

**STRUCTURAL INSIGHTS INTO AGC-KINASE INHIBITOR BINDING
FROM STUDIES WITH THE WORKHORSE PKA**

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PKA is a prototype enzyme for the protein kinase family with respect to many functional and structural aspects. PKA also is a representative of the AGC-kinase subgroup which comprises many pharmacologically relevant members, such as PKB/AKT, PKC, ROCK, PDK1, SGK, GRK6 and others. Cross selectivity of small molecule protein kinase inhibitors within the group of AGC-kinases, however, is a commonly observed phenomenon. The close relationship and strong homology of the catalytic domains of kinases therefore suggests the use of wild type or mutated PKA enzymes as easily to be crystallised substitutes for other members of the AGC-kinase group in order to elucidate the binding properties of various protein kinase inhibitors. We solved crystal structures of PKA in complex with Rho-kinase inhibitors, such as Fasudil, H-1152P or Y-27632 [1]. Fasudil was the first protein kinase inhibitor which received clinical approval (1995 in Japan). H-1152P is a high affinity binding derivative of Fasudil with two additional methyl groups. The inhibitor binding site of Rho-kinase is unique in the kinase family and differs from that of PKA in four residues, which are involved in a large number of all inhibitor/enzyme contacts. In the case of H-1152P the inhibitor clashes with residues which have a larger spatial requirement in PKA, but are exchanged to smaller residues in Rho-kinase, explaining the stronger affinity of this inhibitor for Rho-kinase. In PKA, H-1152P also occupies an additional inhibitor binding site removed from the ATP-pocket, and contacts the activation loop and the helix C, two critical sites of kinase activity regulation.

Because of the close relationship of PKA and other AGC-kinases, some features of the ATP-binding pocket can be structurally mimicked by site-directed mutagenesis, as verified by the high convergence of PKA mutant and PKB crystal structures (Breitenlechner, C.B. *et al.* Crystal structures of the catalytic subunit of cAMP-dependent Protein kinase in complex with Rho-kinase inhibitors Y-27632, Fasudil (HA-1077) and H-1152P, submitted). Aspects of PKC selective inhibitor binding have been explored by cocrystal structures of bisindolyl maleimide compounds with mutants of PKA. In one of these structures PKA adopts the most open conformation observed so far for this enzyme, showing concomitant shortening of secondary structural elements, including helix C. Comparison of the bisindolyl maleimide and staurosporine inhibitor binding properties reveals significant differences with respect to

binding contacts, conformation of the inhibitor and induced conformational changes of the enzyme. Molecular modelling of the clinical phase III inhibitor LY333531 into the electron density of the bisindolyl maleimide molecule reveals features of its probable binding mechanism in accord with the differences of the catalytic sites of PKC and PKA and the chemical structures of the inhibitors (Gaßel, M. *et al.* Crystal structure of an altered catalytic subunit of cAMP-dependent protein kinase in complex with the PKC-kinase inhibitor bisindolyl-maleimide in two different conformations – implications for selectivity, in preparation).

REFERENCE

1. Gaßel, M., Breitenlechner, C.B., Rüger, P., Jucknischke, U., Schneider, T., Huber, R., Bossemeyer, D. and Engh, E.A. Mutants of protein kinase A that mimic the ATP binding site of protein kinase B (or AKT). **J. Mol. Biol.** (in press).