

05-Molecular Modelling and Design for Proteins and Drugs

isozymes, are widely used in the treatment of several types of physiological disorders. Individual data sets for three sulfonamides, namely, Acetazolamide, Methazolamide and Amsulf complexed to Human Carbonic Anhydrase I (HCAI) enzyme were collected using both conventional X-ray generators with photographic films and Photon factory synchrotron source with image plates. Starting from the refined phases of the native HCAI model without the solvent molecules, the structures of the three complexes were refined at 2Å resolution to R-factors of 0.179, 0.186 and 0.192 respectively using molecular dynamics / simulated annealing method of XPLOR. These refined structures show differences in the orientations of the sulfamido group of the drug molecules bound to the essential Zinc ion in the active site of the enzyme. The aromatic ring of these molecules is always found to be sandwiched between the active site loop region residues Leu198 and His200 resulting thereby in large shifts of the imidazole ring and indicating thereby the importance of this loop in the catalytic activity / inhibition of this enzyme. Since differences in the orientation of the sulfamido group indicate corresponding differences in the inhibitory power of these drugs, correlation of inhibition constants with the structures was investigated on the basis of computed energetics of sulfonamide-HCAI interactions. From the force fields used for refinement above, the various energy terms were estimated for the refined structures of the drug complexes. A series of twenty different thiophene/benzene based sulfonamide drug molecules with known inhibition constants for HCAI were docked into the active site of HCAI using TOM/FRODO package on IRIS-4D/20 graphics workstation after anchoring the sulfamido and the aromatic groups approximately around the positions found from the three refined structures above. These starting models were energy minimised using different trial force fields in XPLOR. The set of parameters which resulted in models of the drug molecules having minimum differences with the common parts of the refined structures was accepted as the correct one for the corresponding model complex. Various energy terms were computed again from these reparametrized force fields for each of the model complexes and variations of these terms plotted against the nature of the substituents on the aromatic rings. These were then correlated with the known inhibition constants of the corresponding drug molecules, details of which will be discussed.

PS-05.03.17 SUCCESSFUL RATIONAL SELECTIVE INHIBITION OF A TRYPANOSOMAL ENZYME

Christophe L.M.J. Verlinde and Wim G.J. Hol*

The Biomolecular Structure Program, Department of Biological Structure, University of Washington, SM-20, Seattle, Washington 98195, USA

The bloodstream form of the trypanosome, the causative agent of sleeping sickness, relies upon glycolysis to the stage of pyruvate as its sole source of energy supply. Therefore, the trypanosomal

glycolytic enzymes are attractive targets for designing selective inhibitors which should obviously exhibit minimal affinity for the equivalent enzymes of the human host. The design of selective active site inhibitors is difficult because the active site of an enzyme is usually well conserved in the course of evolution. In contrast, selective inhibition may be easier in case an enzyme makes use of a large cofactor. A substantial part of such a cofactor is not directly involved in the catalytic reaction, and as a consequence, its environment is less conserved. We wondered whether the adenosine part of NAD would be a good lead for trypanosomal glyceraldehyde phosphate dehydrogenase (GAPDH) and inhibitor design.

Careful comparison of the *Trypanosoma brucei* (*T.b.*) GAPDH structure, obtained from Laue diffraction study at 3.2 Å, and a 2.4 Å resolution human GAPDH structure reveals: (1) the presence of a small hydrophobic pocket next to the adenine C2-position in *T.b.* GAPDH versus the presence of Asn in human GAPDH; (2) the proximity of a hydrophobic canyon next to O2' of the adenosine ribose in *T.b.* GAPDH versus this region being occupied by protein atoms in human GAPDH due to a different loop conformation; (3) the presence of a hydrophobic patch near the adenine C8-position in both *T.b.* and human GAPDH.

After one design-synthesis-testing cycle starting from adenosine the following preliminary results were obtained: (1) a 2-methyl adenine substituent improves inhibition 43-fold in *T.b.* and 16-fold in human GAPDH; (2) 2'-benzamide-2'-deoxy-adenosine selectively inhibits parasite GAPDH and shows a 16-fold better inhibition; (3) an 8-thienyl substituent enhances inhibition 100-fold for parasite GAPDH and 20-fold for mammalian GAPDH. More recently, a meta-methoxy benzamide derivative of 2'-deoxy-2'-amino adenosine appeared to have a 170-fold *higher* affinity for the parasite enzyme and a 3-fold *lower* affinity for the human enzyme, compared to adenosine. In one step selectivity was hence improved over 500-fold.

We would like to thank our colleagues for their numerous contributions to this work. Fred Opperdoes, Paul Michels, Mia Callens and colleagues in Brussels for providing protein material and carrying out kinetic studies; Fred Vellieux, now in Grenoble, and Randy Read, now in Edmonton, for their X-ray studies leading to the three-dimensional structures of GAPDH; and Piet Herdewijn and Arthur van Oirschot for the synthesis of the new inhibitors.

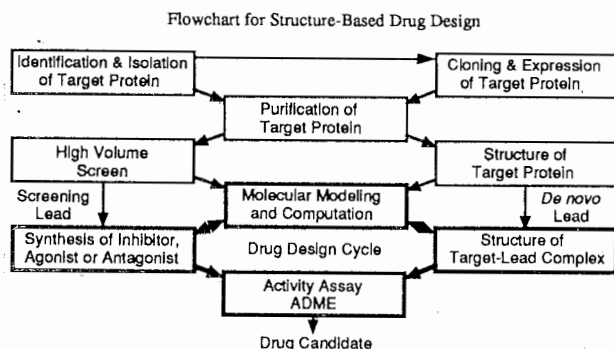
PS-05.03.18 STRUCTURE-BASED DRUG DESIGN IN THE PHARMACEUTICAL INDUSTRY: SOME LESSONS LEARNED. Noel D. Jones, Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285-0403, USA.

Since 1980 nearly fifty pharmaceutical companies worldwide have established protein crystallography laboratories, most with an emphasis on structure-based drug design. This iterative process uses experimentally determined or modeled structures of macromolecules and their complexes with potential drug molecules as a guide to the design of enzyme inhibitors and receptor agonists and antagonists. The goal is to develop tighter binding, more selective drugs with improved pharmacokinetics. Several companies are now entering clinical trials with compounds derived from this process.

Experience in several laboratories indicates that: (1) Structure-based drug design reduces substantially but does not replace the need for traditional screening and SAR studies, (2) Improving the properties of lead compounds obtained from screening is generally more efficient than *de novo* drug design, (3) Rapid and early access to enzyme or receptor assay and ADME data is crucial, (4) It is more productive to start with leads which already have acceptable pharmacokinetics and then improve their binding and selectivity, (5) Success in structure-based drug design and the speed with which a drug candidate can be developed depend greatly on the degree of integration and interaction between scientists performing the functions shown in the flowchart above.

05-Molecular Modelling and Design for Proteins and Drugs

163



PS-05.03.19 INHIBITOR DESIGN FOR HUMAN RHEUMATOID ARTHITIC SYNOVIAL FLUID PHOSPHOLIPASE A₂. By Qiaolin Deng*, Luhua Lai, Yu Luo and Xiaojie Xu, Department of Chemistry, Peking University, Beijing, China

It is known that the phospholipases A₂ (PLA₂) associates with localized and systemic inflammatory process and tissue injury. In synovial fluids of patients with rheumatoid arthritis, high level of soluble PLA₂ (s-PLA₂) was detected. We used structure-based method to design the inhibitors of s-PLA₂.

The crystal structure data of bovine pancreatic PLA₂ (1BP2, 2.5Å) and *Crotalus atrox* PLA₂ (1PP2, 1.7Å) were used as the basis to construct the 3-D structure of s-PLA₂ by applying homology protein structure prediction program developed in this laboratory. The sequence of s-PLA₂ has about 37% homology with that of 1BP2 and 44% homology with that of 1PP2. After energy minimization with CHARMM, DOCK program developed by I. D. Kuntz was used to search the CSD for molecules which have structural complementarity to this enzyme. According to their spatial complementarity and chemical properties, several molecules were chosen as the inhibitor templates. The synthesis and activity tests are under development.

PS-05.03.20 STRUCTURAL STUDIES OF DEXMEDETOMIDINE HYDROCHLORIDE, A NEW DRUG SUBSTANCE. By R. Rajala and E. Laine*, Department of Physics, University of Turku, Finland.

Dexmedetomidine hydrochloride (Recommended International Non-proprietary Name, Rec.INN), (+)-(S)-4-[1-(2,3-dimethylphenyl)ethyl]-1H-imidazole hydrochloride is a novel, selective α₂-adrenoceptor agonist synthesized by Orion Corporation Farnos, Oulu, Finland. Dexmedetomidine hydrochloride appears as a white or

almost white crystalline powder. The molecular weight, true density, melting range of dexmedetomidine hydrochloride are 236.7, 1.17 grams per cubic centimetre and 156.5 - 157.5 °C, respectively.

The preliminary crystal structure determination of dexmedetomidine hydrochloride has been carried out. The crystal belongs to the monoclinic space group P2₁ with cell dimensions a = 7.851(2) Å, b = 7.463(2) Å, c = 11.596(2) Å and β = 98.29(2)°. The diffraction data has been collected on Rigaku AFC5S diffractometer with graphite monochromated MoKα radiation at 23°C. The molecular structure and packing in the unit cell have been solved by using matrix least-squares refinement to an R-value of 6.8% with 870 observed reflections. All the calculations have been performed by using the TEXSAN crystallographic software package by Molecular Structure Corporation.

Dexmedetomidine hydrochloride appears in two different forms which represent the anhydrous (dry) and the hydrous (humid) form. The dry-humid structure transition of dexmedetomidine hydrochloride proved to be thermally reversible. The effect of moisture on the crystal structure of dexmedetomidine hydrochloride has been studied by using Philips PW1820, APD1700 automated powder diffractometer system with heating and humidity chamber and Perkin-Elmer DSC-7 differential scanning calorimeter.