Structural studies on protein kinase inhibitor complexes

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Protein kinase activity is critically involved in human cancer. In the majority of cancer cases overactive kinases can be found. Furthermore, the activity of several protein kinases is a factor in cell survival, cell migration or tumor progression. The selective inhibition of certain protein kinases therefore is a promising approach to fight cancer. The ATP-binding pockets of different protein kinases are highly conserved in their phosphate binding subsite, in other parts, however, they are quite variable and proved to by useful targets for protein kinase inhibitors. Protein kinase A (PKA), a prototype protein kinase and epresentative for the AGC group of Ser/Thr Kinases can be used as a model kinase to study the parameters of small molecule inhibitor binding and selectivity. We constructed a variety of PKA mutants with alterations in the variable portions of the ATP pocket, in order to reveal factors determining binding properties, to detect inhibitor binding associated conformational changes and to analyse the role of single amino acid residues of the binding site in inhibitor selectivity [1-3]. These mutants crystallize as easily as wildtype PKA, but offer certain ATP-site properties of related, pharmacologically important AGC kinases. Our goal is to solve and analyse a large number of high resolution crystal structures from such PKA mutants in complexes with small molecule protein kinase inhibitors of differing kinase specificity. This will improve our understanding of the parameters relevant for inhibitor binding and selectivity and provide a basis for the design of more selective kinase inhibitors for therapeutic purposes.
Various crystals from PKA wildtype and mutant proteins in complex with small molecule inhibitors were measured at Beamline BW6 and data collected between 1.39 and 2.2 Å resolution (Figure 1). The effect of changes in the inhibitor binding pocket on inhibitor binding was monitored by solving cocrystal structures of wildtype and increasingly mutated PKA subunits with the same set of inhibitor derivatives. Accordingly, changes in conformation were observed for sidechains as well as for larger structures within the protein. Most notably, specific polar and van der Waals interactions with the inhibitors differed. In combination with data on inhibition kinetics these results add to our understanding of how the selectivity of certain protein kinase inhibitors among related protein kinases is defined structurally.

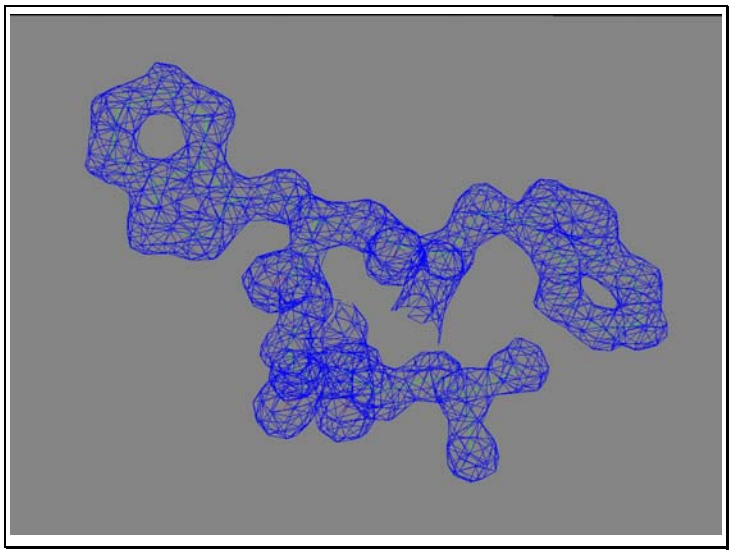

Figure 1: Part of the 2FoFc electron density map of PKA contoured at 2.0 Sigma

References

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