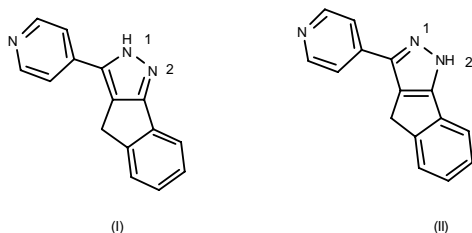


s8a.m1.p41 *De Novo Design and Protein X-ray Analysis of a Protein Kinase Inhibitor.* P. Furet, T. Meyer, A. Strauss, S. Raccuglia and J.-M. Rondeau' *Novartis Pharma Research, 4002 Basel (CH).*

Keywords: enzyme catalysis, protein engineering.

In pharmaceutical research, lead structures are still mainly identified by screening. When the three-dimensional structure of the target protein is known, however, this information can be used to design, *de novo*, molecules fitting the protein binding site. The molecules designed by molecular modeling can then be synthesized or retrieved from a database if they have already been made. A biological activity in the micromolar range or below warrants consideration as a potential lead structure.

Following this approach, we have identified PKF049-365 as a representative of a new class of inhibitors of the cell cycle kinases CDK1/2. The three-dimensional structure of the CDK2/PKF049-365 complex was subsequently determined by protein crystallography and refined to 1.53Å resolution. The X-ray analysis shows that PKF049-365 binds to the adenine pocket of the ATP binding site and interacts with the hinge region of the kinase molecule, as designed by molecular modeling. However, two overlapping - and hence mutually exclusive - binding modes are detected, which are characterized by a different pattern of hydrogen-bonded interactions with the main chain of the hinge region of the kinase. In the first binding mode, which corresponds exactly to the prediction of modeling, N2 of the pyrazole ring of PKF049-365 receives an H-bond from the amide nitrogen of Leu 83 and N1 donates an H-bond to the carbonyl oxygen of the same residue. In the second binding mode, N2 of the pyrazole ring donates a H-bond to the carbonyl oxygen of Glu 81, and N1 receives an H-bond from the amide nitrogen of Leu 83. Therefore, the observed binding modes involve two distinct tautomeric forms of PKF049-365, differing by the presence of a proton either on N1 or N2 of the pyrazole ring of the inhibitor. These two alternate binding modes appear to be equally populated. Furthermore, switching from one to the other binding mode only requires a small translation/rotation of the inhibitor molecule within the same plane and does not induce any significant structural rearrangement of the kinase.



Tautomeric Forms of PKF049-365

s8a.m1.p42 *Dihydroorotate dehydrogenases.* S. Nørager, O. Björnberg*, K.F. Jensen*, S. Larsen. *Centre for Crystallographic studies, Department of Chemistry, University of Copenhagen, Universitetsparken 5, DK-2100 Copenhagen Ø, Denmark.* **Institute for Molecular Biology, University of Copenhagen, Sølvgade 83H, DK-1307 Copenhagen K, Denmark.*

Keywords: dihydroorotate dehydrogenases, pyrimidine nucleotide biosynthesis, reaction mechanism.

Dihydroorotate dehydrogenases (DHODs) catalyse the oxidation of (S)-dihydroorotate to orotate in the *de novo* biosynthesis of pyrimidine nucleotides. The inhibition of DHODs provides potential targets for drugs used in the treatment of immune based diseases. All DHODs have FMN as a cofactor, which is involved in a hydride ion transfer from the substrate to the electron acceptor. Based on the amino acid sequence similarity, two major families of DHODs have by now been identified [1]. Cytosolic enzymes from Gram-positive bacteria belong to family 1, while family 2 consists of membrane associated enzymes from Gram-negative bacteria and eukaryotes. The catalytic active base in family 1 is a cysteine, while in family 2 enzymes this residue is replaced by a serine. Family 1 can be further subdivided into two sub-groups 1A and 1B. Family 1A enzymes use fumarate as natural electron acceptor, while family 1B use NAD⁺. Family 2 enzymes use long-chain ubiquinones as electron acceptors.

The structures of *Lactococcus lactis* DHODA (family 1A) and DHODB (family 1B) have previously been determined [2, 3] in the presence and absence of the reaction product orotate. And recently the structure of the *E.coli* DHOD (family 2) was established as a complex with orotate [4]. This has permitted us to perform a detailed comparison of the structures of the different families. The overall structure and the environment of their active sites are very similar for the three enzymes. Significant small differences are found which are important for the fine-tuning of the catalytic mechanism for each enzyme and adapting them to the use of their specific electron acceptor. Due to the large amount of structural and kinetic data gathered, we have been able to propose a common reaction mechanism for the first half reaction, the oxidation of dihydroorotate to orotate and the hydride transfer to FMN, taking into account the specificity of each enzyme.

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[2] P. Rowland, O. Björnberg, F. S. Nielsen, K. F. Jensen and S. Larsen. "The crystals structure of *Lactococcus lactis* dihydroorotate dehydrogenase A complexed with the reaction product throws light on its enzymatic functions." *Protein Science* (1998), 7, 1269-1279.

[3] P. Rowland, S. Nørager, K. F. Jensen and S. Larsen. "The crystals structure of *Lactococcus lactis* dihydroorotate dehydrogenase B : electron transfer between two different flavins bridged by an iron-sulphur cluster." (submitted).

[4] S. Nørager, O. Björnberg, K. F. Jensen and S. Larsen "Crystal structure of the *E.coli* dihydroorotate dehydrogenase and structural and functional relations between the different families of this class of enzymes." (submitted).