04–Crystallography of Biological Small Molecules

04.01 – Molecular Structure and Biological Activity

MS–04.01.01 THE STRUCTURES OF TRANSMEMBRANE CHANNELS. B.A. Wallace*, D. Doyle, B. Hussain-Bates, and R.W. Janes. Department of Crystallography, Birkbeck College, University of London, London WC1E 7HX, U.K.

Ion transport across biological cell membranes is an area in which structural studies can play an important role. To this end, the availability of a molecule for which high resolution crystallographic studies can be correlated with functioal properties, is needed. Gramicidin, a 16 residue hydrophobic polypeptide synthesized by *B. brevis*, is an ideal model system. Crystallographic studies of its complexes with ions have given us insight into the nature of the interactions between the polypeptide backbone and the transported ligand. Studies of its complexes with lipids give us insight into the nature of its interactions within the membrane. A high resolution structure of the complex of gramicidin with caesium chloride has been determined. A number of other caesium complexes have now been found to produce other crystals forms which have caesiums at different sites along the length of the pore; these structures give rise to a series of "snapshot" pictures of the ion being transported, which are forming the basis of our molecular simulation studies. Recent studies have examined complexes between gramicidin and other monovalent cations of different sizes, and of divalent cations. All of these crystallographic analyses are complemented by our spectroscopic studies which have examined the dynamics of the interactions of gramicidin with ions of different sizes. Thus, this relatively small molecule which acts as an ion channel provides the most complete data to date on structure/function relations for ion transport in membranes. (This work has been supported by grants from the U.S. National Science Foundation and the S.E.R.C. of the U.K.).

MS–04.01.02 STRUCTURAL STUDIES ON BIOACTIVE PEPTIDES. By G. Précigoux*, S. Llido, S. Geoffre and P. Picard, Laboratoire de Cristallographie, Université de Bordeaux I, 33405 Talence, France.

Among the bioactive peptides, one of the widely studied families is constituted of the aspartyl protease inhibitors.

There are two strategies for the design of such inhibitors: the replacement of the scissile peptide bond of a substrate with other nonhydrolysable moities, or the substitution of a usual endogenic aminoacid by an unusual one.

All the aspartic proteases are known to be inhibited by pepstatin A (isovaleryl - Val - Val - Sta - Ala - Sta), where (Sta) is [(4S,3S)-4-amino-3-hydroxy-6-methylheptanoic acid]. Statine has been found to be essential for inhibitory potency of pepstatin and is widely used in the design of inhibitors.

In spite of the great interest of statine, only a limited number of X-ray diffraction studies has been carried out on statine alone and on statine containing peptides. However, the number of conformations observed in the crystal state is large enough to allow a study aimed at determining the main conformational preferences of statine and the conformational role of its two additional main chain carbon atoms.

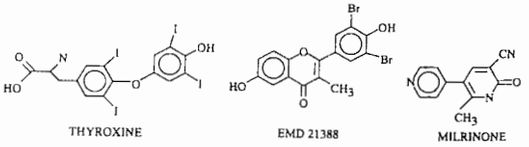
MS–04.01.03 DESIGN, STRUCTURE AND ACTIVITY OF CONFORMATIONALLY SPECIFIC PEPTIDES. By T.P. Singh, Department of Biophysics, All India Institute of Medical Sciences, New Delhi -10029, India.

α,β -dehydro-amino acids have emerged as a very effective tool in the design of specific peptide structures. These residues occur naturally in a variety of peptide antibiotics and in some proteins. The peptides can be prepared in the laboratory with substitutions of α,β-dehydro-residues at desired sites. Our investigations suggest that a dehydro-residue adopts three sets of site specific ϕ,ψ values: $80,0^{\circ}$ if dehydro-residue is at (i+2) position, $-60, 140^{\circ}$ while at (i+1) and $\pm 60 \pm 30^{\circ}$ in a sequence of dehydro-residues separated by one or two saturated residues. Therefore, a β-turn II, β-turn III and a 3_{10} -helical conformations can be produced very specifically. The dehydro-Ala with only methylene group at the C^{β}-position adopts an extended chain conformation and in a peptide sequence gives rise to a mixed β-strand structure similar to those observed in large loops of proteins. These studies, thus, offer a highly promising and effective principle of peptide design.

MS–04.01.04 MOLECULAR STRUCTURE AND BIOLOGICAL ACTIVITY: TRANSTHYRETIN-INHIBITOR BINDING INTERACTIONS AS A TARGET SITE MODEL. Vivian Cody, Medical Foundation of Buffalo, 73 High St., Buffalo, NY 14203 USA.

Recent structure activity data show that many pharmacological agents are strong competitors for thyroxine (T_4) binding to transthyretin (TTR), a serum thyroid hormone transport protein. Furthermore, the marked similarity in the structural features required for relative binding affinity to TTR and activity of thyroid-responsive enzymes such as iodothyronine deiodinase (ITD), Ca^{2+}-ATPase or membrane T_3 transporter suggests homology between the TTR hormone binding site and these enzyme active sites. To understand how diverse classes of molecules such as iodothyronine analogues, plant flavones, inotropic bipyridines and benzodiazepines can act as inhibitors of TTR binding, computer graphic modeling studies of inhibitor structures were carried out. Crystallographic analysis of thyroid hormones reveals that the tyrosyl 3,5-iodines cause the diphenyl ether to adopt a skewed conformation, whereas removal of this bulk releases this constraint. Flavonoids, a broadly distributed class of hydroxy substituted phenyl benzopyrones or benzofurones plant pigments, are also potent inhibitors of TTR hormone binding and ITD activity. Although these structures have less conformational flexibility and are in general planar, computer graphics modeling data suggest homology between the hormone phenolic ring and that of the flavones and reveal that the flavones can bind in the TTR hormone site. From these studies the bromoflavone, EMD 21388, was designed as a potent ITD and TTR inhibitor. To test this model, the structures of TTR-flavone complexes were undertaken and reveal a complex binding pattern which indicates the flavones have multiple binding modes to TTR. Milrinone (2-methyl-5-cyano(3,4'-bipyridin)-6-(1H)-one) and amrin-

one, its desmethyl 5-amino parent compound, are members of a new class of oral nonglycosidic cardiac positive inotropic agents developed for the treatment of congestive heart failure. Structure activity correlations reveal that milrinone, but not amrinone, stimulates rabbit myocardial Ca^{2+}-ATPase activity as does thyroxine. To further develop this correlation, competive binding studies were carried out which showed that in a similar manner, milrinone, but not amrinone, was a strong competitor for T_4 binding to TTR. Comparison of a series of bipyridine structures revealed homology between the phenolic ring of T_4 and the substituted ring of the bipyridine and a model which defined the conformational features required for activity was developed. The crystal structure of the milrinone-TTR complex was carried out and these data confirmed the model. Structure activity data for a membrane bound T_3 transporter showed that benzodiazepine derivatives were potent inhibitors of T_3 transport. Conformational comparison of their structures revealed homology with T_3 and resulted in a model which incorporates key features of the benzodiazepine subclasses. Thus, these analyses revealed that TTR can be used as a prototypic model to explain the relative potency of these enzyme inhibitors. Based on these results, inhibitors can be designed with selective actions at their respective target sites.



THYROXINE EMD 21388 MILRINONE

Supported in part by DK 494001 and Boots Pharmaceuticals.

MS–04.01.05 Structural Determination of Natural Products. By Angela Y. Lee and Jon Clardy, Department of Chemistry, Cornell University, Ithaca, N.Y. 14853-1301, USA.

Natural products have been a wonderful source of chemically novel and biologically active molecules, but they have also been a severe technical challenge to crystallographers. Most of our research has involved characterizing a variety of biologically active compounds, and more recently, their complexes with cellular receptors. The ultimate goal is to understand not only the structure but also the biochemical function of these compounds. We have worked with marine peptides for many years, and recently have become interested in a novel cyclic peptide from a marine sponge called cyclotheonamide. We never worked on this structure directly because of the inability to grow crystals. Cyclotheonamide is a serine protease inhibitor with K_I ~0.2 nM with trypsin and ~1.0 nM with thrombin. The three-dimensional structure of cyclotheonamide A complexed with bovine β-trypsin has been successfully determined at 2.3 Å resolution. This reveals not only the structure of cyclotheonamide but also its mode of action. The strong electron density and low thermal parameters allowed the conformation of bound cyclotheonamide A to be determined unambiguously—a result that has also clarified the stereochemistry.

Key words: natural product, novel protease inhibitor, mechanistic studies.

MS–04.01.06 POLYMORPHYSM AND BIOINEQUIVALENCE OF 6-METHYLURACIL. By N.B.Leonidov*, S.I.Uspenskaya, Institute "Bioeffect" of Ministry of Science, High School and Technical Policy of Russian Federation. Moscow, Russia; P.M.Zorky & A.E.Masunov, Chemical Department, Moscow State University, Russia.

New perspective trend in drug design is obtaining polymorphic modifications with different molecular conformations. Under consideration has been influence of polymorphism of drugs on their biological activity. Examples of influence of conformational polymorphism of drugs on changes in its biological characteristics in solutions are given. One of the explanations of this phenomenon is the difference between molecular agglomerates which are present in the crystals. The cooperative effect of molecular packing can fasten the details of molecular conformation. After dissolution of a substance such agglomarates or some fragments of them can occur in the solution for relatively long period of time. Specifically two crystal forms of 6-methyluracil differ in the antioxydative and wound-healing effect of their solutions.

X-ray data show that the cyclic dimers formed by a pair of NH...O=C bonds occur in both of them. However, in the form I the dimers are uniteed by single H-bonds and wavy layers arise as a result. In the form II the dimers are joined into ribbons using pairs of H-bonds. It is very likely that hydrated dimers predominate in the solution of the crystals I, but rather long fragments of ribbons occur in the solution of the crystal II. Slightly different molecular conformations are to exist in the agglomerates of these two types. Thus, we obtain a probable explanation of the biological inequivalence of two polymorphs.

MS–04.01.07 A STUDY ON STRUCTURE-ACTIVITY RELATIONSHIPS IN 16- AND 17-SUBSTITUTED ESTRANES AND ANDROSTANES. By S. Stankovic*[1], D. Miljkovic[1], R. Kovacevic[1], D. Lazar[1], Lj. Medic-Mijacevic[2], V. Pejanovic[2] and Ch. Courseille[3], [1]Faculty of Sciences, University of Novi Sad, Trg D. Obradovica 4, 21000 Novi Sad, Yugoslavia, [2]ICN Galenika Institute, 29. novembra 111, 11000 Beograd, Yugoslavia, [3]Laboratoire de Cristallographie et de Phisique Cristalline, Faculté de Scienes, Université de Bordeaux I, Talance, France.

A series of synthetic estranes (1-5) and androstanes (6-9) have been prepared and tested for anti-estrogenic and anti-androgenic properties. These steroids have been subjected to X-ray structural analysis to permit structure-activity relationship studies.

In the first series (1-5) a novel chemical rearrangement during acid-catalized hydrolysis of 16-oximino to 16-carbonyl group has been noticed. Namely, the benzyl moiety at C-17 changes its orientation from α- to β-position. The new β-orientation of the benzyl moiety affects the chemical and biological properties of the C-17 centre.

Among the compounds from the first type (1-3) an expected phenomenon was noticed: β-orientation of the C-17 hydroxyl group produced higher biological activity. The compounds from the second type (7-9) showed differences in biological activity. Namely, the benzyl-derivative 7 possesses an anti-estrogenic activity, while the corresponding phenyl analogue 9 showed no activity at all. The phenyl analogue 8, with 17-OH group, showed in vitro an inhibitory effect on steroidogenesis.