INHIBITION MECHANISMS OF STAUROSPORINE AND H7 TO cAMP-DEPENDENT PROTEIN KINASE THROUGH DOCKING STUDY

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Inhibition mechanisms of staurosporine and H7 to cAMP-dependent protein kinase have been investigated through docking studies. For each molecule, the energetically most stable docking model was searched by using the conformationally flexible automatic docking program ADAM without any presumptions. The results explain well the observation that staurosporine does not bind to the enzyme competitively with H7, even though the two compounds competitively inhibit ATP binding.

KEY WORDS cAMP-dependent protein kinase; staurosporine; H7; inhibitor; docking study

Protein kinases play important roles in signal transduction leading to a variety of cellular functions, often by phosphorylating a particular hydroxyl group in the target protein, under the control of a small-molecular or macromolecular messenger.[1] As regards the cAMP-dependent protein kinase, the active form of the enzyme is generated upon binding of cAMP to the cAMP-binding domain of the precursor protein.[2] Crystal structures of the enzyme are available from the Protein Data Bank as a binary complex with a peptidyl inhibitor (2cpk)[3] and as a ternary complex with ATP and a peptidyl inhibitor including Mn^{2+} ions (1atp)[4].

(+)-Staurosporine **1** and H7 **2** are non-peptidyl inhibitors, having the structures shown in Fig. 1. Staurosporine, a microbial product, is a strong nonselective inhibitor of many protein kinases including cAMP-dependent protein kinase.[5-6] H7, a synthetic isoquinolinesulfonamide, inhibits protein kinase C and cyclic nucleotide dependent protein kinases nonselectively.[7] Recently, Herbert *et al.* have reported that staurosporine binds to cAMP-dependent protein kinase and other protein kinases competitively with ATP, but not with H7.[8] This result indicates that staurospoine and H7 occupy different regions of the ATP binding site of the kinases. In order to explain consistently the observations described above, we have performed docking simulations of these inhibitors to cAMP-dependent protein kinase based on the crystal structure of the enzyme.

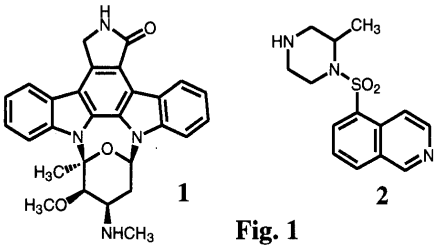

Fig. 1

Atomic coordinates of cAMP-dependent kinase from 1atp were mainly used. The allowed region for accommodating the two inhibitors was designated so as to cover the binding sites of ATP and the peptidyl inhibitor molecule, by removing those molecules. The structure of staurosporine was modeled based on the crystal structure taken from the Cambridge Structural Database (STAURO10).[9] The structure of H7 was modeled by the conventional modeling procedure and was optimized by MOPAC Ver. 6.0 (AM1 method).[10] As there was no information about which enantiomer of H7 is active, both enantiomers (R and S) involving methyl group of H7 were tested independently. Other conformational flexibilities were treated in the docking processes.

Docking study was performed using the automatic docking program ADAM developed by us.[11] The program covers all possible binding modes and ligand conformations, searching for the most stable docking models automatically and reliably. For each inhibitor molecule, several stable models were generated from the program, using inter- and intramolecular interaction energies as criteria in all pruning steps and by optimizing

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relative positions and conformations repeatedly. Only the most stable docking model for each molecule, which was selected from among the candidates after energy minimization taking into account protein flexibilities and a sufficient number of water molecules, was used for later discussion. The AMBER force field was used for all energy calculation and minimization,[12] and the dielectric constant was assumed to be 4.0R (R = distance in Å).

The active site of the enzyme is shown as a bird-cage diagram in Fig. 2, which also shows the locations of the ATP and peptidyl inhibitor molecules and two Mn^{2+} ions in the crystal structure. As can be seen from Fig. 2, the active site is thin and narrow at the bottom and becomes wider near the entrance. The enzyme structures in 1atp and 2cpk were very similar, but 1atp has a rather thinner cavity due to the induced fit.

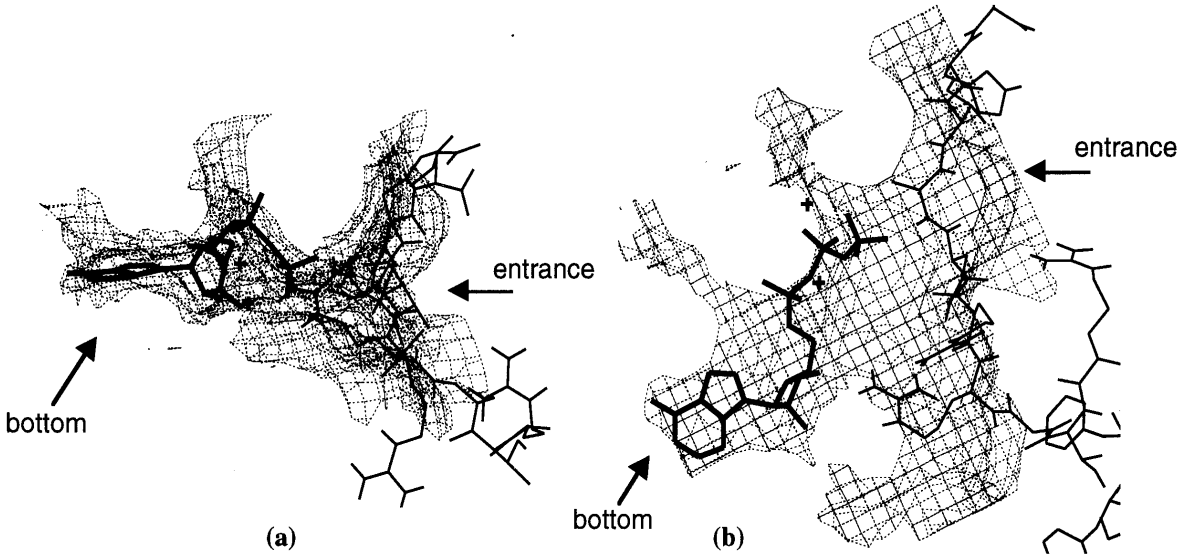

Fig. 2. Side View (**a**) and Over View (**b**) of the Catalytic Site of 1atp Containing ATP (Bold Line), Mn^{2+} Ions (Cross Marks) and Peptidyl Inhibitor (Thin Line)
The allowed region for the centers of ligand atoms is displayed as a bird-cage representation. Entrance and bottom of the cavity are also shown.

The most stable docking model of (+)-staurosporine with the intermolecular energy of –39.52 kcal/mol is shown in Fig. 3. The molecule was located at the entrance of the cavity, overlapping the binding location of the phosphate group of ATP. In the binding mode, a H-bond was formed between γ-lactam carbonyl oxygen atom of staurosporine and Nζ of the Lys^{168} residue, which exists in the catalytic loop of the enzyme and plays an important role in the phosphorylation process.

The most stable docking model of H7 is shown in Fig. 4. The model for the R-enantiomer of H7 was a little more stable than that of the S-enantiomer, forming an H-bond between the isoquinoline nitrogen atom of H7 and the main chain NH group of the Val^{123} residue. The enantiomers occupy similar positions in the active site, fully overlapping the binding location of the adenosine moiety of ATP. The isoquinoline ring fitted well at the bottom of the pocket, where the planar adenine ring of ATP would be bound. The intermolecular energy difference (–48.02 kcal/mol for R and –47.11kcal/mol for S) was very small and is unlikely to be significant.

Thus, the docking results clearly imply that the two inhibitors would bind to the enzyme competitively with ATP, though they occupy different regions of the ATP-binding site. The noncompetitive binding of staurosporine and H7 is explained by their different binding locations. H7 can coexist with staurosporine, which binds at the entrance of the cavity, as shown in Fig. 5.

The most stable docking models of staurosporine and H7 to cAMP-dependent protein kinase were rationally estimated by using our automatic docking program ADAM. The observed binding characteristics (competitive or noncompetitive) of the inhibitors with respect to ATP and to each other inhibitor could all be well explained by the estimated locations of the molecules in the active site.

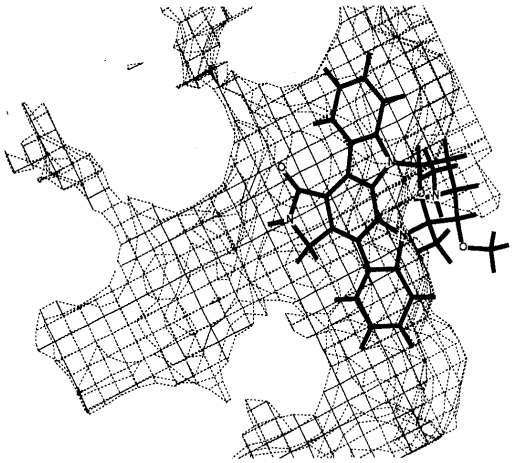

Fig. 3. Structure of the Most Stable Final Docking Model for Staurosporine

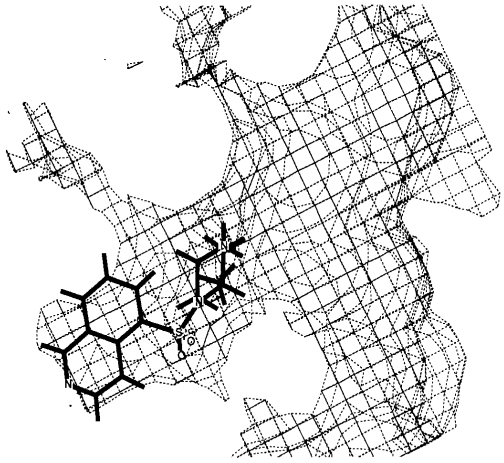

Fig. 4. Structure of the Most Stable Final Docking Model for H7

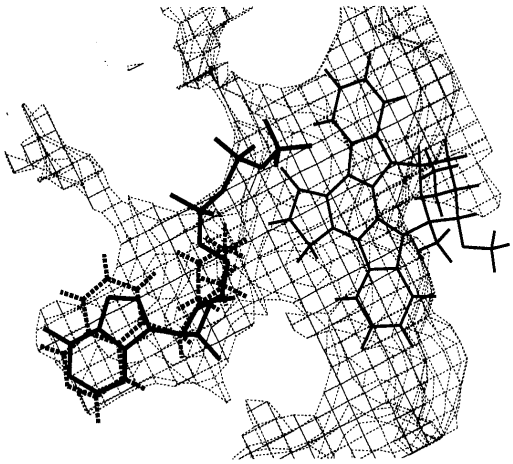

Fig. 5. Superposition of the Most Stable Final Docking Models for Staurosporine (Thin Line) and H7 (Dashed Line) at the Catalytic Site of 1atp, Showing the Binding Location of ATP (Bold Line)

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