STI571 (IMATINIB): STRUCTURAL AND ENZYMATIC STUDIES OF INTERACTIONS WITH ABL KINASE AND RESISTANCE MUTANTS

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In chronic myelogenous leukemia (CML), a reciprocal balanced chromosomal translocation in haematopoietic stem cells produces the BCR-ABL hybrid gene, which encodes a constitutively activated kinase. STI571, marketed as imatinib, is a potent ATP-competitive inhibitor of c-Abi (IC50 188 nM). Despite the efficacy of the drug in stable-phase CML, in advanced leukemias, where there is a high frequency of DNA mutation, many patients relapse with point mutations in the kinase domain. We have determined the crystal structure of STI571 in complex with human Abi kinase (spacegroup P2221) and determined the inhibitory activity of STI571 towards many of the Abi kinase mutants. The structure shows that STI571 stabilises an inactive conformation of the protein, thus confirming Schindlers findings (Science 2000, 289:1938-1942) for murine Abi-kinase in complex with a structurally-related inhibitor (spacegroup P222). These studies allow us to rationalise the decreased sensitivity of mutant Bcr-Abi to STI571. Point mutations such as T315I in the ATP-binding site result in loss of a hydrogen bond and steric hindrance to the binding of STI571 (IC50 111) >10 µM). Mutations in the glycine-rich loop such as E255V (IC50 (STI571) 3.5 µM), E255K and Y253H (IC50 (STI571) >10 µM), destabilise the unusual conformation that this loop adopts in the inactive state, as does H396P in the activation loop. The variety and spread of these and other mutants makes it a challenge to design drugs to overcome resistance.

Keywords: KINASES DRUG DESIGN ONCOLOGY

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DISCOVERY OF A NEW BINDING POCKET OF FKBP52-N AND DESIGN OF TWO NOVEL NEUROTROPHIC LIGANDS OF FKBP12

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FKBP (FK506 binding protein) family is a kind of immunophilin possessing peptidylprolyl isomerase (PPlase) activity, which can be inhibited by the immunosuppressant drug FK506. FKBP52 is a high molecular mass immunophilin belonging to FKBP family. It can be divided into two PPlase domains, one tetratricopeptide repeats (TPR) domain, and one calmodulin binding domain. The first PPlase domain (FKBP52-I), which is homologous with FKBP12 is mainly responsible for the FK506 binding and the PPlase activity of the whole protein. Recent studies have showed that FKBP52, rather than FKBP12, can mediate the neurotrophic activity of FK506 and its analogues. The 2.5 Å structure of the FKBP52-I has been solved and given us more detailed insight into the function mechanism of the FKBP52-I and FKBP52 full-length protein. Most interestingly, besides the FK506 binding pocket, a new hydrophobic pocket is detected at the N-terminus, which does not exist in FKBP12. This pocket is a potential new binding site of small molecules and other proteins related with FKBP12 for drug design. FKBP12 is now well known to be a target protein related with the nerve regeneration. Ligands that bind to the protein FKBP12 have attracted attention in recent years due to their neuroregenerative abilities. Aided by the structure-based drug design, two novel FK506 analogues have been synthesized. Both of them showed higher affinity to FKBP12 and much better neurotrophic effection than GPI-1046, which is undergoing second phase clinical test as a neurotrophic drug. Furthermore, two 1.8 Å crystal structures of human FKBP12 complexed with the two ligands respectively have been determined, which gave a strong structure proof at atomic level to our neurotrophic ligands design.

Keywords: FKBP NEUROTROPHIC DRUG

THE STRUCTURE OF AN ALDOSE-REDUCTASE INHIBITOR COMPLEX SHOWS FLEXIBILITY OF THE INHIBITORS CARBOXYLATE AS A FUNCTION OF pH

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Aldose Reductase, an enzyme involved in diabetes complications, is the target of an extended effort in inhibitor development. Previous work has showed the existence of an anchoring point in the active site to which the carbonylate head of inhibitors can be bound. The carbonyl group mimics the transient enolate state of the substrate, and binds the two catalytic residues, Tyr 48 and His 110. Different orientiations of the carbonylate have been observed, depending on the pH of the crystallisation conditions and on the inhibitor. In each previous case, the carbonylate has been well ordered, especially in the complex that was crystallized at pH 5 and that led to a subatomic resolution (0.66 Å) structure. We have obtained crystals of the same complex at pH 8 as well, and solved the structure at 1.5 Å resolution. The conformations of most of the inhibitor are very close to each other at pH 5 and pH 8 but clearly shows two alternative conformations of the carbonylate head. These conformations correspond to optimising the hydrogen bond geometry with the His 110 and the Tyr 48 respectively, and could correspond to alternative catalytic mechanisms in which the proton donor is either His10 or Tyr48.

Keywords: ENZYME INHIBITION DRUG DESIGN MACROMOLECULAR CRYSTALLOGRAPHY

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The support of Drug Discovery

Mitochondrial Cyclophilin D (CypD) belongs to a family of cyclophilin-A binding proteins, which catalyses rotation about prolyl peptide bonds. Structures of the three other types of mammalian cyclophilins (A, B and C) are known, but crystallization of CypD has not been reported yet. A truncated version of the three other types of mammalian cyclophilins (A, B and C) are known, but crystallization of CypD has not been reported yet. A truncated version of CypD has now been crystallized and purified to homogeneity. The protein shows a monodisperse size distribution in dynamic light scattering, but crystallization trials gave no crystals. Therefore, site-directed mutagenesis was used to obtain a variant of CypD suitable for crystallization and structure determination. A series of mutants was selected such that crystal contacts seen in the X-ray structures of CypA would be restored or the unusually high solubility and isoelectric point (pI) of 10.3 destabilise the unusual conformation that this loop adopts in the inactive state, as does H396P in the activation loop. The variety and spread of these and other mutants makes it a challenge to design drugs to overcome resistance.

Keywords: DRUG DESIGN LEAD Finding CRYSTAL ENGINEERING

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The structure proof at atomic level to our neurotrophic ligands design.