Pyridinyl Imidazole Inhibitors of p38 Mitogen-activated Protein Kinase Bind in the ATP Site*

Peter R. Young‡‡, Megan M. McLaughlin‡, Sanjay Kumar‡, Shouki Kassis‡, Michael L. Doyle‡, Dean McNulty‡‡, Timothy F. Gallagher‡‡, Seth Fisher‡‡, Peter C. McDonnell‡, Steven A. Carr‡‡, Michael J. Huddleston‡‡, George Seibel§§, Terence G. Porter‡‡, George P. Livi¶, Jerry L. Adams§§, and John C. Lee‡

From the Departments of ‡Molecular Immunology, §Comparative Genetics, ¶Cellular Biochemistry, **Macromolecular Sciences, §§Protein Biochemistry, ¶¶Medicinal Chemistry, and §§Physical and Structural Chemistry, SmithKline Beecham Pharmaceuticals, King of Prussia, Pennsylvania 19406-0939

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The site of action of a series of pyridinyl imidazole compounds that are selective inhibitors of p38 mitogen-activated protein kinase in vitro and block proinflammatory cytokine production in vivo has been determined. Using Edman sequencing, 125I-SB206718 was shown to cross-link to the nonphosphorylated Escherichia coli-expressed p38 kinase at Thr175, which is proximal to the ATP binding site. Titration calorimetric studies with E. coli-expressed p38 kinase showed that SB203580 bound with a stoichiometry of 1:1 and that binding was blocked by preincubation of p38 kinase binding was blocked by preincubation of p38 kinase with the ATP analogue, FSBA (5′-[p-(fluorosulfonyl)benzoyl]adenosine), which covalently modifies the ATP with the ATP analogue, FSBA (5′-[p-(fluorosulfonyl)benzoyl]adenosine), which covalently modifies the ATP binding site. The intrinsic ATPase activity of the nonphosphorylated enzyme was inhibited by SB203580 with a K_i of 9.6 mM. Kinetic studies of active, phosphorylated yeast-expressed p38 kinase using a peptide substrate showed that SB203580 was competitive with ATP with a K_i of 21 nM and that kinase inhibition correlated with binding and biological activity. Mutagenesis indicated that binding of 125I-SB206718 was dependent on the catalytic residues K53 and D168 in the ATP pocket. These findings indicate that the pyridinyl imidazoles act in vivo by inhibiting p38 kinase activity through competition with ATP and that their selectivity is probably determined by differences in nonconserved regions within or near the ATP binding pocket.

Several novel intracellular signaling pathways associated with heat; chemical, osmotic, and oxidative stress; UV; and the proinflammatory cytokines interleukin 1 and tumor necrosis factor have recently been discovered in mammalian cells (1–7). Because these signals are commonly associated with the early stages of host responses to injury and infection, they have generated significant interest for their possible role in various pathological conditions and consequent potential as targets for novel therapeutics.

The key components of these pathways are members of the MAP kinase family of serine-threonine protein kinases (8).

The MAP kinases are proline-directed serine-threonine protein kinases that are activated by phosphorylation on both a threonine and tyrosine in a Thr-X-Tyr motif found in an activation loop proximal to the ATP and substrate binding sites (9). This is accomplished in vivo by a dual specificity MAP kinase kinase, which in turn is activated by phosphorylation in response to an appropriate extracellular or intracellular signal. There are three main classes of MAP kinase, the ERKs, the c-jun N-terminal kinase/stress-activated protein kinases, and the p38 kinases (also known as CSBP, RK, MPK2, and HOG1 (1, 5–7)), which differ in the size of the activation loop and the nature of the X amino acid in the TXY motif. This is Glu in the ERKs, Pro in the c-jun N-terminal kinase/stress-activated protein kinases, and Gyl in the p38 kinases. As a result of this and additional proximal differences, each MAP kinase is recognized and activated by a different set of MAP kinase kinases and in turn has different in vivo substrates (7, 10, 12, 13). These differences are also reflected in the different activation stimuli for each MAP kinase, with the c-jun N-terminal kinase/stress-activated protein kinases and p38 kinases and their homologues (6, 14–18) being particularly associated with stress and inflammatory stimuli (1942).

We recently discovered that a series of pyridinyl imidazoles that inhibited the production of interleukin 1 and tumor necrosis factor from lipopolysaccharide-activated human monocytes bound to and inhibited p38 kinase, which we originally called CSBP (6). These compounds inhibit two splice forms of p38, and a newly discovered homologue of p38 kinase, p38g (16), but they do not inhibit the closely related ERK or c-jun N-terminal kinase/stress-activated protein kinases or other serine-threonine protein kinases (20). The pyridinyl imidazoles, typified by SB203580, have proven useful in investigating the role of p38 kinase in regulating transcription, translation, and cytoskeletal elements in response to various stress and cytokine stimuli, as well as its potential role in animal models of inflammatory disease (21–26). However, there has not been to date a detailed understanding of how SB203580 binds and subsequently inhibits p38 kinase catalytic activity. In the present study we show that the pyridinyl imidazoles inhibit p38 kinase activity by binding to the ATP binding site, a region that until recently was not thought to provide enough specificity for the design of protein kinase inhibitors.

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‡ To whom correspondence should be addressed: Department of Molecular Immunology/EU05-48, SmithKline Beecham Pharmaceuticals, P. O. Box 1539, King of Prussia, PA 19406-0939. Tel: 610–270-7691; Fax: 610–270-7692; E-mail: Peter_R_Young@sbphrd.com.

§ The abbreviations used are: MAP, mitogen-activated protein; MAPKAP, mitogen-activated protein kinase-activated protein; ERK, extra-cellular-regulated kinase; CSBP, CSAID binding protein; FSBA, (5′-[p-(fluorosulfonyl)benzoyl]adenosine); MS, mass spectrometry.

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EXPERIMENTAL PROCEDURES

Preparation of Inhibitors—The synthesis of SB203580, 3H-SB202190, and 125I-SB206718 has been described previously (27, 28). Multiple preparations of the latter consistently afforded material with radiochemical purities of >98% and specific activities ranging from 1670 to 1736 Ci/mmol. The radioactivity label 125I-SB227931 was prepared by reacting 5-nitrophenyl-tolylmethylsycyanide with pyrindine boronate (31-4) and the azido-nitrophenyl was conjugated to p38 kinase using a modification of the van Leusen reaction (29), which afforded 1-[1-(4-iodobenzolyl)aminopropyl-3-yl]-5-(4-pyridyl)-4-(3-nitrophenylinidazole). Conversion of the nitro group to an azide was accomplished in the same manner as with SB206718. Incorporation of radiolabeled iodine was accomplished by first converting the iodide to the corresponding tributylstannane derivative followed by radioiododestannylation using Na125I in the presence of chloramine T, yielding a product with a radiochemical purity of 96.5% and a specific activity of 1670 Ci/mmol.

Expression of Met-Ala-His38 in Saccharomyces cerevisiae—An expression vector encoding Met-Ala-His38 was constructed as follows. A 1.4-kilobase BamHI (Klenow filled)-Asp917 fragment encoding Met-Ala-His38 (6) was subcloned into p138NBU (30) that had been digested with XhoI (Klenow filled) and Asp718I, creating p138NBU-Ala-His38. Briefly, the plasmid contains the URA3-selectable marker, partial 2 μg sequences for maintenance at high copy number, with Met-Ala-His38 expression driven by the copper-inducible CUP1 promoter and terminated by the CYC1 transcriptional terminator. p138NBU-Ala-His38 was introduced into JBY10 (1), a hog1Δ strain of S. cerevisiae, by the lithium acetate method (31). Cells were grown in synthetic complete medium with uracil (30) to 30 °C and 225 rpm to Asat = 1 and induced for expression with 150 μM CuSO4 for 4 h.

Mutagenesis of p38 Kinase—Site-directed mutagenesis was performed on a FLAG-p38 construct (12). The mutants were subcloned into p138NBU and propagated in JBY10, and extracts were prepared as described previously (30).

Purification of Met-Ala-His38 Kinase from Escherichia coli—Met-Ala-His38 p38 kinase was purified from clarified E. coli lysates by chromatography on a Qiagen Ni2+-NTA column equilibrated in 100 mM Tris, pH 8.0, 1 mM phenylmethylsulfonlfyl fluoride at 4 °C and eluted with a 10–250 mM imidazole gradient in the same buffer. The p38 kinase was further purified by sequential passage over a Superdex 75 column equilibrated in 20 mM Tris, 150 mM NaCl, 5 mM dithiothreitol, and a Mono Q column equilibrated in 100 mM Tris, pH 8.0, 1 mM phenylmethylsulfonlfyl fluoride, 5 mM dithiothreitol and eluted by a 0–500 mM NaCl gradient. The purity was greater than 95% and yield was 5 mg/g of E. coli.

Purification of Yeast-expressed Met-Ala-His38 Kinase—Yeast cells were thawed and lysed with glass beads in 100 mM Tris buffer containing 250 mM imidazole, 1 mM phenylmethylsulfonlfyl fluoride, 10 μg/ml pepstatin A, 10 μg/ml leupeptin, 20 mM NaF, 2 mM Na3VO4, 2 mM sodium orthovanadate, 0.1 M Tris, pH 7.4, and the lysate was clarified by centrifugation. After addition of an equal volume of buffer A (20 mM Tris, pH 7.4), the supernatant was purified by sequential chromatography on Q-Sepharose Fast Flow and Ni2+ -NTA columns (Qiagen) and elution in both cases with 250 mM imidazole in buffer A. Purified p38 kinase was exhaustively dialyzed against 20 mM HEPPS, 2 mM NaF, 0.2 mM Na3VO4, pH 7.4, and stored frozen at −70 °C. The purity was greater than 95% and yield was 20 μg/mg of yeast.

Analysis of Protein Phosphorylation—50 pmol recombinant E. coli-expressed Met-Ala-His38 was reduced, pyridylethylated, digested with trypsin (6 h at 37 °C), and analyzed using negative ion liquid chromatography electrospray mass spectrometry and stepped collision energy scanning for selective identification of phosphorylated peptides (33). No phosphorylated peptides were detected using the phosphate-selective scan, and the putative phosphorylation-sitesite peptide (His182-Thr188). Thr-Asp-Asp-Glu-Met-Thr-Gly-Tyr-Val-Ala-Thr-Arg) was observed in its nonphosphorylated state. Approximately 300 pmol Met-Ala-His38 expressed in yeast was reduced, pyridylethylated, and desalted on a C18 guard column. The sample was then dried, reconstituted in 25 mM NH4HCO3, 25 mM β-glycerophosphate, 25 mM β-glycerophosphate, 25 mM β-glycerophosphate, 25 mM β-glycerophosphate, and trypsin (1 μg) was added to the digest containing approximately 50 pmol Met-Ala-His38 was mixed on期刊号]{SB203580} with approximately 30 nanomoles/min (34). A number of experiments were carried out on this single loading of approximately 10 pmol sample, including a full scan negative ion electrospray mass spectrometry, precursor scan of m/z 79 (phosphate-selective scan), full scan positive ion electrospray mass spectrometry to locate parents of phosphopeptides for sequencing by tandem MS (MS/MS), and MS/MS of the phosphoform of the peptide HTDEMDGTYVATGR from the digest. Signals were observed for this peptide with zero, one, and two phosphates. MS/MS of the phosphoform of the peptide HTDEMDGTYVATGR from the presumed sequence. MS/MS of the monophosphorylated parent indicated that this species was primarily the peptide phosphorylated on Thr188. We were not able to obtain useful MS/MS data on the putative di-phosphorylated species.

Sequencing of Radiophotoaffinity-labeled p38 Kinase—Purified E. coli-expressed Met-Ala-His38 was UV cross-linked with 125I-SB206718 as described previously (6) and was fragmented using cyanogen bromide (Pierce) and lysyl endopeptidase (Wako Biochemicals). Cyanogen bromide digests were performed in 70% formic acid using 10 μl of a fresh 70 mg/ml cyanogen bromide solution added per 10 μg of protein digested. Digests with lysyl endopeptidase were performed in 20 mM Tris-HCl, pH 9.0, at 1:20 (w/w) enzyme:substrate. Digest fragments were separated by 15% separating Laemmli SDS-polyacrylamide gel electrophoresis gels and electroblotted onto Problott Membrane (Applied Biosystems) for sequencing.

Protein sequence analysis was performed on a Beckman LFS 3400 TC gas-phase protein sequencer equipped with a Beckman 126/166 system for on-line phenylthiohydantoin analysis. For determining the site of radiolabeled cross-linking, a modified sequencing protocol was used in which the anillothiohaxanthone derivatives were collected in 5% ice (ethyl acetate) directly for 125I determination on a Beckman Gamma 8500 scintillation counter. The cross-linking site of the related compound, 125I-SB227931, could not be determined but was clearly distinct from that of 125I-SB206718.

Measurements of p38 Kinase Activity—Kinase activity was determined by measuring the incorporation of 32P from γ32PATP into an epimerization growth factor receptor-derived peptide (Te699) having the following sequence: KRELVPLTSPGAEPNQALLR. 30 μl reactions contained 25 mM HEPES, pH 7.4, 8 μM MgCl2, 10 μM Na3VO4, 1 mM EDTA, 20 ng of purified p38 kinase and 0.4 mM peptide. Compounds were preincubated for 20 min at 4 °C with enzyme and peptide. Reactions were initiated by the addition of γ32P-ATP to a final concentration of 0.5 μC and 170 μM (unless indicated otherwise) and incubated for 10 min at 30 °C before being stopped by addition of 10 μl of 0.3 M phosphoric acid. The phosphorylated peptide was captured on phosphocellulose filter paper (PS1), washed with 75 mM phosphoric acid, and counted in a liquid scintillation counter.

For the immune-complex kinase assay, cells were solubilized with SB203580 and its concentration was determined from absorbance at 280 nm using an extinction coefficient of 1.12 (mg/mL) cm1 and a modified procedure was used to perform the immune complex assay. p38 kinase was expressed as described (12, 35) The amount of radioactivity was quantitated in a B603 Betascope blot analyzer (Betagen). The amount of p38 kinase present in the immunoprecipitates and its extent of activation was determined by immuno blot using anti-phosphotyrosine (PY20, Santa Cruz Biotechnology) and rabbit polyclonal antibodies generated against recombinant p38 kinase (6).

Isothermal Calorimetry—Isothermal titration calorimetry experiments were performed with a Microcal Inc. iMCS microcalorimeter (36). Data were analyzed with an equilibrium binding model having a single equilibrium constant and enthalpy change. p38 kinase was dialyzed against the experimental buffer just prior to the titration with SB203580, and its concentration was determined from absorbance at 280 nm using an extinction coefficient of 1.12 (mg/mL) cm1 and a modified procedure was used to perform the isothermal titration calorimetry experiments. SB203580 was dissolved to 5% in 100 mM DMSO and then diluted into experimental buffer to 100 μM (2% DMSO final).

Reaction of p38 kinase with FSBA was carried out under conditions similar to those reported for cAMP-dependent protein kinase (37, 38). p38 kinase was incubated overnight at room temperature with 1 mM FSBA, 0.1 mM Tris, 150 mM NaCl, 0.1 mM MgCl2, at pH 7.4, and dialyzed against experimental buffer prior to titration calorimetry.
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Confirmation that p38 kinase reacted with FSBA was done by evaluating its conformational stability. The thermal stability of p38 kinase was evaluated by monitoring the circular dichroism spectral signal at 220 nm as a function of temperature. p38 kinase was unfolded as a sharp, homogeneous transition before and after reaction with FSBA, but the FSBA-reacted p38 kinase showed enhanced thermal stability ($T_m = 51 \degree C$) over the unreacted ($T_m = 46 \degree C$).

RESULTS

Binding to Unactivated p38 Kinase—To determine where the pyridinyl imidazole inhibitors bind to p38 kinase, we determined the cross-linking site of the radiophotoaffinity compound. $^{125}$I-SB206718 (Fig. 1), previously used to identify and purify p38 kinase. Sequencing of peptide fragments from cross-linked, E. coli-expressed p38 kinase showed that radioactivity was associated predominantly with Thr$^{175}$ and at much lower levels with Leu$^{171}$ and Asp$^{177}$ (Fig. 2), all residues in the activation loop of p38 kinase. This suggested that the compounds may be inhibitors of ATP or substrate binding.

In support of this, the previously demonstrated binding of $^3$H SB202190 to p38 kinase in the lysates from unstimulated THP-1 cells (6) was competed by ATP with an IC$_{50}$ of approximately 4 mM (data not shown). To obtain more quantitative data, we examined compound binding and ATP competition to purified p38 kinase via microcalorimetry. SB203580 bound to E. coli-expressed p38 kinase at 30 °C with a $K_d = 15$ nM and a $\Delta H = -12$ kcal (Fig. 3, left panel). The molar binding ratio was equal to 0.75 ± 0.1 SB203580/p38 kinase, which is within experimental error of a 1:1 molar binding stoichiometry. When p38 kinase was preincubated with FSBA (5'-[p-(fluorosulfonyl)-benzoyl]adenosine), an ATP analogue that covalently modifies -[p-(fluorosulfonyl)adenosine], an ATP analogue that covalently modifies p38 kinase was preincubated with FSBA (5'-[p-(fluorosulfonyl)-benzoyl]adenosine), an ATP analogue that covalently modifies -[p-(fluorosulfonyl)-adenosine], an ATP analogue that covalently modifies p38 kinase nevertheless had an intrinsic ATPase activity in the absence of substrate. Using a spectrophotometric coupled-enzyme assay (39), the Michaelis-Menten equation was found to be 0.911. Combined with the previously noted correlation of compound binding affinity with inhibition of proinflammatory cytokine production from lipopolysaccharide-activated monocytes (6), this indicates that one mechanism by which these pyridinyl imidazoles act is through inhibition of the catalytic activity of the activated form of p38 kinase.

To evaluate further the competition between pyridinyl imidazole binding and ATP observed for the unactivated form of p38 kinase above, the mode of inhibition of p38 kinase by SB203580 with respect to ATP was determined. The resulting Lineweaver-Burke plot (Fig. 6) shows that SB203580 inhibits p38 kinase in a manner competitive with ATP with a $K_i$ of 21 nM. The $K_m$ [ATP] was increased from 200 to 1992 μM in the presence of 100 nM SB203580 (Fig. 6).

To determine potential sites important for compound binding, we examined the effect of selective mutations of p38 kinase expressed in yeast on the binding of two radiophotoaffinity cross-linkers, $^{125}$I-SB206718 and a related cross-linker $^{125}$I-SB227931, which has the azide group localized to a different region of the pyridinyl imidazole, closer to the active pharmacophore (27) (Fig. 1). Interestingly, mutation of the Thr$^{175}$
cross-linking site of 125I-SB206718 to Ala did not eliminate cross-linking to either compound, suggesting that hydroxyl group of the Thr is not required for cross-linker attachment (Fig. 7). Mutation of other loop residues (Thr180 and Tyr182) also had no effect on cross-linking but did abrogate kinase activity. On the other hand, mutation of the catalytic residues Lys53 and Asp168 resulted in a reduction of both kinase activity and 125I-SB206718 and 125I-SB227931 cross-linking, indicating that these compounds bind in the catalytic site occupied by ATP. These mutations do not lead to gross misfolding because both mutants were phosphorylated on Tyr182 by yeast Pbs2 as shown in Fig. 7 (see also Ref. 30), and D168A p38 kinase is still able to interact with its downstream substrate MAPKAP kinase-3 (12). Interestingly, the equivalent mutation to K53R in the closely related ERK (K52R) was shown not to affect ATP binding, but only the catalytic activity (41). Hence, this mutation may specifically affect the binding of the pyridinyl imidazoles and not ATP. In contrast, mutation of A34V, which results in a reduction in kinase activity (30), had no effect on cross-linking by either compound, once again suggesting differences in the binding of ATP and the pyridinyl imidazoles.

Finally, we wanted to know whether the binding of a pyridinyl imidazole to the unactivated form of p38 kinase prevented its activation by MAP kinase kinases in cells. HeLa cells were pretreated with increasing concentrations of SB203580 or SB202474 (a structurally related but inactive compound (6)) prior to addition of 0.4 mM sorbitol, which increases osmolarity. An in vitro kinase assay of immunoprecipitated p38 kinase isolated from these cells and washed free of compound showed that SB203580 had no effect on the p38 kinase activity stimulated by high osmolarity. Similarly, SB203580 has no effect on tyrosine phosphorylation of p38 kinase (Fig. 8). However, under these conditions, activation of the downstream in vitro substrate of p38 kinase, MAPKAP kinase-2, as measured by in vitro phosphorylation of hsp27, is inhibited by SB203580 (Fig. 8). In contrast, p38 kinase activity is inhibited if SB203580 is added to the in vitro reaction (data not shown (6, 35)). It is unlikely that any activation is occurring in vitro by autophosphorylation because mutagenesis has shown that both tyrosine and threonine phosphorylation are required for activation of p38 kinase activity (30, 42). These data suggest that SB203580 binding inhibits p38 kinase activity but does not prevent its activation by MAP kinase kinases. SB202474, a structurally

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**Fig. 3.** Titration calorimetry analysis of the effect of FSBA on the binding of SB203580 to p38 kinase. p38 kinase was titrated with SB203580 in the absence (left panel) and presence (right panel) of FSBA. Conditions were 5 mM HEPES, 150 mM NaCl, 20 mM MgCl2, 2% DMSO, pH 7.4, at 30 °C.

**Fig. 4.** Inhibition of intrinsic ATPase activity of p38 kinase by SB203580 as measured by isothermal titration microcalorimetry. Data shown are μcal/s versus the ratio of SB203580/p38 kinase. Heat produced per second is proportional to the amount of p38 kinase-catalyzed ATP hydrolysis. The conditions used were 2.5 μM p38 kinase in 5 mM HEPES, 150 mM NaCl, 20 mM MgCl2, 1 mM ATP, pH 7.5, 2% DMSO for 30 °C. 8.3-μl injections of 100 μM SB203580 were made into a 1.4-M solution of p38 kinase in the calorimeter. Curve is the best fit to a competitive inhibition Michaelis-Menten model with an upper limit to Ki determined as K_i ≈ 100 nM.

**Fig. 5.** Potency of pyridinyl imidazole compounds in p38 kinase binding and kinase activity. Expression of p38 kinase in yeast, purification of the active form, and the peptide phosphorylation kinase assay were as detailed under “Experimental Procedures.” Competition binding to p38 kinase in cytosol of THP.1 cells was performed as described previously (6). Kinase and binding IC50 values were calculated from % inhibition data obtained using serial dilutions of each compound.
related but inactive compound, has no effect on either the activity or the activation of p38 kinase or MAPKAP kinase-2 (6, 35).

**DISCUSSION**

We have shown that the pyridinyl imidazoles typified by SB203580 inhibit the catalytic activity of p38 kinase by binding to the ATP site. This immediately raises questions about why the inhibitors are as selective in vitro and in vivo as they appear to be (20, 27, 35). Part of the explanation may be the high $K_{m}$ of p38 kinase for ATP (200 $\mu$M), which is considerably higher than several other protein kinases, such as cAMP-dependent protein kinase ($K_{m}$ $\approx$ 190–350 $\mu$M depending on substrate (41)).

The published x-ray crystal structures of the unactivated forms of ERK2 and p38 show an open conformation that does not create a competent active site (44, 45). Comparison of these structures to the active structure of cAMP-dependent protein kinase (46, 47) suggests that the phosphorylated and active form of p38 kinase will differ substantially in conformation from the unactivated form. However, a unique feature of SB203580 and related inhibitors is their ability to bind to both the unactivated and activated forms of p38 kinase. Because the binding affinity of pyridinyl imidazoles to unactivated p38 kinase correlates with inhibition of the catalytic activity of activated p38 kinase, this suggests that the inhibitors must bind similarly to both forms of the enzyme. Indeed, the binding affinities of individual inhibitors to the two forms of the enzyme appear comparable. It is thus possible that the inhibitors lock p38 kinase into the conformation seen in the unactivated, non-phosphorylated kinase and hence prevent it from adopting the active conformation.

It is not known whether binding to the unactivated form itself plays a role in the biological effects of the inhibitors. Based on evidence presented here, SB203580 does not prevent...
activation by endogenous MAP kinase kinases because tyrosine phosphorylation of p38 kinase is unaffected, and the isolated enzyme, washed free of inhibitor, is fully active. This is consistent with the recent finding that both Thr180 and Tyr182 are surface-exposed in the x-ray crystal structure of unactivated p38 kinase (45). However, the present experiments do not address whether the observed preformed complexes of unactivated p38 with MAPKAP kinases 2 and 3 (12) might influence activated p38 kinase (45). However, the present experiments do not address whether the observed preformed complexes of unactivated p38 with MAPKAP kinases 2 and 3 (12) might influence activated p38 kinase (45). This suggests that the compound binds with the x-ray crystallography is the conformation of the activation loop being investigated.

One distinction between ERK2 and p38 kinase revealed by x-ray crystallography is the conformation of the activation loop being investigated. This model, consisting of the Thr and Tyr that are phosphorylated by MAP kinases, is the conformation of the activation loop being investigated.

The recent discovery of several potent inhibitors of protein kinases complexed with inhibitors have provided direct interactions, or they might cause subtle local conformational changes. Certainly it is hard to see how these two residues could provide the selectivity of the inhibitors observed with respect to other protein kinases and especially MAP kinases (20, 27, 35). It is likely that other residues in or near the ATP pocket might be involved. For example, there are differences in several noncatalytic residues in the ATP binding site in addition to the different activation loop residues and conformation noted above. Some recent reports of the structures of other protein kinases complexed with inhibitors have provided direct evidence for the recognition of noncatalytic and non-ATP-binding residues by inhibitors (48–50). The residues in p38 kinase responsible for binding the pyridyl imidazoles are currently being investigated.

The recent discovery of several potent inhibitors of protein kinases that retain some measure of specificity in vitro and in vivo, despite competing at the ATP site (48–50), bodes well for the development of therapeutic agents directed toward blocking protein kinases (e.g. see Ref. 11). Further understanding of the basis for this specificity will come from an understanding of the detailed interactions between enzyme and inhibitors through x-ray structural studies and further mutagenesis of p38 kinase.

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