Chemical Inhibitors of Cyclin-Dependent Kinases: Insights into Design from X-Ray Crystallographic Studies

Martin E. M. Noble and Jane A. Endicott*

LAboratory of Molecular Biophysics, Department of Biochemistry and Oxford Centre for Molecular Sciences, University of Oxford, The Rex Richards Building, South Parks Road, Oxford, OX1 3QU, UK

ABSTRACT. Cyclin-dependent kinases (CDKs) are a family of protein kinases that regulate progression through the eukaryotic cell cycle. Aberrant CDK activity or function is a common defect in human tumours, resulting in unrestrained cellular proliferation. X-ray crystallographic analysis of monomeric CDK2 and CDK2 complexes has revealed how phosphorylation and cyclin binding mediate enzyme activation and how this activity can be regulated by further protein association. Current research aims to improve the selectivity and/or potency of small molecule CDK inhibitors, both to develop specific probes to study the roles of the different CDK family members in coordinating cell cycle progression, and as lead molecules for the design of therapeutically useful drugs. This design process has been assisted by the availability of a number of CDK2/ inhibitor structures determined using X-ray crystallography. These structures have shown that molecules related to ATP can be accommodated in the ATP-binding site in a number of orientations, utilising interactions observed between CDK2 and its natural ligand, as well as novel interactions with CDK2 residues that lie both within and outside the active site cleft. This site can also bind inhibitors that are structurally unrelated to ATP. These results suggest that it may be possible to design pharmacologically and pharmaceutically important ATP-binding site-directed ligands that act as specific and potent inhibitors of CDK activity. Pharmacol. Ther. 82(2–3):269–278, 1999. © 1999 Elsevier Science Inc. All rights reserved.

KEY WORDS. Cell cycle, cyclin-dependent kinases, drug design, small molecule inhibitors, protein phosphorylation, X-ray crystallography.

CONTENTS

1. INTRODUCTION .................................................. 269
2. CYCLIN-DEPENDENT KINASE REGULATORY MECHANISMS: STRUCTURAL INSIGHTS ................. 270
3. CYCLIN-DEPENDENT KINASE 2-INHIBITOR INTERACTIONS AT THE ADENINE BINDING SITE .................. 271
4. EXPANDING THE PURINE RING: OPPORTUNITIES WITHIN THE ATP-BINDING SITE CLEFT .................. 273
5. EXPLOITING INTERACTIONS AT THE CYCLIN-DEPENDENT KINASE 2 RIBOSE-PHOSPHATE BINDING SITE ........ 275
6. NOVEL INTERACTIONS OUTSIDE THE ATP-BINDING SITE POCKET ........................................... 275
7. CONCLUSIONS ................................................... 277
Acknowledgements .................................................. 277
References .......................................................... 277

ABBREVIATION. CDK, cyclin-dependent kinase.

1. INTRODUCTION

The cyclin-dependent kinases (CDKs) are required for the correct timing and order of the events of cell growth and cell division (reviewed in Morgan, 1997). They are the downstream targets of a number of biochemical pathways that coordinate cell cycle passage through “checkpoints,” which ensure that critical steps are successfully completed before cells enter the next cell cycle stage (reviewed in Elledge, 1996; Paulovich et al., 1997). Monomeric, non-phosphorylated CDKs have no detectable kinase activity. CDK activation requires two components. One component is the binding of a positive regulatory molecule, a cyclin. The timing of cyclin expression largely dictates CDK activity (reviewed in Sherr, 1996; Fines, 1995). For example, D-type cyclins associate with and activate CDKs 4 and 6 during G1 phase. CDK2 associates with cyclin E to promote progression through G1 and subsequently relocates into complexes with cyclin A that are essential for entry into S phase. Once cells have passed through S phase, both entry into and exit from mitosis is controlled by CDC2 (CDK1) in complexes with cyclin A and cyclin B. The second component required for CDK activation is phosphorylation of the kinase on a threonine residue (Thr160 in CDK2) located in a surface loop termed the activation segment (reviewed in Draetta, 1997; Morgan, 1997). CDK/cyclin complexes are then subject to multiple additional mechanisms of regulation, including further protein association, most notably with specific and characteristic members of the CDK inhibitor families (reviewed in Elledge and Harper, 1994; Sherr and Roberts, 1995), and regulated proteolysis (reviewed in Hershko, 1997). This elaborate regulation re-
fects the need for orderly progression and the requirement of the cell to integrate a number of positive and negative regulatory signals.

Aberrant CDK control and consequent loss of cell cycle checkpoint function have been directly linked to the molecular pathology of cancer (Kamb, 1995). For example, cyclin overexpression (e.g., cyclin D), loss of function of endogenous CDK inhibitors (e.g., p21 secondary to p53 malfunction), and CDK substrate alterations (e.g., retinoblastoma gene mutations) all have been documented in human tumours. These CDK-related events are amongst the most common genetic changes found in human tumours, and, clinically, they confer a poor prognosis. Currently, it is not possible to pharmacologically probe the role and importance of individual CDKs in these events because of the paucity of specific and potent CDK inhibitors (Meijer, 1995, 1996). Selective CDK inhibitors would be invaluable tools for structural, cell, and molecular biologists working in the cell cycle field. Furthermore, the characterisation and development of low molecular weight CDK inhibitors, which could restore normal cell cycle control in tumour cells, would also have considerable potential as cancer therapeutics.

A number of selective, small molecule CDK inhibitors have been identified over the past few years. All these compounds target the CDK ATP-binding site. The development of these compounds has been aided by detailed knowledge of their binding mode to one member of the CDK family, CDK2, derived from X-ray crystallographic studies of CDK2-inhibitor complexes. These complexes have been prepared either by crystallising CDK2 in the presence of the inhibitor or by soaking CDK2 crystals in inhibitor solutions prior to data collection. This article reviews the CDK2-inhibitor structures that are currently available. A brief description of the structure of CDK2 and the effects on CDK2 conformation of cyclin binding and phosphorylation, as revealed by X-ray crystallographic studies, is provided as an introduction. Monomeric CDK2 undergoes considerable conformational change on cyclin binding and activation. These changes affect the CDK2 active site and in particular, the locations of residues that are essential for correctly orienting ATP for catalysis. It may be important to consider these structural changes when designing ligands based on knowledge of their binding mode to inactive monomeric CDK2.

2. CYCLIN-DEPENDENT KINASE REGULATORY MECHANISMS: STRUCTURAL INSIGHTS

Crystallographic studies on CDK2 have provided a detailed understanding of the basis of CDK2 activation by cyclin A binding and phosphorylation (De Bondt et al., 1993; Jeffrey et al., 1995; Russo et al., 1996). CDK2 encodes little more than the protein kinase catalytic core, which is composed of multiple conserved subdomains found in all protein kinases (Hanks and Hunter, 1995). It adopts the characteristic protein kinase fold: a smaller (≈80 residue) N-terminal domain formed principally from β-sheet with one helix, the C-helix, and a larger C-terminal domain (of ≈210 residues), which is predominantly α-helical (Fig. 1a). The ATP-binding site is situated at the domain-domain interface. In the structure of inactive, monomeric CDK2, residues at the ATP-binding site are wrongly disposed and are unable to promote the correct alignment of the triphosphate moiety for catalysis, although the inactive monomer can bind ATP (De Bondt et al., 1993). The inactive conformation arises mainly from the organisation of two key elements of structure. These are the C-helix, which contains the PSTAIRE motif (single-letter amino acid code, residues 45–51), and the activation segment, which includes residues that lie between the conserved DFG and APE motifs (residues 145–147 and 170–172, respectively) (Fig. 1b). The position of the C-helix in the inactive CDK2 monomer results in the loss of the stabilising interaction between Glu51 (the E of the PSTAIRE sequence) and Lys33, which is important for correct localisation of the ATP triphosphate. The activation segment conformation places Thr160 away from solvent, close to the conserved glycine-rich loop.

On formation of the CDK2/cyclin A complex, there are no changes in the structure of cyclin A (Brown et al., 1995), but there are substantial changes in CDK2 that complete the ATP triphosphate recognition site (Jeffrey et al., 1995) (Fig. 1b). Briefly, the C-helix swings towards the active site cleft and the αL12 helix in the monomeric structure melts to form a short β-strand, β9 (Fig. 1b). Both these regions include conserved residues important for ATP binding. The C-helix contains Glu51, which together with Lys33, now coordinates to the α-phosphate of ATP; β9 includes the “DFG” motif (single-letter amino acid code, residues Asp145, Phe146, Gly147), which is conserved in all protein kinases and again, is crucial for orientation of the ATP phosphate groups for phosphotransfer. CDK2/cyclin A exhibits about 0.2% of the activity of the fully activated phosphorylated binary complex.

The role of phosphorylation in the activation of CDK2 seems to be to allow the formation of the peptide substrate binding site. In monomeric unphosphorylated CDK2, this site is occluded by the conformation of the activation segment. Phosphorylation of monomeric CDK2 disrupts this inappropriate conformation, thus allowing substrate to bind (Brown et al., 1999). Phosphorylation of the CDK2/cyclin A binary complex completes the structural rearrangement of the activation segment to correctly form a peptide binding site (Russo et al., 1996). Thr160, now phosphorylated, turns in to contact three arginine residues; one (Arg50) from the C-helix PSTAIRE motif within the N-terminal domain, a second (Arg126) that is adjacent to the catalytic aspartate (Asp127), and a third (Arg150) from the start of the activation segment. The phosphothreonine group, therefore, acts as an organising centre, coordinating residues responsible for substrate binding and catalysis.
3. CYCLIN-DEPENDENT KINASE 2-INHIBITOR INTERACTIONS AT THE ADENINE BINDING SITE

ATP binds to monomeric CDK2 in the active site cleft that lies between the N- and C-terminal domains (De Bondt et al., 1993). ATP makes a number of hydrogen bonds to the backbone groups of residues that constitute the hinge between the two domains (Fig. 2a). The N6 amino group acts as a hydrogen-bond donor to the backbone carbonyl of Glu81, and N1 accepts a hydrogen bond from the backbone nitrogen of Leu83. Analysis of the apo-CDK2 structure shows the presence of two water molecules with low temperature factors in positions corresponding to those of N1 and N6 of ATP, a result that supports the model that CDK2 has a strong preference to bind polar atoms at these positions (Schulze-Gahmen et al., 1996; Lawrie et al., 1997). In addition to hydrogen-bonding, there are a number of hydrophobic and van der Waals contacts between CDK2 and the adenine ring of ATP. Formation of the CDK2/ATP complex buries a large hydrophobic surface area contributed both by ATP and conserved hydrophobic amino acids within the active site cleft. In total, $573 \, \text{Å}^2$ is buried, of which $265 \, \text{Å}^2$ is apolar, $175 \, \text{Å}^2$ contributed by CDK2 and $90 \, \text{Å}^2$ contributed by ATP.

The majority of published CDK inhibitors have been developed from molecules that are substituted purines and so, it was expected that they would resemble ATP in their binding to CDK2. X-ray crystallographic analysis, however, has shown that dependent on the substitutions made on the ring system, the CDK2 ATP-binding site is tolerant of a number of purine ring orientations (Fig. 2). Only a hydrogen bond acceptor group equivalent to that of the N1 nitrogen of ATP is universally conserved. Although all the binding modes involve interactions between the purine ring and CDK2 residues 80–84 that constitute part of the hinge between the N- and C-terminal domains, there is no significant change in relative orientation of the CDK2 N- and C-terminal domains on inhibitor binding.

The CDK2 inhibitors roscovitine (Fig. 2b) and olomoucine are bound such that there is no equivalent interaction to that of ATP with the backbone carbonyl of Glu81 (De Azevedo et al., 1997; Schulze-Gahmen et al., 1995). Instead, using the program O (Jones et al., 1991). Monomeric CDK2 is coloured as described in (a). The CDK2 structure from the phosphorylated CDK2-cyclin A complex (Russo et al., 1996) is coloured grey. The relative shift in orientation of the N-terminal domain that accompanies cyclin A binding is seen. Both the PSTAIRE helix (gold in the monomeric CDK2 structure) and the activation loop (coloured cyan) undergo considerable structural rearrangement. ATP bound to each structure is shown in “ball-and-stick” representation. The orientation of ATP bound to monomeric CDK2 is profoundly different from the conformation it adopts in the active CDK2-cyclin A complex. In particular, the $\alpha$, $\beta$, and $\gamma$ phosphate groups are realigned into a conformation that presumably is more appropriate for the phosphotransfer reaction.
N7 acts as a hydrogen-bond acceptor from the backbone nitrogen of Leu83 and a novel interaction is made between N6 and the peptide oxygen of Leu83. Roscovitine and olomoucine have measured IC$_{50}$ values in vitro against CDC2-cyclin B of 0.65 μM and 7 μM, respectively, and the cellular effects of these compounds have been documented in vitro in a large number of cell lines (Glab et al., 1994; Vesely et al., 1994; Meijer and Kim, 1997; Meijer et al., 1997).

Crystal structures of CDK2 in complex with 2-[bis-(hydroxyethyl)amino]-6-(4-methoxybenzylamino)-9-isopropylpurine (CVT-313), another specific CDK2 inhibitor with a measured IC$_{50}$ in vitro of 0.5 μM (Brooks et al., 1997), or a series of C2 alkynylated purines (measured IC$_{50}$ ~200 nM) (Legraverend et al., 1998) have not been reported, but the nature of the substitutions on the purine ring would suggest that their binding modes would resemble that of olomoucine.

FIGURE 2. ATP, roscovitine, NU2058, and staurosporine binding to CDK2. Conserved hydrogen bonds between the CDK2 backbone at residues Glu81 and Leu83 and ATP (a), roscovitine (b), NU2058 (O6-benzyl guanine) (c), and staurosporine (d) are illustrated by thin lines. Only CDK2 residues Phe80 to Leu83 and Lys33 are included for clarity. CDK2 residues are labelled at their Cα positions. In addition to the three hydrogen bonds with backbone carbonyl and amide groups of CDK2 residues in the hinge region, members of the O6-substituted guanine series of inhibitors interact via a fourth hydrogen bond with the terminal amino group of Lys33. In the other three structures illustrated, Lys33 is swung away from the active site.
Crystalllographic Studies of CDK2-Inhibitor Complexes

cine. The recently designed 2,6,9-trisubstituted purine, purvalanol B (measured IC_{50} in vitro against CDC2-cyclin B is 6 nM), has a binding mode to CDK2 similar to that of roscovitine and olomoucine. Unlike these two compounds, however, purvalanol B is able to hydrogen-bond to the backbone oxygen of Glu81 via the acidic C8 atom of the purine ring (Gray et al., 1998).

This ability to achieve all three hydrogen bonds that can potentially be made between the active site ligand and the CDK2 residues in the hinge region had only previously been observed in a series of O6-substituted guanine inhibitors. These compounds adopt a third binding mode similar to that reported for the inhibitor isopentenyladenine (Schulze-Gahmen et al., 1995) (Fig. 2c). The purine ring orientation positions N9 to act as a hydrogen-bond donor to the carbonyl oxygen group of Glu81, N3 to accept a hydrogen bond from the NH group of Leu83, and N2 to act as a donor to the backbone oxygen of Leu83. A fourth novel hydrogen bond is observed between the inhibitor guanine ring N7 and the terminal amino group of Lys33 (Fig. 2c). Lys33 does not adopt this conformation in the CDK2-ATP complex, other CDK2/inhibitor complexes, or in the structure of active CDK2/cyclin A (Figs. 2a, 2b, and 2d). In these structures, it swings out from the active site cleft to accommodate, for example, the additional groups present in roscovitine or staurosporine, and in the CDK2-ATP complex to coordinate to the α-phosphate of ATP.

In addition to the purine-based inhibitors, crystal structures for monomeric CDK2 in complex with two other compounds with different backbones have been reported. The first is a flavone, (−)-cis-5,7-dihydroxyphenyl-8-[4-(3-hydroxy-1-methyl)piperidinyl]-4H-1-benzopyran-4-one hydrochloride hemihydrate (L868276) (De Azevedo et al., 1996), a chlorinated derivative of which, flavopiridol, is in clinical trials (Senderowicz et al., 1996). The second is the potent, but nonspecific, protein kinase inhibitor staurosporine (Lawrie et al., 1997). The flavone L868276, like ATP, interacts with the carbonyl oxygen of Glu81 and the backbone nitrogen of Leu83 via the C5 hydroxyl and C4 carbonyl groups, respectively. Staurosporine also shares these interactions: staurosporine N1 mimics ATP’s N6, and O5, like the N1 atom of ATP, acts as a hydrogen bond acceptor group (Fig. 2d). That the staurosporine lactam amide group could mimic the hydrogen-bonding interactions of the ATP adenine ring was predicted in what has now been shown to be an accurate modelling study of staurosporine binding to protein kinase A (Furet et al., 1995).

The disposition of the hydrogen-bonding groups with respect to a large hydrophobic surface area may be features that are sufficient to distinguish the ATP-binding site in the protein kinase family from that of other ATP-utilising enzymes. For example, the importance of the presence and disposition of both hydrogen-bond donor and acceptor groups in the maleimide ring in the bisindolylmaleimides to the binding of these compounds to protein kinase C had been recognised previously (Davis et al., 1992). Another reported specific CDK inhibitor, butyrolactone I, for which no crystal structure in complex with a CDK has been published, also shares these structural characteristics (Kitagawa et al., 1993; Meijer, 1995).

The CDK2 ATP-binding site cleft is lined with conserved hydrophobic residues, most notably Ile10, Val18, Ala31, Phe80, and Leu134 (Fig. 3). Both the shape and charge distribution within the active site cleft complement these characteristics of the ATP purine ring, the burial of which upon ATP-binding would be expected to make a considerable contribution to the energetics of CDK2/ATP association. This complementarity of shape is illustrated in two views of the model of the CDK2-staurosporine complex, where flexibility in side chain positions and relative domain orientation act to sandwich the large, planar staurosporine molecule into the cleft (Figs. 3a and 3b). Formation of the CDK2-staurosporine complex buries 658 Å^2, of which 545 Å^2 is apolar surface area (274 Å^2 contributed by CDK2, 271 Å^2 contributed by staurosporine). The characteristic hydrophobicity of the ATP-binding site is illustrated in Fig. 3c.

Unlike other reported CDK2/inhibitor structures, the binding of staurosporine to CDK2 causes a slight relative opening of the two domains (Lawrie et al., 1997). Notably, cyclic AMP-dependent protein kinase is similarly induced into an open conformation on staurosporine association (Prade et al., 1997; reviewed in Toledo and Lydon, 1997). Different protein kinase structures have been reported to adopt different relative dispositions of their N- and C-terminal domains (Johnson et al., 1996). This change is particularly striking in CDK2, as described above, on its association with cyclin A (Jeffrey et al., 1995).

4. EXPANDING THE PURINE RING: OPPORTUNITIES WITHIN THE ATP-BINDING SITE CLEFT

The crystal structure of CDK2 in complex with staurosporine shows that ring systems larger than that of ATP can be accommodated within the ATP-binding site (Lawrie et al., 1997). The inherent flexibility of protein kinases both in the amino acid side chains that line the active site cleft and in the relative orientations of their N- and C-terminal domains appears to offer more possibilities for inhibitor design than originally might have been anticipated. The crystal structure of p38 mitogen-activated protein kinase in complex with the 3′-iodo pyridinyl-imidazole derivative of SB 203580 showed that it is possible to identify potent and specific inhibitors by exploiting differences between kinases, despite the high level of conservation of the ATP-binding site (Tong et al., 1997). In this structure, Thr106 (the residue equivalent to Phe80 of CDK2) opens up a hydrophobic pocket into which the inhibitor 3′-iodo 4-phenyl group binds. The interactions of this substituent with p38 have been proposed to contribute to the observed specificity of this series of compounds towards p38 (Tong et al., 1997).

1Newell, D.R. Submitted for publication.
FIGURE 3. The shape and surface properties of the CDK2 ATP-binding site cleft as seen in complex with staurosporine. The Connolly molecular surface of CDK2 is rendered in orthogonal views in (a), (b), and (c) (Connolly, 1985). (a) and (b) demonstrate the complementary shapes of staurosporine and the CDK2 active site, with an extended flat surface offered by the N-terminal domain of the protein (above), to the flat “upper” surface of staurosporine, and a “stepped” surface offered by the C-terminal domain to receive the glycosyl moiety of staurosporine. (c) is looking through the staurosporine molecule towards the N-terminal domain, and is coloured such that regions showing a preference for hydrophobic interactions (as calculated by the program GRID; Goodford, 1996) are coloured from yellow (strong preference) to grey (no particular preference). The extended hydrophobic surface presented by the N-terminal domain complements the equivalent surface of staurosporine.

FIGURE 4. Interactions available around the isopropyl group of roscovitine when it is bound to CDK2. The structure of the roscovitine-CDK2 complex is shown with green carbon atoms and a gold-coloured Connolly surface (Connolly, 1985). The isopropyl group of the inhibitor (upper) packs against Phe80 and extends towards a hydrophobic pocket beyond this residue. The superimposed structure of CDK2, as seen in the phosphorylated CDK2-cyclin A complex, is shown with yellow carbon atoms. Rearrangement of the PSTAIRE helix in this structure has brought residue Glu51 (upper central part of the figure) into a position such that access to the hydrophobic pocket is blocked.
Crystallographic Studies of CDK2-Inhibitor Complexes

The purvalanol B 1-(R)-isopropyl-2-hydroxyethylamino group hydrogen bonds to the backbone oxygen of Gln131 (Gray et al., 1998) (Fig. 5b). The olomoucine 2 hydroxyethyl side chain, although in van der Waals contact both with Asp86 and the backbone oxygen of Gln131, hydrogen bonds only to the Gln131 side chain amide group (Schulze-Gahmen et al., 1995). The C2 substituent of roscovitine (1-ethyl-2-hydroxyethylamino) makes no hydrogen bonds to CDK2 (De Azevedo et al., 1997).

Studies using staurosporine derivatives suggest that substitutions of the charge of the molecule in this region can affect potency and selectivity towards a number of different kinases (Osada et al., 1990; Meggio et al., 1995; Nakanishi et al., 1986; Kase et al., 1987). Substitutions of the olomoucine C2 hydroxyethylamino group have been shown to affect the ability of the compound to inhibit purified CDC2 kinase (Vesely et al., 1994), and this analysis has been extended in the recent isolation of purvalanol B (Gray et al., 1998). C2 alkynylated purines show improved IC50 values against CDK1/cyclin B as compared with olomoucine and IC50 values comparable with roscovitine (Legraverend et al., 1998).

A superposition of the structure of ATP bound to monomeric CDK2, with ATP bound to the active phosphorylated CDK2-cyclin A complex, shows that although the positions of residues in the CDK2 C-terminal domain remain largely unchanged on cyclin A binding, the N-terminal domain undergoes considerable rearrangement (De Bondt et al., 1993; Russo et al., 1996) (Fig. 1b). This structural change results in ATP adopting a different position and conformation within the active site cleft. The ribose-phosphate group is re-oriented to allow phospho-transfer (Fig. 1b). This result provides grounds for caution when attempting to predict potential interactions between inhibitors and CDK2 in this part of the CDK2 structure. The determination of structures of inhibitors bound to an active CDK-cyclin complex will show whether these interactions can be exploited to improve the potency or selectivity of CDK inhibitors.

6. NOVEL INTERACTIONS OUTSIDE THE ATP-BINDING SITE POCKET

The conservation of residues within the ATP-binding site suggests that inhibitors with improved selectivity would be achieved by exploiting interactions outside the catalytic cleft. The compounds olomoucine and roscovitine were shown to have good selectivity against members of the CDK family, and it was proposed that this resulted from interactions of their N6 benzylamino substituent with residues in the CDK2 C-terminal domain (Schulze-Gahmen et al., 1995; De Azevedo et al., 1997). There are extensive van der Waals contacts between the benzyl ring and the side chains of residues Ile10, Phe82, and His84 (Fig. 6). Protein kinase alignments show residues Phe82 and His84 are not well conserved (Hanks and Hunter, 1995). Other hydrophobic substituents can be accommodated at this position.
with essentially similar inhibitory efficiencies (De Azevedo et al., 1997). The phenyl ring of the flavonoid L868276 exploits similar interactions (De Azevedo et al., 1996).

The development of purvalanol B, which has a 3-chloro-4-carboxyanilino group at N6, shows how interactions at this site can help generate a potent CDK2 inhibitor. Purvalanol B has a measured IC50 against CDK2/cyclin A that is 1000-fold lower than that of olomoucine (Gray et al., 1998). In addition to the advantageous hydrophobic effect of the aromatic ring burying the side chains of Ile10 and Phe82, the presence of the chlorine atom acting as an electron-withdrawing group attached to the ring must enhance the π-stacking interactions between the ring and the planar peptide backbone between residues 84 and 85 (Fig. 6). The model has two alternative conformations for the N6 substituent, which in each case, leaves the p-carboxylate...
group in a position to interact with the terminal amino group of Lys89. This interaction has been shown not to contribute to potency against CDK2/cyclin A, but rather to decrease the potency of the inhibitor against CDK4/cyclin D1 (Gray et al., 1998). Of the residues shown to contact this group, Ile10, Asp86, and Gln85 are conserved between CDK2 and CDK4. However, there are sequence differences at the contacting residues Phe82, His84, and Lys89 of CDK2, that are replaced by a histidine, an aspartate, and a threonine, respectively, in CDK4. This result suggests that substituents that probe this surface region might offer not only the potential to develop general CDK inhibitors, but also inhibitors selective amongst different members of the CDK family.

7. CONCLUSIONS

The observed alterations in the control of the cell cycle that can occur in tumour cells suggest CDKs as potential targets for the design of novel anticancer therapies. The success of this strategy relies on the development of specific CDK inhibitors. This goal is complicated by the high degree of conservation within the ATP-binding site of the vast family of protein kinases. Structural data on the binding of a growing range of kinase inhibitors bound to CDK2 are now helping to identify the determinants of binding mode and binding potency. Allied with the techniques of high-throughput screening and combinatorial chemistry, it is hoped that structural data will help to inform the design of suitable compounds.

Acknowledgements–We acknowledge with gratitude the help of the beamline scientists at DESY, Hamburg; Elettra, Trieste; ESRF, Grenoble; and at the SRS, Daresbury for their assistance during the authors’ data collection visits to these synchrotrons. We would like to thank S.H. Kim, L. Meijer, and N. Pavletich for providing coordinates prior to their release from the PDB. We would also like to thank our colleagues at the LMB, Oxford, N. Brown, E. Garman, A. Lawrie, J. Tucker, P. Tunnah, and L. Johnson; members of the Anticancer Drug Discovery Initiative at Newcastle University; T. Boyle and P. Jewsbury at Zeneca Pharmaceuticals; and L. Meijer for much stimulating discussion and assistance throughout. This work was funded by the MRC, BBSRC, and the Royal Society.

References
kinase activities, blocks plant cells at the G1 to S and G2 to M cell cycle transitions. FEBS Lett. 353: 207–211.


