Main Lectures

ML–03.02 VIRUS STRUCTURE AND VIRUS INFECTIVITY. By M. G. Rossmann[1]*, P. R. Kolatkar[1], M. A. Oliveira[1], R. Zhao[1], T. S. Baker[1], N. H. Olson[1], R. H. Cheng[1] and J. M. Greve[2]; [1]Department of Biological Sciences, Purdue University, West Lafayette, IN 47907, USA; [2]Institute for Molecular Biologicals, Pharmaceutical Division, Miles, Inc., West Haven, CT 06516-4175.

A brief description of the crystallographic techniques used to determine virus structure and a short review of known virus structures will be followed by a presentation of recent results on the interaction of rhinoviruses with their receptors.

Cryoelectron microscopy has been used to determine the structure of human rhinovirus 16 complexed with its cellular receptor, intercellular adhesion molecule-1. The receptor binds into a 12 Å deep "canyon" on the viral surface, confirming the prediction that the viral-receptor attachment site is in a cavity inaccessible to the host's antibodies. The atomic structure of human rhinovirus 16 and the structure of CD4 (homologous to intercellular adhesion molecule-1) have both been determined and could, therefore, be fitted into the cryoelectron microscopy density, thus establishing the interacting amino acids in the virus-receptor complex.

A pocket close to the site of receptor attachment can contain a cellular cofactor which can be replaced by antiviral agents. These antiviral compounds can inhibit viral attachment or uncoating. The overlapping binding sites of receptor and cofactor regulate the ability of a stabilized form of the virus to be transmitted between cells or for a destabilized form to enter the cell and thereby initiate infection.

ML–03.03 CRYSTALLOGRAPHYOF RIBOSOMES

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Ribosomes are the universal cell organelles responsible for the translation of the genetic code into proteins. A typical bacterial ribosome contains more than a quarter of a million atoms and is of a molecular weight of 2.3 million daltons. It sediments with a coefficient of 70S and is composed of 3 chains of RNA, of a total of about 5500 nucleotides, and about 57 different proteins. These are arranged in two independent subunits of unequal size (1.45 and 0.85 million daltons) which associate upon initiation of protein biosynthesis.

Intensive systematic exploration of crystallization conditions, combined with individual seeding, led to reproducible formation of crystals of ribosomes, their complexes with components of protein biosynthesis, and natural, mutated and chemically modified (with an undecagold cluster) subunits. In all cases the crystallization conditions are chosen to be as close as possible to the natural environment of the ribosomes, and the crystalline ribosomal particles retain their integrity and biological activity for long periods in spite of the natural tendency of ribosomes to disintegrate and in contrast to the short life time of isolated ribosomes in solution. The most suitable crystals are of ribosomal particles from extreme halophilic and thermophilic bacteria. The highest resolution obtained so far is 2.9 Å.

The large unit cell dimensions, the extremely weak diffracting power, the relatively large mosaicity and the shape of the crystals (very thin plates or needles) dictates the performance of all steps in X-ray crystallographic analysis with intense and highly collimated synchrotron radiation. At ambient temperature, all ribosomal crystals decay upon the first instance of X-irradiation. To overcome the severe sensitivity of these crystals to the X-ray beam, we developed cryo data collection techniques. These involve the determination of appropriate freezing conditions for each crystal form, shock cooling in liquid propane at liquid nitrogen temperature and data collection from crystals at about 90 K. Under these condition the crystals can be irradiated and stored for periods long enough for the collection of more than one data set.

The strategy of data collection and evaluation, the specific problem of these experiments and the quality of the results are addressed separately (Agmon et al., this volume). In general, the crystallographic data of these crystals is of a reasonable quality, with R merge(I) = 5-9% and adequate completeness.

A monofunctional reagent was prepared from a multi-heavy-atom cluster, an undecagold to which a very short, rigid and non-chiral arm has been attached. This was used for quantitative specific binding to an isolated ribosomal protein which was, in turn, reconstituted into a mutated ribosome, lacking this protein. The modified particles have been crystallized, and crystallographic of reasonable quality have been collected. Biochemical procedures for detaching selective ribosomal proteins from non-denatured ribosomal cores, for reconstitution of halophilic and thermophilic crystallizable ribosomes are currently in progress. In parallel, genetic procedures are being exploited for the insertion of exposed SH- moieties on the surfaces of these ribosomes.

This cluster was also attached to base 47 on $tRNA^{phe}$ from E. coli. The derivatized molecule was visualized by cryo-STEM electron microscope. It was shown that this modification does not impair the amino-acylation of the tRNA and allows its binding to ribosome and to small ribosomal subunits. We aim to used the modified tRNA for phasing crystals of complexes of ribosomes mimicking distinct states in protein biosynthesis (containing mRNA and tRNA), some of which have been crystallized.

Approximate models have been reconstructed at 30-47 Å, using diffraction information obtained from tilt series of two-dimensional sheets examined by electron-microscopy. These show the overall shape of the ribosome as well as separation between the two subunits; the presumed location for protein biosynthesis; a possible binding site for m- and t-RNA and a tunnel which may be the exit path of the nascent protein chain. These models led to further biochemical studies, focusing on the fate and the initial folding of nascent proteins and of homopolypeptides. These, in turn, stimulated modelling attempts.

The reconstructed models were also used for initial phasing at low resolution. Represented as pseudomolecules, they were employed in rotation and translation searches as well as anchoring features in maps constructed from X-ray or neutron crystallographic data, phased by non-MIR methods. These led to a few distinct packing arrangements, some of which could be further refined as rigid-bodies or exposed to solvent flattening techniques.

ML–04.01 CRYSTALLOGRAPHIC ENVIRONMENT AS AN APPROACH TO MOLECULAR RECOGNITION AND DRUG-DESIGN. By Claudine Pascard I.C.S.N.-C.N.R.S. Gif-sur-Yvette, France

The molecular recognition process involved in drug-design has been extensively studied over the last ten years. Thus, the structural information contained in large libraries such as the Cambridge Structural Database has been crucial in the process of finding new "lead" molecules. When the receptor site is known (by the X-ray structure determination of an inhibitor-enzyme complex), the aim is to find a new ligand complementary with the binding site. With an unknown receptor, the strategy is to identify the pharmacophore, to use its stereo and physicochemical features for mapping the receptor site, and a search is then made for other ligands using recently developped 3D-search programs, in conjunction with crystallographic data banks. Examples of these strategies which have accelerated the research for more potent drugs will be presented.

However, crystallographic results must not be reduced to a source of three-dimensional coordinates. The solid state medium is as rich in structural informations as both the solution state and *ab initio* calculations. Very valuable informations on desirable active conformation can be retrieved from various crystal structures of a given family of active compounds, wherein the different interactions molecule-solvant, and/or molecule-ion can be compared. These points will be discussed.

