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Crystallography in Drug Design*

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Crystallography is traditionally one of the monitoring methods used in pharmaceutical industry. X-ray powder diffraction allows fast analysis of individual chemicals and their mixtures. Crystallography considers the habit of a given compound and its internal structure (crystalline, amorphous), and it gives a detailed description of polymorphism, molecular adducts, enantiomorphism, *etc.* Hazards of teratogenic activity of some drugs led to the inclusion of molecular structure, as determined by X-ray methods, into the list of standard characteristics demanded by the Drug Master Files.

More recently, crystallography has also taken an active role in drug design. Atomic coordinates of thousands of bioactive molecules are now available from the Cambridge Structural Databank, Protein Data Bank and other files. This information is a good starting point for mechanistic drug design, especially if the structures of the effector and of the target are already known on the atomic level. However, structural information about the target molecule is frequently unavailable. In the case of enzyme-target, advantage can be taken of the fact that enzymes with similar functions often retain similar structural features and catalytic sites and may be modelled by using the structural information from a known enzyme. Receptor-target molecules represent a more difficult problem because they are larger and frequently membrane-associated. However, a knowledge of the effector structure is a good tool for mapping an active site of the receptor. Crystallography, in combination with other methods (NMR, IR, 3-D graphics, *etc.*) makes it possible to deal with such an ambitious problem in this field.

INTRODUCTION

The very first drugs were natural products whose effects were detected by observation. As an example, we can mention aspirin or acetylsalicylate, present in several plants, or the antimalarial drug, artemisinin, used already 2000 years ago. Next step in drug design was based on clinical experience and pharmacological modelling.

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A characteristic feature of this phase was a good knowledge of the active agent. However, no information about the structure of the target molecule was available. The last period in drug design utilized the knowledge of the structures of target proteins or related macromolecules. Knowledge of the structures of nucleic acids, proteins and related receptors plays a decisive role in drug design. A combination of the use of structural databases, based on X-ray crystallography, with highly sophisticated graphics, potential energy calculation and QSAR is a very potent tool in the drug design of today. The X-ray structure is now an obligatory part of documentation of any new drug.[1]

The need for new drugs is indicated by several factors. One is the negative response, in terms of side effects, of the patient to a lasting use of the same pharmaceutical. Viruses can easily adapt to a drug agents and change their defensive system. Due to the changing environment, our population is exposed to new diseases and/or modified old diseases, assumed to have been overcome. This is a challenge to pharmaceutical companies to develop new drugs utilizing the most advanced research and technology (R&D) available. Increasing demands on the quality and safety of new products have a significant influence on the period of testing and the price of any new product. Vagelos[2] shows that, on average, it takes 12 years, from synthesys to regulatory clearance, to bring a prescription drug to market. The average cost, which includes discovery and development, of a prescription medicine is US$ 231 million. Creation of a new drug involves more than 10 000 compounds to be studied at the beginning. Only 20 of them enter animal studies and 10 enter clinical (human) trials – but only one gaines final approval.

Crystallography, in close cooperation with other branches of science, can significantly contribute to the speeding up of R&D in this area and lowering of drug cost.

DATABASES AND COMMUNICATION

R&D is always based on information. The exponential growth of papers, monographs and industrial reports requires sophisticated storage, processing and dissemination. Printed material rapidly gets obsolete. For example, the current content of Chemical Abstracts contain more than 11 million entries with an increment of 0.5 million/year. The full edition spans one room in a library. Therefore, the computer is the only solution and most of the datafiles are available in computer-readable form now.

The Powder Diffraction File (PDF) on CD-ROM medium can store up to 680 megabytes of data, an equivalent to more than 150 000 pages of text. The main product of the International Centre for Diffraction Data, the PDF-2 Database,[3] is a collection of 58 000 single-phase X-ray powder diffraaction patterns with an increment of 2 000 entries/year.

The Cambridge Structural Database (CSD)[4] currently holds information on over 100 000 organo-carbon crystal structures with an increment of 6 000 entries/year. Search systems have been devised that employ the graphical language of chemistry for query construction and for display of hits. The system also permits a systematic analysis of geometric structure, a proces leading to acquisition of new structural knowledge from the data accumulated in the CSD. Studies of conformational preferences, mapping of structural interconversions and reaction pathways and their relation to the potential energy hypersurface, as well as systematic studies of hydrogen-bonded and non-bonded interactions, are supported by the system.

The Protein Data Bank (PDB)[5] is a collection of more than 2 000 structures of biological macromolecules with an increment of 100 structures/year. It is available on magnetic tape and CD-ROM. The database has been incorporated into a number of commercial molecular graphics, simulation, databases, and computer-assisted molecular design packages.

The Nucleic Acid Database (NDB)[6] is a relation database, designed to facilitate retrieval of nucleic acid structures with specific primary or derived structural features. Currently, the NDB consists of tables describing 215 DNA, RNA, and *t*-RNA structures contained in the database. The NDB includes coordinates for 149 of these structures. Approx. 50 new entries are included every year.

Quick access to 3-D structural information is vital for molecular modelling. This requires specially designed databases and database management systems, which allow an interactive search and retrieval of substructural information on both small molecules and macromolecules. Müller and coworkers[7] have developed such a system, known as ROCSD (Roche Cambridge Structural Data Base) and ROPDB (Roche Protein Structural Data Base).

Kabsch and Sanders[8] have introduced a system for pattern recognition of H-bonding and geometrical features operating on PDB.

A side-chain rotamer library, published by Ponder & Richards,[9] is used for the analysis of tertiary templates for proteins.

The database ISIS is a system developed under the auspices of the SERC Protein Engineering Club to bring together the protein structure[10] and sequence data. Several novel approaches have been taken to accomodate the structural data in a form which is more generally useful than the PDB files.[11]

Another relational database is SESAM,[12] which contains structures and sequences of macromolecules. Presently, it is implemented within the commercial package SYBASE. The database allows full integration of raw data on protein structure, sequence, ligands, and heterogroups, obtained from the PDB, with pure sequence information available from other databanks, such as SWISS-PROT.

Prestrelski & Liebman[13] described a library of conformationally equivalent structure fragments. The library contains features that reproduce the major secondary structure classes and define conformations previously described only as random or undefined conformations.

Crystallographic data may be accessed and retrieved from databases, file transfer archives and electronic bulletin boards. The most popular way of communication among academic institutions is the e-mail which passes through all networks and which may be used to adress a query to a server.

Standard protocols through the global Internet have given rise to several tools for drawing together library resources and various archive sites.[14,15]

Gopher provides a hierarchy of menus alllowing structured access to data categories. Each entry in the menu may represent data stored in a different machine, anywhere in the world. The PDB ftp (file transfer protocol) site can be accessed via Gopher allowing retrieval of complete structure files.

The WAIS (Wide-Area Information Server) system is a standard way of indexing the textual content of files stored at an archive site. WAIS can be used to supply index service to a data collection initially accessed through Gopher. Also PDB is accessible *via* this server.

Despite the data boom registered in various structural data banks, there are many researches that keep secret the data and results on which their scientific conclusions are based. In the case of structure laboratories springing up in many pharmaceutical companies, there is a strong incentive to keep information secret to cash in on its commercial value. Frustration over difficulty in obtaining coordinates drove Rossmann & Argos to devise and publish a computer program designed to extract coordinate information from the stereo drawings that are often included in structure papers. The advent of such programs has driven some researchers to obscure their results even further, generating structure drawings too small and snarled to be easily interpreted.[17] Moreover, some really »hot« areas are kept completely closed to publication, even if the dead end has been reached. This problem is a global one and is currently being considered by ICSU.[18]

A similar story can be traced in the world of programs for data retrieval, processing and system dedicated to computer-aided molecular modelling (CAMM) and, particularly, drug design (CADD). Besides free program systems[19] like AMBER, there are utility programs which are part of rented databases (CAMAL, *etc.* for CSD)[20,21] and company-produced commercial systems (INSIGHT, SYBYL, QUANTA). However, most pharmaceutical companies have their own systems, which are kept secret and priced in millions of US$.

CRYSTAL STRUCTURE

Crystal Habits

The performance of different drugs depends on, among other parameters, the habit and crystalline modifications of active drugs. The same drug produced by two different companies can significantly differ in its properties, such as solubility, bioavailability, stability, and possibility of tabletting, despite the fact that both have the same melting point and the same X-ray pattern. Crystal habit, the outer appearance of the crystal, plays a significant role here. We can distinguish two basic classes: (a) anhedral (allotriomorphic) which are irregularly shaped, and (b) euhedral (idiomorphic) which are bound by plane faces. In this group, we find tabular, platy, prismatic, needle-like (acicular), blade crystals, and others. Factors that may affect crystal habits are the supersaturation of the mother liquor, the rate of cooling and the degree of solution agitation, the nature of crystallizing solvent, the presence of cosolutes, cosolvents and adsorbable foreign ions, and the constancy of conditions.[22]

Crystal habits may influence several pharmaceutical characteristics. For example, a suspenssion of plate-shaped crystals may be injected through a small needle with greater ease than that of needle-shaped crystals of the same overall dimensions. Similarly, the tabletting behaviour is influenced by crystal habits by way of the powder material texture. In the same way dissolution of drug crystals and bioavailability of the drug may be function of crystal habits.

Internal Structure

(a) Amorphus form can be prepared by lyophilization, rapid quenching, precipitation, and other techniques. Amorphous drugs show thermodynamic instability and tend to revert to a more stable form. As an example, we can mention novobicin[23] which is ten times more soluble in amorphous form than in crystalline form. Similarly, injectable prompt insulin zinc suspension USP, which consists of amorphous

insulin complex, is readily absorbed when injected and has a relatively short duration of action. Crystalline, extended insulin zinc suspension USP is absorbed very slowly and has a longer duration of action. The size of the particles also plays a role here. Extended insulin is made up of large particles, and *vice versa*. Properties of the final insulin product can be tuned up by mixing these two crystallographically different forms.[24]

(b) Single-entity crystalline solid-polymorphism very frequently occurs in pharmaceuticals. For example, barbiturates are about 70% polymorphic. More than 42 polymorphic steroid hormones and 30 polymorphic sulfonamides[25] have been published. X-ray single crystal structure determination revealed absolute configuration of many frequently used drugs. For example, *p*-chlorophenol[26,27] exists in alpha-form (stable modification) and in beta-form (metastable modification). A similar situation was found for estrone[28] and chloramphenicol palmitate.[29,30] The very popular aspirin[31,32] is at least dimorphic. Polymorphism influences the tabletting behaviour of powders, physical and chemical stability of the drugs and biochemical availability. Tagamet (cimetidine), an antiulcer drug can be produced in four modifications. However, only the alpha modification, soluble in stomach, is effective.[33]

(c) Stoichiometric adducts-solvates form another group of important drugs. Estradiol forms solvates with more than 30 solvents.[34] Many antibiotic-antibacterials can also form solvates, *e.g.* erythromycin,[35] chloramphenicol,[36] *etc.* The dissolution rate of a solvate may be many times greater than that of the anhydrous form. On the other hand, the dissolution rate of some hydrates is less than that of the anhydrous form. These properties also control the bioavailability.

(d) Nonstoichiometric inclusion compounds like channel, layer and cage (clathrate) compounds are of great importance. There are several areas in pharmaceutical industry where clathrates have a potential application. They can be used for purification (benzene contaminated with thiophene),[37] separation of rare gases (argon from neon),[38] separation of optical isomers (tri-*o*-thymotide),[39] storage of inert gases (hydroquinone),[40,41] handling of dangerous materials (dimethylmercury),[42] and control of the action of anesthetics.[43,44]

X-ray diffraction is one of the best methods available for differentiating crystalline modifications like polymorphs, solvates or clathrates.

MOLECULAR STRUCTURE

Perun and Propst[45] described the situation in drug design saying that, with general acceptance of the molecular basis of a disease, we have come to realization that the disease process can be understood at the chemical level and, consequently, the disease can be interrupted chemically. This has led to the mechanistic approach in the develoment of drugs to treat diseases.

The mechanistic approach to drug discovery begins with the knowledge of the desease process itself. It also requires that we know chemical structures of the interacting molecules. Since substrates, ligands, or drugs that mimic them (effectors) and their enzymes or receptors (targets) interact *via* a look-and-key type mechanism, knowledge of their 3-D structures is of critical importance. Once these are known, scientists can begin to design new chemical entities to influence the targets involved in the desease process.

Structure of Small Molecules

Solution of the 3-D structure of a single compound is the very first step. An important situation appears when an asymmetric carbon atom is present in the molecule. A warning case is the thalidomide (CONTERGAN), a sedative from the early sixties. It was responsible for thousands of fetal malformations in Europe. The pharmaceutical marketed was a mixture of left- and right-handed, mirror image molecules. The *S*-(-)-enantiomer is teratogenic, causing malformation, whereas its mirror image, *R*-(+)-enantiomer, is not. Had this been known at that time, great anguish and human loss could have been prevented.[46,47]

However, more information is available when series of similar structures are retrieved from crystallographic structural databases. Cluster analysis, principal component analysis and other statistical methods support our effort to find a pharmacophore, *i.e.* the geometric arrangement of key atoms or groups responsible for their biochemical activity.[48,49] Further modification can increase the effectiveness of the lead drug and suppress its unwanted side effects. Experimental work is supported here by methods based on computer-generated 3-D structures.[50-55] Scatterplots of hits in a relevant parameter space show variability in the geometry of the molecular fragment under study. Flexible molecules, for example, are not appropriate for mapping drug receptors. Moreover, in some cases, we can visualize the reaction pathways describing conformational interconversion and/or chemical reactions.[56,57]

Structure of Macromolecules

Targets for mechanistic drug design usually fall into three categories: enzymes, receptors, and nucleic acids. A knowledge of their structure is fundamental for further drug design. Molecular structures of proteins and nucleic acids are systematically studied by X-ray methods since increasing number of such compounds are prepared in crystalline form. Also, the advent of computer-aided molecular modelling (CAMM) methods can be observed in this area. The long standing theory that 3-D protein structure is totally determined by the amino acid sequence is supported by recent experimental results. As an example, we can mention the syntheses of two forms of human immunodeficiency virus ((HIV) protease – one using naturally occurring *L* amino acids, and the other using non-natural *D* amino acids. The two enzymes turned out to be structural mirror images of each another.

Based on this principle, computerized methods have been designed for construction of secondary and/or tertiary structures of proteins from the amino acid sequence given by the primary structure.[59-63]

However, more reliable methods are based on the detection of common 3-D substructures of already known protein structures and the structure under investigation.[64-70]

Drug – Macromolecular Target Interaction

There are four possible situations depending on our knowledge of the 3-D structure of both drug and target.

First, we should mention the case when macromolecular structure information is available. Methods of computer manipulation, like docking and intercalation, are of frequent use here.[71-81] For example, Lavery *et al.*[82] published a paper on DNA mi-

nor groove binders such as netropsin. Detailed modelling structures with ruthenium triphenanthrolines is given by Haworth *et. al.*[83]

Enzymes as targets have been extensively studied in the last decades. Just for illustration, we can mention dihydrofolate reductase,[84,85] angiotensin-coverting enzyme,[86] chymatrypsin,[87,88] and serine proteinase.[89]

A more challenging case is the situation when no 3-D structures of the target and not even of the drug are available. Then, we have to start with the diagram of biological pathway and choose which step, such as synthesis of a particular reagent, we aim to block. Then the energy profile of the biochemical reaction must be calculated. From these calculations, transition states or intermediates must be specified. As catalytic enzymes lower transition states, the stable transition state analogues should bind to and inhibit the enzyme and, thus, disrupt the biochemistry.

Once the structure of transition state is specified, we need to design a stable molecule that »looks like« the transient structure. A combination of methods, based on CAMD, QSAR and crystallographic structural databases, is necessary here.[45,85,90,91]

CONCLUSION

The scientific effort in pharmaceutical R&D is remarkable. To illustrate the status of R&D in this area we can mention that the Merck Co., (approx. 5% worldwide market share) pays more than 4500 people to do research. The company's spending[2] for R&D over the 20-years span rose up from an index of 100 in 1969 to more than 1200 in 1989. Academic research, located at many universities and non-profit institutions, also contributes to this area of medicine.

Crystallographic community is getting increasingly engaged in biochemistry and, especially, in drug design. At the 15-th Congress of the IUCr (Bordeaux, 1990) approx. 20 % of all the contributions were dedicated to bioscience. At the 16-th Congress of the IUCr (Beijing, 1993) 24% of microsymposial contributions and 42% of main lectures were bio-oriented.

Specific academic environment is a very fruitful ground for fundamental research also in areas not of immediate market interest. International scientific centres, and close cooperation of scientists around the globe is our response to the social needs of millions of humans.

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SAŽETAK

Kristalografija u oblikovanju lijekova

Karel Huml

Rendgenska difrakcija na praškastim uzorcima omogućuje brzu identifikaciju spojeva i smjesa. Difrakcija pak rentgenskih zraka na monokristalima odraz je svojstava i strukture spoja te može pružiti potanki opis polimorfizma, enantiomorfizma i dr. (npr. podaci o molekulskim aduktima). Kako bi se spriječilo nepoželjno teratogeno djelovanje nekih lijekova, za Drug Master Files uz uobičajene karakteristike svakog lijeka zahtijevaju se i podaci o molekulskoj strukturi određeni metodom rentgenske strukturne analize. Od nedavno, kristalografija ima također aktivnu ulogu u oblikovanju lijekova. Atomske koordinate struktura tisuća bioaktivnih

molekula dostupne su preko Cambridge Structural Databank, Protein Data Bank i drugih banaka podataka. Te su informacije dobra polazna točka za oblikovanje lijekova, posebno ako su poznate molekulske strukture aktivne male molekule kao i makromolekulske komponente. Međutim, vrlo često struktura makromolekule nije poznata. Ako je vezna makromolekula enzim, moguće je pri modeliranju koristiti se spoznajom, da enzimi sličnih funkcija imaju i analognu strukturu uključujući i katalitičko mjesto. Upravo receptori predstavljaju najsloženije slučajeve, jer su kompleksni i često vezani uz membrane. Poznavanje strukture aktivne male molekule vrlo je koristan pribor u prepoznavanju aktivnog mjesta receptora.

Kristalografija u kombinaciji s drugim metodama (NMR, IR, 3-dimenzijska grafika i dr. čine ovaj ambiciozan pothvat mogućim).