4). Furthermore, aberrant Pim-1 expression is detected in human cancer patients.

Pim-1 is ubiquitously expressed, with the highest expression being found in both hematopoietic tissues and testes (5). The expression of Pim-1 is induced by a variety of cytokines, growth factors, and mitogens. Pim-1 is believed to be involved in cell growth control and survival (6). The overexpression of Pim-1 may enhance cellular survival by protecting cells from apoptosis. Cells deficient for all Pims were defective in response to proliferation signals (7). How Pim-1 exerts its influence on cell proliferation remains obscure. Several proteins are reported to be Pim-1 substrates in vitro, e.g. Cdc25A (8), p21Cip (6), SOCS (9), BAD (10) et al. How Pim-1 is activated and concomitantly regulated also remains obscure. Limited mutagenesis and biochemical data exist that may identify particular Pim-1 residues critical for substrate selection, ATP binding, and kinase activity, except that the K67M mutation results in the loss of Pim-1 kinase activity (11).

It is well established that some kinases can switch on and off through conformational changes in the activation loop (A-loop). The structural nature of these conformational changes is also well understood for these kinases (12). The phosphorylation of 1–3 residues in the A-loop leads to salt bridges being formed to basic residues in both the catalytic loop (C-loop) and the N-lobe, thereby resulting in an active kinase (13). Other kinases are constitutively active, as phosphorylation is not necessary for their kinase activities. These kinases, including CK2 (14), phosphorylase kinase (PhK) (15), and checkpoint kinase-1 (CHK-1) (16), adopt a unique conformation in which acidic residues in the activation loop form a salt bridge with a conserved arginine residue in the C-loop; both loops are well ordered and do not block the ATP binding pocket, whereas the glycine-rich loop (G-loop) conformation varies. In this paper, such conformation is loosely termed as a constitutively active conformation.

ATP traditionally makes two hydrogen bonds to the kinase hinge region using its adenine moiety; its phosphate moiety interacts both with two lysine residues and two bound magnesium ions, which, in turn, coordinate to Asx or Glx side-chain oxygen atoms. The specific binding interactions and the associated residues are highly conserved among kinases, and the presence of two bound magnesium ions also remains invariant (12). The two ribose hydroxyl groups also form hydrogen bond interactions in cAMP-dependent protein kinase (cAPK) (17), but this not always observed in other kinases. To catalyze the transfer of ATP γ-phosphate to substrate, kinases adopt an active conformation in which the ATP pocket is open and the highly conserved residues, both binding to ATP and transferring the ATP γ-phosphate during catalysis, are correctly positioned relative to one another.

Whether the Pim-1 kinase must be phosphorylated for activ-

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1 The abbreviations used are: A-loop, activation loop; AMP-PNP, 5′-adenylate-β,γ-imidodiphosphate; cAPK, cAMP-dependent protein kinase; CHK-1, checkpoint kinase 1; C-loop, catalytic loop; G-loop, glycine-rich loop; PhK, phosphorylase kinase.
Crystal Structures of Human Pim-1 Kinase at 2.1-Å Resolution

6131

Crystallographic data and refinement statistics

| Data set       | Apo-1 | Apo-2 | AMP-PNP |
|----------------|-------|-------|---------|
| Space group    | P6₃   | P6₃   | P6₃     |
| Unit cell (a, c; \(\beta = 120^\circ\)) | 95.8, 79.8 | 95.9, 80.0 | 96.7, 80.0 |
| Mol. per a.u.  | 1     | 1     | 1       |
| Resolution (Å) | 50–2.5 | 50–2.1 | 50–2.1 |
| Completeness   | 99.8 (99.6) | 99.7 (99.6) | 91.0 (79.6) |
| (last shell) (%) | 10.4 (3.38) | 17.8 (3.09) | 10.1 (2.14) |
| \(R_{free}\) (last shell) | 0.213 (0.516) | 0.106 (0.511) | 0.075 (0.323) |
| R.m.s.d. Bond (Å) | 0.007 | 0.007 | 0.007 |
| Angle (°) | 1.252 | 1.315 | 1.205 |

Ramachandran regions (°) | 86.6 | 87.1 | 84.6 |

Additional allowed | 11.3 | 11.3 | 13.8 |

Average B-factor Protein | 30.6 | 38.3 | 39.1 |

Ligand | 35.7 |

Water | 27.6 |

No. of water molecules | 274 | 277 | 27 |

Crystallographic data and refinement statistics

| Resolution (Å) | 50–2.5 | 50–2.1 |
|----------------|--------|--------|
| Completeness   | 99.8 (99.6) | 99.7 (99.6) |
| (last shell) (%) | 10.4 (3.38) | 17.8 (3.09) |
| \(R_{free}\) (last shell) | 0.213 (0.516) | 0.106 (0.511) |
| R.m.s.d. Bond (Å) | 0.007 | 0.007 |
| Angle (°) | 1.252 | 1.315 |

| No. of water molecules | 42 | 64 |

Materials and Methods

Cloning, Expression, Purification, and Crystallization—Details of cloning, expression, purification, and crystallization have been published (36). The full-length Pim-1 protein was cloned and fused with a glutathione S-transferase tag. The protein was expressed in Escherichia coli in a fermenter. The cell lysate was applied to a glutathione S-transferase resin column. The resin was subsequently washed and digested with thrombin “on-resin.” The raw Pim-1 sample eluted after thrombin digestion was loaded directly to a Mono-Q column and eluted with 0–1 M NaCl gradient followed by size exclusion chromatography (Superdex-75) with a buffer containing 20 mM Tris, pH 8, 200 mM NaCl, 2 mM DTT, and 5 mM β-mercaptoethanol. The Pim-1 protein at this stage was determined to be highly pure by SDSE-PAGE and to be monodisperse by the dynamic light-scattering technique. Crystals were grown using the hanging drop method with the Pim-1 protein at a concentration of 4.4 mg/ml (\(A_{280} = 6.6\)). The final crystallization buffer contained 30% polyethylene glycol 1500, 0.1 M Tris, pH 7.5, and 300 mM NaCl.

Data Collection—The statistics of data collection and refinement are summarized in Table I. The AMP-PNP co-complex crystal was prepared by adding ~1% volume of a 100 mg/ml aqueous AMP-PNP stock solution to the crystallization drop and then soaking overnight. Both the apo Pim-1- and AMP-PNP-soaked crystals were frozen in liquid nitrogen and then transferred into a cryo-nitrogen gas stream for data collection using an RAXIS IV detector mounted on an RU-300 x-ray generator (Rigaku/MSC). The diffraction data were processed and scaled using HKL software (30), revealing a hexagonal P₆₃ or P₆₃ space group. The 2.5-Å data set was collected on a crystal of size 40 × 40 × 400 μm. The 2.1-Å data sets for both apo and the AMP-PNP co-complex were collected on crystals of size ~100 × 100 × 1000 μm. All crystals have presented the same space group and similar unit cell dimensions.

Refinement, Model Building—Molecular replacement (Amore in the CCP4 suite) (31) was carried out on a 2.5-Å Pim-1 data set using a Pim-1 homology model built from the cAPK crystal structure (32) (1BKX). The homology model was truncated to residues 20–290 as a search model in molecular replacement using data from 20- to 3.5-Å resolution. The molecular replacement solution was found for the P₆₃ space group, with one peak having a correlation of 0.247 and an \(R_{factor}\) of 0.480. All other peaks showed a correlation <0.180 and an \(R_{factor}\) >0.498.

RESULTS

Structural Overview—Both the apo Pim-1 and the Pim-1-AMP-PNP co-complex structures have been determined to 2.1-Å resolution, with the AMP-PNP co-complex being obtained by soaking apo crystals. The Pim-1 kinase structure adopts a typical two-lobe kinase fold with a deep, intervening cleft (Fig. 1A), as described for cAPK (17). The N-terminal lobe comprises primarily anti-parallel β-sheets, and the C-terminal lobe is composed of α-helices. The two domains are connected through the hinge region (residues 121–126). There is no ion interaction between the two lobes in the Pim-1 structure, even though an ion pair, comprising a lysine residue in the N-lobe and an acidic or phosphorylated residue in the C-lobe, is commonly observed for many different kinases and is believed to be critical in maintaining structural stability for kinase activity (12). Although Pim-1 adopts a typical kinase fold, its kinase domain structure does contain an extra pair of anti-parallel β-sheets (βH1 and βH2) that connects the β3-strand and the αC helix. This may represent a unique structural feature.
among kinases. In other kinases, this region usually adopts a helical structure, as observed in cAPK, or is disordered as in other kinases. A twist within the β1-strand in Pim-1 is probably caused by the presence of P42. A pair of small, anti-parallel β-sheets (β7 and β10) resides in the C-terminal lobe between the A-loop and the C-loop. This structural feature is similar to that reported for CHK-1 (16) but not for other kinases. AMP-PNP binding into the cleft induces conformational changes that are dramatic in the G-loop but insignificant in the phosphate binding and hinge regions. Most residues in the Pim-1 structures are well resolved, except for the N-terminal residues 14–31 and the C-terminal residues 309–313. The G-loop is poorly resolved in the apo structure but somewhat resolved in the AMP-PNP co-complex, consistent with the flexibility in this loop as inferred from other kinase structures. Note that the first 13 residues in Pim-1 are removed by thrombin digestion at an endogenous cleavage site and are therefore not present in the crystallized Pim-1 protein.

**Pim-1 Kinase Adopts a Constitutively Active Conformation**—The Pim-1 kinase has been expressed in E. coli purified and found to not be phosphorylated based on results from parent ion mass spectrometry and N-terminal amino acid-sequencing analysis. However, the same protein sample has been shown to be active in our kinase activity assay. Because all A-loop residues are clearly seen in both Pim-1 crystal structures reported herein, one can state categorically that no A-loop residue in this crystallized Pim-1 is phosphorylated. A detailed analysis of the crystal structures reveals a solid structural basis that Pim-1 remains active even in the absence of A-loop phosphorylation.

Sequence alignment suggests that the conserved Pim-1 residues Lys67, Glu89, Asp167, and Asp186 would bind ATP or its analogs and that Asp167 would catalyze phosphate transfer. These residues are located within the shaded area in Fig. 1a, which is both the ATP binding pocket and the major portion of the cleft as found in the apo enzyme structure. The ATP pocket in Pim-1 is either open in the apo enzyme structure or bound with AMP-PNP in the co-complex structure. That the two active sites are structurally similar suggests that the Pim-1 kinase active site may be maintained in an active conformation. To further strengthen this hypothesis, residues Lys67, Glu89, Asp167, Lys169, Asn172, and Asp186 in both Pim-1 structures have been superimposed upon the structurally equivalent residues in PhK, which is known to be a constitutively active kinase (15). Fig. 1b shows that the Pim-1 residues critical for both ATP binding and catalysis overlay well with those of PhK, with a backbone atom root mean square deviation of 0.57 Å. In Pim-1, residue Lys67 in the β3-strand forms a salt bridge with Glu89 in the helix αC, equivalent to the Lys68-Glu73 salt bridge in PhK. A salt bridge in this location is known to be critical for kinase activity (13, 19). The phosphate binding residues Lys8, Asp186, Asn172, and Lys169 all adopt the same relative conformation as that found in PhK. The Pim-1 catalytic residue Asp167 is found to be almost identical in conformation to that of Asp149 in PhK. These comparative results further support the hypothesis that Pim-1 is a constitutively active kinase.

**Structural Basis for Constitutively Active Conformation**—How kinases maintain a constitutively active conformation is not clear from a structural standpoint, even though the structures for at least six constitutively active RD kinases (12) have been determined. A detailed examination of the Pim-1 kinase structure reveals an extensive network of interactions between the A-loop and C-loop that may be critical for maintaining its active conformation. A similar network of interactions is also seen in two other constitutively active kinases, PhK (15) and CHK-1 (16).

The active conformation of a monomeric kinase, in the absence of a bound regulatory subunit or domain, depends critically on the conformations of the A- and C-loops (12, 13, 19). These loop conformations are usually stabilized by a salt bridge between a conserved arginine residue immediately N-terminal to the catalytic aspartate and a phosphorylated residue (S/T/Y) in the A-loop. Formation of this A-loop to C-loop salt bridge both opens up the ATP pocket and helps to stabilize the induced changes in both loop conformations, thereby activating the canonical kinase. The clear electron density shown in Fig. 2a establishes that no A-loop residue in Pim-1 is phosphorylated, and, therefore, the constitutively active Pim-1 conformation is not stabilized through phosphorylation.

The conserved Arg166 residue in the Pim-1 C-loop does form
a salt bridge with Asp\textsuperscript{200} in the A-loop. The Pim-1 crystal structures (Fig. 2, \textit{a} and \textit{b}) show that the same guanidino amino group of this residue makes a bifurcated interaction with Asp\textsuperscript{200}, forming both a salt bridge with the Asp\textsuperscript{200} side-chain carboxyl group at a distance of 2.5Å and a hydrogen bond with the Asp\textsuperscript{200} main-chain carbonyl oxygen. This non-phosphate salt bridge and hydrogen bond in Pim-1 might be functionally equivalent to the one involving a phosphorylated residue. Two additional hydrogen bonds within the A-loop involving residue pairs Leu\textsuperscript{192}-Tyr\textsuperscript{198} and Lys\textsuperscript{194}-Val\textsuperscript{197} should help further in stabilizing its active conformation (Fig. 2\textit{a}).

Additional hydrophobic interactions that should stabilize both loops are shown in Fig. 2\textit{b}. Residues Leu\textsuperscript{193}, Tyr\textsuperscript{198}, and Phe\textsuperscript{201} in the A-loop are seen to pack into a hydrophobic cluster with residue Leu\textsuperscript{164} from the C-loop and residues Trp\textsuperscript{212} (\alpha\textit{EF}) and Tyr\textsuperscript{218} (\alpha\textit{F}). This hydrophobic cluster serves to anchor both loops to the kinase core. Corresponding residues in PhK are also hydrophobic, \textit{i.e.} Leu\textsuperscript{174}, Leu\textsuperscript{180}, and Leu\textsuperscript{183} in the A-loop, Leu\textsuperscript{146} in the C-loop, and Ile\textsuperscript{194} and Tyr\textsuperscript{206} in the kinase core. A similar hydrophobic cluster also exists in CHK-1. That this hydrophobic cluster is conserved in at least three constitutively active kinases suggests that it may play a significant role in maintaining this general subgroup of kinases in an active conformation.

**Determination of Hinge Region Alignment**—The ATP adenine usually forms two hydrogen bonds with the kinase hinge region. Maintaining this hydrogen bond motif is an important consideration in aligning a novel kinase hinge region. Although hinge regions can usually be aligned in a straightforward manner, the Pim kinases are somewhat more complicated because of their ERPXXP consensus sequence in the hinge region. Two Pro residues present at least two distinct challenges in building a homology model for Pim-1. First, Pro residues may adopt either a \textit{cis}- or a \textit{trans}-peptide bond. The propensity for a \textit{cis}-peptide bond may be increased when at least two Pro residues are together in sequence and lie in a region of an not well defined secondary structure. The second challenge can be illustrated as follows.

A BLAST search indicates that Aurora B (1MUO), PhK, CHK-1 (1IA8), and cAPK (1BKX) are most homologous to Pim-1, with sequence identities in the range of 25–29%. Aligning Pim-1 to these reference proteins (Fig. 3\textit{a}) has initially identified Leu\textsuperscript{120} as the gatekeeper residue and Pro\textsuperscript{123} as the hydrogen bond donor residue to the adenine of ATP. Because a
Pro residue cannot act as a hydrogen bond donor, a different alignment has been sought for the Pim-1 hinge region. In this second alignment, Pro123 in Pim-1 is aligned to Pro108 in Aurora-B, making Glu124 the hydrogen bond donor residue to the ATP adenine moiety. However, the gatekeeper residue resulting from this alignment scenario, Glu1241, does not match the non-polar profile of other gatekeeper residues. Therefore, both possible sequence alignments for the Pim-1 hinge region produce partially acceptable results with no strong indication as to which, if either, is the correct one.

The conformation of the hinge region is well determined (Fig. 3b) in both the apo Pim-1 and the AMP-PNP co-complex structure, and so is that of AMP-PNP (Fig. 4b). The locations of its two chelating magnesium ions are also well determined in the co-complex structure. The co-complex structure clearly shows that the adenine amino group of AMP-PNP makes a hydrogen bond to the Glu124 backbone carbonyl oxygen. This structural registration of AMP-PNP to the Pim-1 hinge region places Leu120 at the gatekeeper position and Pro123 at the position where a hydrogen bond donor residue is generally expected. This positioning of Pro123 is rather surprising, because this position is almost always occupied by a residue, other than proline, that is capable of donating a hydrogen atom to the adenine of ATP.

**Novel Hinge Region Architecture**—The unique hinge region sequence in Pim-1 also gives rise to an equally unique three-dimensional architecture. As discussed above, Pro123 is positioned but yet unable to donate a hydrogen bond to the AMP-PNP adenine moiety, as illustrated in Fig. 3b. The backbone amide groups of both Glu124 and Val126 which point away from the ATP pocket, are >4 Å away from any adenine atom. As a result, there is no suitable residue at all in the Pim-1 hinge region to form this normally critical hydrogen bond.

Furthermore, a structural comparison to cAPK and PhK reveals three additional unique aspects to the Pim-1 hinge region architecture. Pro125 exists in a cis-peptide conformation (Fig. 3b), creating a sharp kink within the hinge region (Fig. 3c). The Pim-1 hinge region is also expanded horizontally by ~4 Å to the left (Fig. 3c). Therefore, the conformation of the Pim-1 hinge region produces an ATP pocket that is both larger than and of a different shape to that of cAPK and PhK. The Pim-1 β5-strand is also shifted ~2.2 Å higher than that in cAPK and PhK. This difference would create a bigger separation between the two lobes of Pim-1 and consequently widen the ATP binding cleft in Pim-1.

**A Novel Binding Mode of an ATP Analog**—The electron density shown in Fig. 4a demonstrates that the entire AMP-PNP molecule and the two bound magnesium ions are well determined, as are the Pim-1 residues interacting directly with both AMP-PNP and the two magnesium ions (electron density not shown). Some nucleotide binding features observed in Pim-1 are common to those observed in other kinases; others are unique to Pim-1 (Fig. 4b). Lys67, known to be critical for Pim-1 activity (11), forms a salt bridge directly with an α-phosphate oxygen. Asp186, from the conserved DFG motif, not only makes a hydrogen bond to Lys67 but also coordinates to a magnesium ion that, in turn, coordinates to a β-phosphate oxygen. Asn172, together with Asp186, coordinates to the second magnesium ion, which, in turn, coordinates to oxygen atoms from both the α- and γ-phosphate groups. The ribose moiety does not hydrogen bond to the kinase, contrasting with what is found in cAPK.

There are also two unique aspects as to how AMP-PNP binds to Pim-1. In stark contrast to kinase binding dogma, AMP-PNP does not make the usually critical hydrogen bond acceptor interaction with the Pim-1 hinge region, because there is neither available nor accessible a hydrogen bond donating group in these residues. The conserved residue Lys67 does not form a salt bridge to the AMP-PNP phosphate group that is observed in other kinases (15). The lack of this salt bridge is explained by the additional 2.2 Å separation between the N- and C-lobes of Pim-1, as illustrated in Fig. 3c. As a result, Lys67 and Lys67 are not within the proper distance range to form the salt bridges with an AMP-PNP phosphate group.

**Induced Fit upon AMP-PNP Binding**—The root mean square deviations of backbone atoms between the apo and co-complex Pim-1 structures are summarized for various regions in Table II. The β1-strand undergoes a noticeable conformational change; however, the G-loop changes conformation dramatically. All other regions remain relatively unchanged, with root mean square deviation values <0.3 Å. The conformational change in the β1-strand, which has no direct interactions with AMP-PNP, may result simply from this dramatic conformational change in the G-loop because the two regions are directly connected. Otherwise, only limited conformational changes occur within either kinase lobe.

Fig. 5 shows some representative Cα traces of each kinase lobe. For this trace, the two Pim-1 structures have been superimposed on their C-loops. Most residues in the N-lobe move ~0.6 Å toward AMP-PNP, indicating a concerted shift upon AMP-PNP binding to bring the N-lobe closer to the C-lobe. This concerted shift causes a contraction of the binding pocket around AMP-PNP. In the apo structure, the G-loop is located near the β- and γ-phosphate binding sites. The binding of AMP-PNP displaces the G-loop from this location by up to 7 Å, as measured from the Cα atoms of Phe19, which resides at the tip of the G-loop. Although the G-loop in other kinases makes discrete interactions with the ATP phosphate groups (12), this is not the case in Pim-1. The Pim-1 G-loop makes no interactions at all with the phosphate groups of AMP-PNP. This comparison suggests that the apo Pim-1 structure is not really frozen in the ATP bound conformation but adopts such a conformation readily for its constitutive activity.
DISCUSSION

Crystal structures of non-phosphorylated Pim-1 kinase demonstrate that the catalytic region adopts a conformation that is consistent with its constitutive activity. This establishes that phosphorylation of Pim-1 is not necessary for its activity and suggests that the phosphorylation reported earlier may not play a role in regulating Pim-1 kinase activity (18). Ser189 in the crystal structures, which is equivalent to Ser190 in X. laevis Pim-1, is neither phosphorylated nor located within the A-loop, thereby undermining its putative role as an activating residue. The active conformation of Pim-1 kinase is thought to be stabilized through both the salt bridge and the hydrogen bond interactions between Arg^{166} and Asp^{200}. The relative importance of these two distinct interactions cannot be discerned presently. An analysis of Pim-1 proteins bearing the respective D200N and D200E mutations may be useful in this regard. It is also not known how much the active conformation is additionally stabilized by the hydrophobic cluster, especially as it tethers both the A-loop and the C-loop to the kinase core.

The catalytic activity of kinases is usually regulated either through a conformational change in the A-loop or through the binding of an endogenous or exogenous regulatory moiety (20). Given that Pim-1 is a constitutively active kinase, how might Pim-1 activity be regulated in cells? One possible mechanism is that its overall enzymatic activity is regulated at the level of protein transcription, translation, or protein degradation. Consistent with this hypothesis is the observation that Pim-1 transcription is regulated by the Janus kinase/signal transducers and activators of transcription (JAK/STAT) and CD40-NF-κB pathways (21, 22). Upon CD40 engagement, the increase in Pim-1 kinase activity is similar in magnitude to the increase in the Pim-1 protein level (23). A similar association between protein level and overall kinase activity has also been reported for Pim-2 (24).

FIG. 4. The atoms are colored as gray, blue, red, and yellow for carbon, nitrogen, oxygen, and phosphor, respectively and panels a and b were produced using Quanta. a, stereo view of electron density (2F_o − F_c coefficients, 1.0 σ level) for AMP-PNP with two bound magnesium ions. b, the binding interactions of AMP-PNP phosphates with Pim-1. Lys^{169} does not bind AMP-PNP in Pim-1, contrasting with what is observed in other kinases. Single letter amino acid abbreviations are used with position numbers.
Conversely, how might Pim-1 activity be down-regulated in cells? Down-regulation is usually achieved either through dephosphorylation (12) or, as in the case of the constitutively active PhK and CHK-1 kinases, through a steric autoinhibitory mechanism (19). PhK is a multimeric protein whose kinase activity is normally inhibited by other subunits within the holoenzyme (25). The CHK-1 N-terminal kinase domain is ~20-fold more active than the full-length protein, suggesting an autoinhibitory function to its C-terminal domain. As Pim-1 kinase does not contain a regulatory domain and has been autoregulated of its kinase activity would not appear possible, as Pim-1 kinase lacks the typical salt bridge (12) between Lys<sup>H11011</sup>, a conserved residue in kinases, and the nucleotide phosphate moiety. The combined lack of both this salt bridge and one of the two hinge region hydrogen bonds might be construed to reduce the binding affinity of ATP for the Pim-1 kinase and for Pim-2 as well. The <i>K<sub>m</sub></i> of ATP for Pim-2 is 3–6 μM, which is within the normal range for protein kinases (data not shown). The rigid structure around the ATP pocket referred from Pim-1 might have entropically compensated the binding loss for Pim-2 to maintain a normal <i>K<sub>m</sub></i> value for ATP. However, whether or not the <i>K<sub>m</sub></i> would be even stronger for Pim-2 with that additional hydrogen bond and the salt bridge is not currently understood. To that end, it would be interesting to see whether swapping to a "typical" hinge region sequence that results in an additional hydrogen bond to ATP will lead to a more potent <i>K<sub>m</sub></i> value for Pim-1 or Pim-2.

Most kinase inhibitors also form two hydrogen bonds with kinase as ATP does. The lack of the heretofore universal hydrogen bond interaction between kinase inhibitors (acceptor) and the kinase hinge region (donor) may have even greater implications for structure-based drug design on Pim family kinases. Cancer drugs like Gleevec and a host of drug candidates targeting kinases bind in the ATP pocket and utilize this critical hydrogen bond interaction to achieve both the requisite molecular potency and, possibly, therapeutic efficacy (28, 29). The Pim-1 structures reported herein suggest that previous kinase inhibitor classes may bind only weakly to the Pim family kinases and that a novel interaction will be required to boosts the molecular potency to the desired level.

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Crystal Structures of Human Pim-1 Kinase at 2.1 Å Resolution

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Structural Basis of Constitutive Activity and a Unique Nucleotide Binding Mode of Human Pim-1 Kinase

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