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Modelling study of protein kinase inhibitors: Binding mode of staurosporine and origin of the selectivity of CGP 52411

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Summary

A model for the binding mode of the potent protein kinase inhibitor staurosporine is proposed. Using the information provided by the crystal structure of the cyclic-AMP-dependent protein kinase, it is suggested that staurosporine, despite a seemingly unrelated chemical structure, exploits the same key hydrogen-bond interactions as ATP, the cofactor of the protein kinases, in its binding mode. The structure–activity relationships of the inhibitor and a docking analysis give strong support to this hypothesis. The selectivity of the dianilinophthalimide inhibitor CGP 52411 towards the EGF-receptor protein tyrosine kinase is rationalized on the basis of the model. It is proposed that this selectivity originates in the occupancy, by one of the anilino moieties of the inhibitor, of the region of the enzyme cleft that normally binds the ribose ring of ATP, which appears to possess a marked lipophilic character in this kinase.

Introduction

Protein kinases catalyse the transfer of the gamma-phosphate group of adenosine triphosphate (ATP) to the hydroxyl function of serine, threonine or tyrosine residues belonging to specific protein substrates. These enzymes play a key role in cellular signal transduction and over-expression of their activity has been associated with cancer [1]. Therefore, inhibition of this class of enzymes constitutes an attractive strategy, currently under intensive investigation [2], for developing new antitumor agents.

Among the different types of chemical structures reported to possess kinase inhibitory activity, the microbial alkaloid staurosporine [3,4] occupies a prominent position due to its high potency and ability to inhibit a wide spectrum of enzymes of both the serine/threonine and tyrosine kinase families [5]. In addition, staurosporine has been the starting point for recent medicinal chemistry efforts that have resulted in the discovery of selective inhibitors of certain targeted protein kinases. These include bisindolylmaleimide inhibitors of protein kinase C (PKC) [6] and dianilinophthalimide derivatives that selectively inhibit the kinase domain of the epidermal growth factor receptor (EGF-R) [7,8].

In this context, it is of much interest to gain insight into the structural basis of the inhibitory activity of staurosporine. The determination of the structural requirements for binding to the protein kinases and the understanding of the relationships between the structure of the ligands and selectivity are crucial for the design of new inhibitors with improved biological properties. In this report, we contribute to such an endeavour by proposing a model for the mode of binding of staurosporine to protein kinases. The model is then used to discuss the origin of the selectivity displayed by the dianilinophthalimide compound CGP 52411 towards the EGF-R protein tyrosine kinase.

Methods

Staurosporine was modelled in its correct absolute configuration, which has been recently determined by X-

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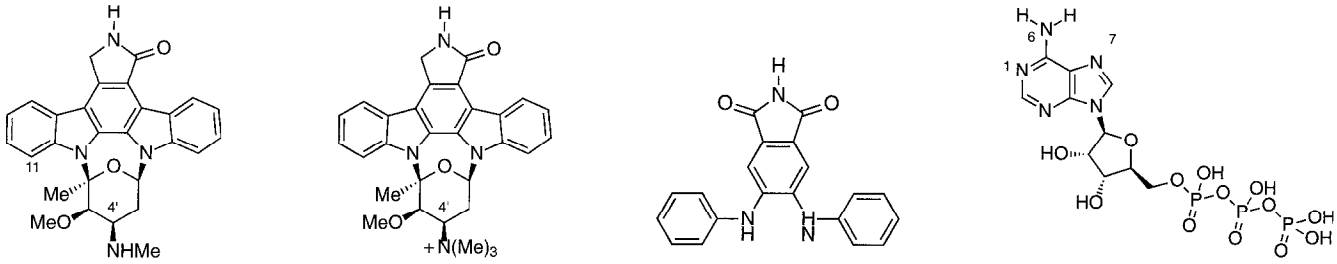

Fig. 1. Chemical structures of the molecules under investigation.

ray crystallography [9]. The following methods, implemented in MacroModel v. 4.0 [10], were used. (i) Energy minimization with the AMBER force field [11]; (ii) the GB/SA water solvation model [12]; and (iii) the Monte Carlo/energy minimization procedure [13].

For the Monte Carlo search, only those residues of the protein (coordinates of the cyclic-AMP-dependent protein kinase complexed with Mg-ATP and a peptide inhibitor [14]) within 8 Å of the initial position of staurosporine were included in the energy calculations (the substrate-derived peptide inhibitor and the magnesium cations present in the structure were removed).

The ligand as well as the residues within a distance of 5 Å were allowed to move freely upon energy minimization, while those at a distance between 5 and 8 Å were constrained by application of a parabolic force constant of 50 kJ/Å^2. Residues beyond 8 Å were ignored. A dielectric constant of 4r was used in the electrostatic part of the force field.

The propeller-shaped conformation observed in the crystal structure [7] was used to model CGP 52411. Molecular mechanics calculations confirm that this conformation and its mirror image conformation are the only realistic low-energy conformational minima of the molecule.

Results and Discussion

Bioactive conformation of staurosporine

Staurosporine presents two main conformational states, corresponding respectively to a chair and a boat conformation of the tetrahydropyran ring (Fig. 2). Davis et al. [15] have shown that in solution the preferred conformation depends on the protonation state of the amine function in the 4' position. While in the free base the chair conformation is predominant, the cationic form prefers the boat conformation, which allows a better solvation of the ammonium group.

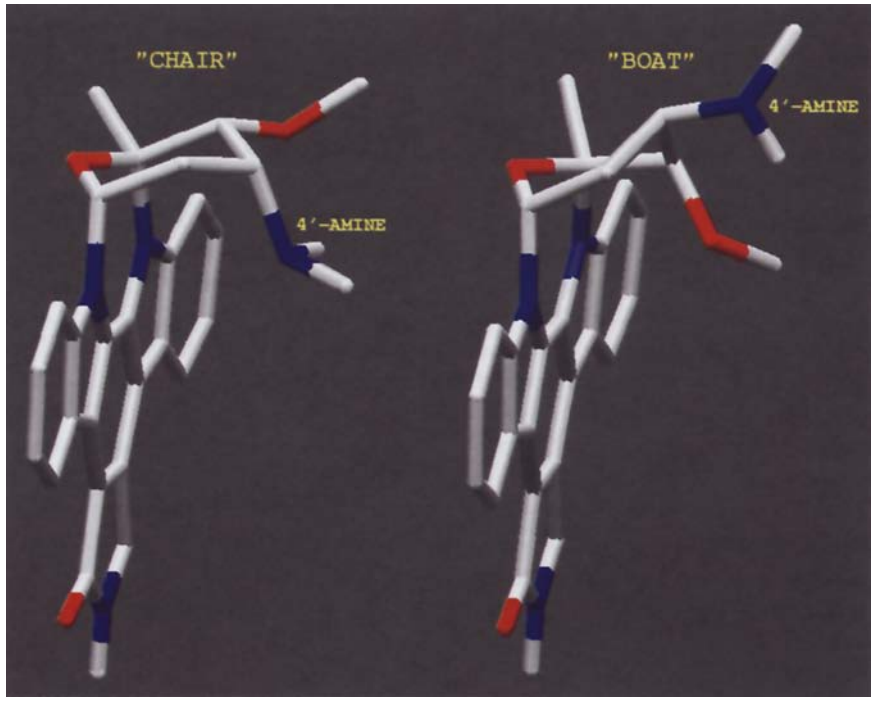

Fig. 2. The two possible conformations of the tetrahydropyran ring of staurosporine.

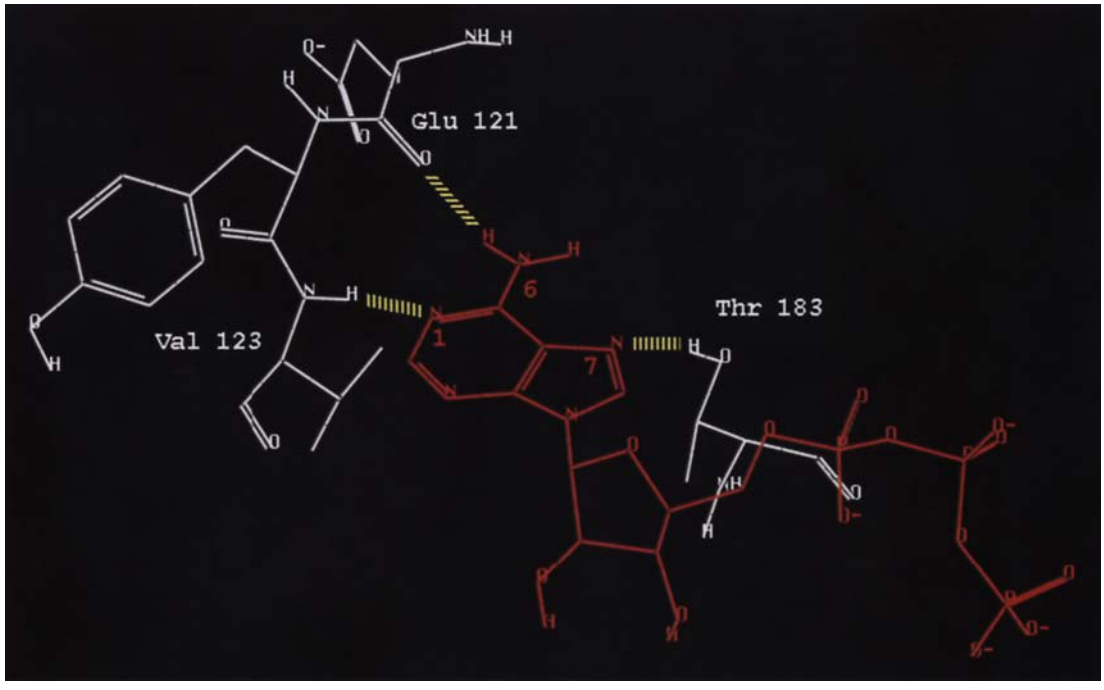

Fig. 3. Hydrogen bonds anchoring the adenine moiety of ATP in the active site of PKA [14]. The 6-amino group and the N1 nitrogen form two hydrogen bonds in a bidendate manner with the backbone carbonyl of Glu^{121} and the amide hydrogen atom of Val^{123}. This mode of binding has been observed in all kinase structures determined so far. The third hydrogen bond, involving the N7 nitrogen, seems less important.

The results of our molecular mechanics calculations support these observations. The calculations corresponding to a gas phase environment indicate that, as expected, the chair conformation is intrinsically more stable (by 3 and 7 kcal/mol in the free base and the protonated forms, respectively). However, inclusion of a water solvation model in the calculations reverses this order. With the solvation model, the boat conformation is computed

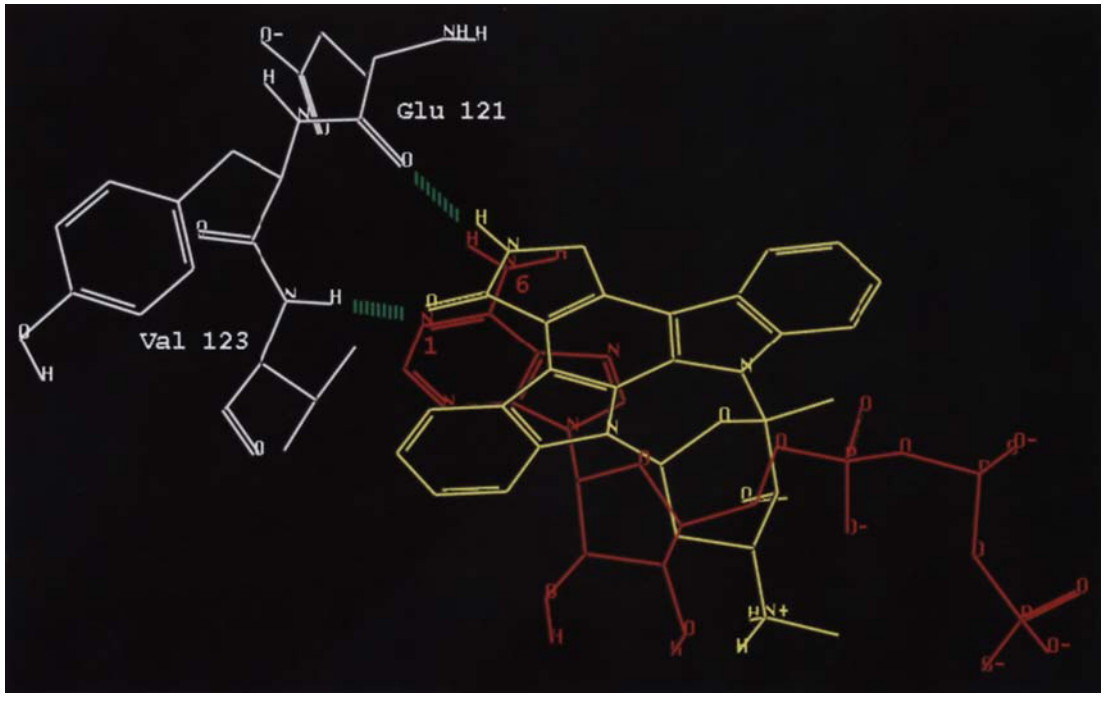

Fig. 4. Superposition of staurosporine (yellow) and ATP (red), showing the alignment of the lactam amide group of the inhibitor on the 6-amino–N1 hydrogen bond donor–acceptor system of the cofactor. This is the possible common binding mode of the two ligands.

to be more stable than the chair conformation by 1.3 kcal/mol in the protonated form.

The question of the bioactive conformation of staurosporine is obscured by this dependence of the chair/boat conformational equilibrium on the protonation state and environment. Nevertheless, some light can be shed on this matter by considering analogues of staurosporine, in particular the 4'-trimethyl ammonium derivative described in Ref. 5 (Table 1, entry 3), which is also a good inhibitor of various protein kinases, including the cyclic-AMP-dependent protein kinase and PKC.

Due to a severe steric clash between the trimethyl ammonium group and the indolecarbazole nucleus, the chair conformation is highly strained (its energy is calculated to exceed 10 kcal/mol compared to the boat conformer with the solvation model) in this cationic analogue of staurosporine. The possibility that it binds to the kinase enzymes in the chair conformation can therefore be excluded. Since the structural modification is minimal and no significant loss of inhibitory activity is observed, one can reasonably extend this conclusion to the parent compound.

The kinase-bound form of staurosporine is thus very likely to be protonated, with the tetrahydropyran ring adopting a boat conformation. The same conclusion was reached by Davis et al. [16] on the basis of modelling comparisons between staurosporine and their bisindolylmaleimide protein kinase C inhibitors. The high potency and conformational analysis of 11-hydroxystaurosporine reported by Kinnel et al. [17] also plead in favor of a bioactive conformation of the boat type.

Binding mode of staurosporine

A compound can inhibit the catalytic activity of a protein kinase by competing for binding either with the peptide substrate undergoing phosphorylation or with the cofactor ATP. The mode of inhibition of staurosporine is considered to be of the second type [18,19], which suggests direct interaction of the inhibitor with the binding site of ATP.

On this premise, we sought to determine in which way staurosporine may mimic ATP in its binding interactions with the protein kinases. As detailed below, this modelling study relied on the analysis of key structure–activity relationships of the ligands and the structural information provided by the X-ray crystal structure of the cyclic-AMP-dependent protein kinase (PKA) in complex with Mg-ATP and a substrate-derived peptide inhibitor [14,20–22].

Basic alignment

The X-ray structure of PKA reveals a bilobal shape of the protein, with a large cleft at the interface of the two lobes that forms the binding site for ATP. The adenine ring of the nucleotide is deeply buried in the predominantly hydrophobic bottom of the cleft, while its sugar and triphosphate moieties extend towards the opening of the cleft where the phosphate transfer is assumed to occur. The same binding mode has been observed in several recently published protein kinase structures (the cyclin-dependent kinase 2 [23], the MAP kinase ERK2 [24], the phosphorylase kinase [25] and the casein kinase-1 [26]), in agreement with the high degree of sequence conservation displayed by the kinase catalytic cores [27].

Prior to the determination of these X-ray structures, analogue studies [28] had shown the important role played by the adenine moiety in the recognition of ATP by protein kinases. In particular, preserving the hydrogen-bond donor capability of the amino group at position 6 was found to be essential for maintaining affinity. The structural basis of this finding is now understood. As illustrated in Fig. 3 for PKA, the adenine ring of ATP is anchored to the enzyme by three hydrogen bonds. Two of these involve precisely the 6-amino group and the N1 nitrogen, which interact in a bidentate manner with backbone atoms of two residues located at the bottom of the cleft (Glu^{121} and Val^{123}). This bidentate interaction, reminiscent of the Watson–Crick mode of base pairing of adenine nucleotides, seems thus to be determinant in the recognition of ATP. The third hydrogen bond, which is formed between the adenine N7 nitrogen and the side chain of Thr^{183}, may not be as important since, according to sequence alignments [27], the latter residue is not conserved within the protein kinase family and corresponds in many kinases to a residue with no side chain hydrogen-bonding capability.

In our search for structural similarities between ATP and staurosporine, we realized that the lactam amide group of the inhibitor could also function as a bidentate hydrogen bond donor–acceptor system. This notion was supported by structure–activity relationships indicating a significant role for the lactam ring in the kinase inhibitory activity of staurosporine and its derivatives. Thus, chemical reduction of the lactam carbonyl produces a large decrease of activity (U. Trinks, unpublished results), while this is completely abolished by alkylation of the nitrogen atom [5,29]. This led us to further investigate, by using docking analyses, the possibility that staurosporine in fact uses the same key bidentate hydrogen-bond interactions as the adenine ring of ATP in its binding mode, as represented in Fig. 4.

Docking

To test our binding-mode hypothesis, staurosporine in its assumed bioactive conformation (protonated, boat type) was positioned in the active site of PKA (coordinates of the ternary complex [14]) according to the alignment shown in Fig. 4. Good complementarity with the cleft of the enzyme was immediately apparent. With the exception of minor steric clashes with the side chains of residues Phe^{327}, Met^{120} and Lys^{72} that were readily removed

upon energy minimization, and a more severe clash with the side chain of Thr^{183} that could be avoided by changing the threonine χ_1 value from a t to a g^+ rotameric state, staurosporine in this orientation nicely fitted the binding site of ATP with many good van der Waals contacts.

Figures 5 and 6 show the resulting energy-minimized complex. As can be seen, the large aromatic system of the inhibitor sits in the hydrophobic environment provided by residues Ala^{70}, Met^{120}, Val^{123}, Phe^{327}, Leu^{173}, Thr^{183}, Val^{104}, Leu^{49}, Val^{57} and the hydrocarbon part of the side chain of Lys^{72}, while its 4'-amino group, known to contribute to its high potency [5,30], forms hydrogen bonds with the backbone carbonyl of Glu^{170} and the carboxylate group of Glu^{127}, two structural features used by the enzyme to bind the hydroxyl groups of the ribose ring of ATP according to the X-ray structure. Thus, the alignment of Fig. 4 leads to a consistent model in which a high degree of complementarity, both in terms of electrostatic and hydrophobic interactions, exists between staurosporine and the binding site of the enzyme's cofactor ATP.

To check if other binding modes could be compatible with the structure of the protein, staurosporine was subjected to a Monte Carlo/energy minimization search [13] inside the cleft, taking its translational and rotational degrees of freedom as variables and with the initial positioning of Fig. 5. Thousands of iterations failed to produce new, different low-energy binding modes. Alternate binding modes were generated, in which the inhibitor was flipped 180° with respect to the orientation of Fig. 5 or moved to other regions of the active site. However, these were significantly higher in energy (above 8 kcal/mol compared to the global minimum) and in none of them the lactam nitrogen and carbonyl were simultaneously engaged in hydrogen bonds, as strongly suggested by the structure–activity relationships. This reinforced the plausibility of the model described above.

An overall picture of the model of PKA complexed by staurosporine is given in Fig. 7. The orientation of staurosporine in the ATP binding site is such that the 4'-amino group is turned towards the opening of the cleft, where the phosphate transfer between ATP and the substrate occurs. In fact, staurosporine does not occupy the entrance of the cleft, which normally serves to anchor the triphosphate chain of ATP. The model therefore suggests that there is room left in the binding site for possible extensions of the structure of staurosporine, starting from the 4'-amino group. In full agreement with this concept, a large variety of substituents is tolerated at this position of the inhibitor without major loss of affinity [5].

Explanation for the selectivity of CGP 52411

A reversal of selectivity has been observed when the indole rings in the indolecarbazole nucleus of staurosporine were opened to give dianilinophthalimide derivatives, exemplified by CGP 52411 [7,8]. These compounds are potent and selective ATP competitive inhibitors of the EGF-R protein tyrosine kinase. CGP 52411 inhibits the EGF-R kinase with an IC_{50} value of 300 nM, while it is less active by at least two orders of magnitude on a panel of protein kinases that includes PKA, the phosphorylase and casein kinases, most of the enzyme isoforms of PKC and the v-abl, c-lyn and c-fgr tyrosine kinases. We were interested in trying to understand the origin of this selectivity using our model for the binding mode of staurosporine.

The imide ring of CGP 52411 displays the same behavior as the lactam ring of staurosporine with respect to modifications that alter its hydrogen-bonding capability. Thus, alkylation of the free nitrogen abolishes activity [7]. It is therefore reasonable to assume that CGP 52411 adopts the same basic binding mode as staurosporine with the two key bidentate hydrogen bonds. This idea is illustrated in Fig. 8 by an overlay of CGP 52411, staurosporine and ATP.

Figure 8 shows that, due to the opening of the five-membered indolo rings, the anilino moieties of CGP 52411 occupy a somewhat different position in space compared to the corresponding rings in the flat indolic system of staurosporine. Of particular interest is the good overlap of one of these moieties with the ribose ring of ATP. This suggests that, in the EGF-R kinase, the part of the cleft forming the ribose environment may have a more lipophilic character than in the other protein kinases, a feature exploited by CGP 52411 and contributing to its selectivity.

To test this hypothesis, we analysed the X-ray structure of PKA to determine which residues of the protein make contacts with the ribose ring of ATP. We then looked for the corresponding residues in the EGF-R kinase, according to the alignment of sequences [27] that has served to build a 3D model of this kinase by homology [31]. This analysis revealed that the environment of the ribose ring is constituted by residues Leu^{49}, Gly^{50}, Val^{57}, Glu^{127}, Glu^{170}, Asn^{171}, Leu^{173} and Thr^{183}. All these residues except for Glu^{127} and Glu^{170}, which, as previously mentioned, form hydrogen bonds with the sugar hydroxyl groups, are conserved in the EGF-R tyrosine kinase. Glu^{127} and Glu^{170} correspond to a cysteine and an arginine residue, respectively, in the latter kinase. Since Glu^{170} is engaged in an interaction with the ribose moiety only through the carbonyl group of its backbone, an amino acid residue with a different side chain at this position is not a dramatic change. However, in the case of Glu^{127}, which interacts through the acid function of its side chain, the change to a more hydrophobic cysteine residue, with poor hydrogen-bonding capacity, is quite dramatic. This supports our hypothesis that a marked lipophilic character of the ribose binding site is a distinctive feature of the EGF-R kinase. Along this line, one can imagine a direct inter-

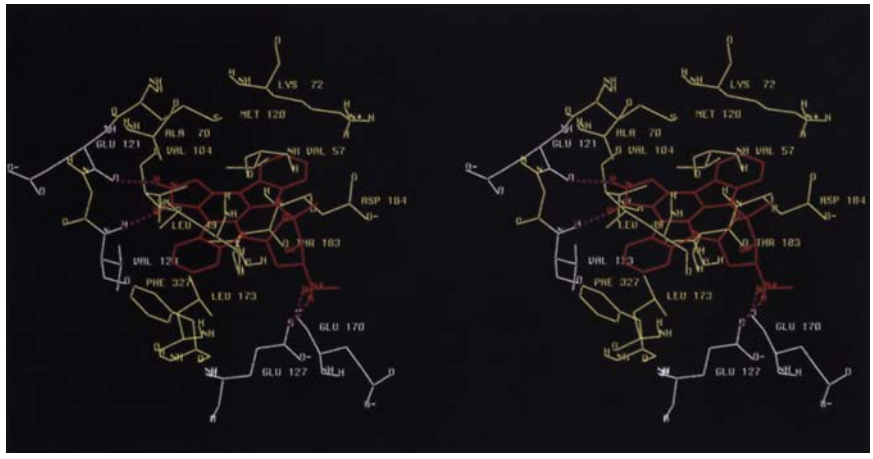

Fig. 5. Stereoview of staurosporine docked in the ATP binding site of PKA. Only the residues closest to the inhibitor are represented, i.e., those that make hydrophobic contacts (yellow) and those forming hydrogen bonds (white).

action of the anilino moiety of CGP 52411 that overlaps the ribose ring in Fig. 8 with the cysteine side chain. Favorable sulfur–aromatic interactions are often observed in proteins [32,33]. Indeed, docking of CGP 52411 in the homology-built model of Knighton et al. [31] fully confirms the plausibility of this hypothesis (P. Furet, unpublished work).

Another fact points to the role of the residue corresponding to position 127 of PKA in controlling selectivity. As presented above, in our model the carboxylate group of Glu^{127} forms a hydrogen bond with the protonated 4'-amino group of staurosporine. By eliminating this stabilizing electrostatic interaction, the replacement of the glutamic acid residue by a cysteine should weaken the binding of staurosporine. This view is supported by the observation that staurosporine inhibits the EGF-R kinase less potently (IC_{50} = 630 nM [34]) than it does PKA (IC_{50} = 15 nM [5]) and other kinases, like PKC (IC_{50} = 6 nM [5]) or the tyrosine kinase domain of the insulin receptor (IC_{50} = 61 nM [34]), which also possess an acidic residue at the position homologue of Glu^{127}. The crystal structure of the latter kinase has recently been solved [35].

Conclusions

By analyzing some structure–activity relationships in light of the crystal structure of a protein kinase complexed with ATP, a consistent model for the binding mode of staurosporine has been developed. In essence, the model suggests that the bidentate hydrogen bonds that serve to anchor the adenine moiety of the cofactor are also used by the inhibitor and thus seem to be key determinants of ligand recognition by the protein kinases. This is an interesting finding that needs to be considered in the design of new kinase inhibitors.

A rather striking aspect of our model is that the ATP binding site (in the catalytically relevant closed form it adopts in the PKA ternary complex [14]), seems sufficiently large to accommodate the bulky aromatic system of staurosporine. In fact, the hydrophobic pocket oc-

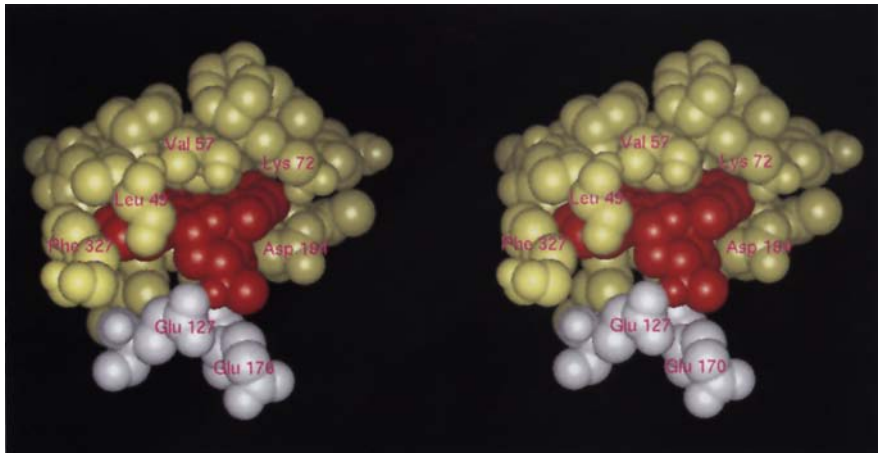

Fig. 6. Spacefilling model of staurosporine docked in the ATP binding site of PKA (the color coding is the same as in Fig. 5).

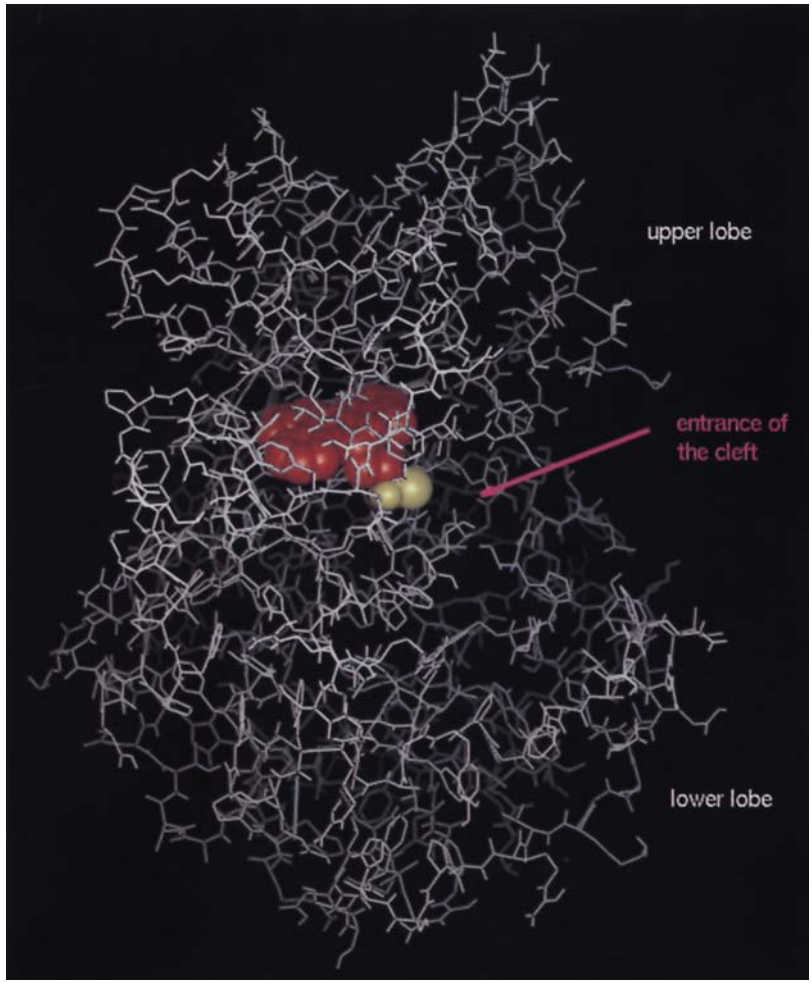

Fig. 7. Full model of the staurosporine–PKA complex. Staurosporine appears in red, except for its 4'-amine function (yellow), which points towards the opening of the cleft, the part of the enzyme that binds the triphosphate chain of ATP and is involved in phosphate transfer.

cupied by the adenine moiety is not fully exploited by the cofactor and its more complete filling by staurosporine very likely contributes to the exceptional potency of this compound. This also should be borne in mind for the design of new inhibitors.

Finally, as illustrated in the discussion regarding the high selectivity of the EGF-R kinase inhibitor CGP 52411, the model provides a rational frame to understand, and in the future hopefully tailor, the selectivity of kinase inhibitors that are ATP competitive.

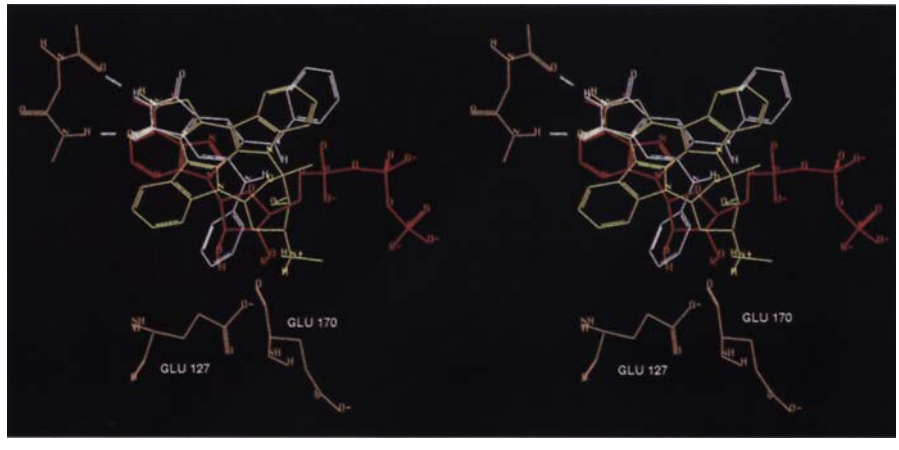

Fig. 8. Stereoview showing an overlay of CGP 52411 (white), staurosporine (yellow) and ATP (red). The PKA residues Glu^{127} and Glu^{170} of the ribose environment, which are not conserved in the EGF-R kinase, are also shown.

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