02.8-1 CRYSTALLOGRAPHY OF INTACT RIBOSOMAL PARTICLES.

A. Yonath* **, K.S. Bartels**, F. Frolow*, H. Hope***, C. Kratky****, I. Makowski*****, S. Weinstein* and H.G. Wittmann*****.

*Weizmann Inst., Israel; ** Max Planck Research Unit, Hamburg, FRG; *** U. of California at Davis; **** U. of Graz, Austria and ***** Max Planck Inst., West Berlin.

Ribosomes are distinct assemblies of protein and RNA chains, on which protein biosynthesis occurs in all organisms. Full functional understanding of this process still requires a detailed molecular model.
Only active ribosomal particles crystallize. In all cases, particles from dissolved crystals are active even after several months, in contrast to the short lifetime of isolated ribosomes.
The best crystals are of the large (50S) ribosomal subunit. These particles have no internal symmetry and consist of about 32 different proteins and two RNA chains, with total molecular weight of 1.6×10^6 . The largest crystals are of 50S particles from (I) Halobacterium marismortui (0.6x0.6x0.1mm) and (II) Bacillus stearothermophilus (1.5x0.3x0.2mm). Using synchrotron radiation, cell dimensions of (I): 369x680x920Å ($P2_12_12_1$) and of (II): 214x300x584 ($C222_1$) have been determined.
Although fresh crystals of (II) diffract to 5.5Å, the high resolution terms are lost after irradiation of 2-3 minutes with synchrotron beam, at -2°C. However, for crystals immersed in an inert hydrocarbon drop, mounted on glass rods and exposed to synchrotron beam for 2-3 days at cryotemperatures (i.e. 85°K) no radiation damage was observed. Thus a full data set could be collected from a single crystal.
A mutant (which lacks protein L11) of (II) was obtained. The mutated 50S subunits crystallize isomorphously with the native particles. Protein L11 from the wild type can be reconstituted into the mutated ribosomes. A heavy atom cluster, undecagold, with diameter of 8.5Å was used for derivation by soaking and a full data set of this derivative was collected. This cluster was also modified to contain one chemically active group. It, as well as a radioactive mode compound, N-ethylmaleimide, can be covalently attached to 50S particles or to isolated ribosomal proteins through free sulfhydryl groups.

Three-dimensional reconstruction studies performed at 28Å on ordered arrays of 50S particles from B. stearothermophilus resulted in a model which contains several projecting arms arranged around a cleft, which turns into a tunnel (up to 25Å diameter, 100-120Å long). This tunnel may provide the exit path for the nascent polypeptide chain. A similar image reconstruction study, performed on 70S ribosomes from the same source at 47Å resolution, shows clearly the separation between the two subunits and the location at which the protein biosynthesis reaction takes place.

02.9-1 CRYSTALS OF GRAMICIDIN A. By B.A. Wallace, Department of Chemistry and Center for Biophysics, Rensselear Polytechnic Institute, Troy, New York 12180-3590, United States of America.

Gramicidin A is a linear polypeptide antibiotic that forms ion channels in phospholipid membranes. It crystallizes in a number of different forms in the presence and absence of lipid and/or monovalent cations of various sizes. In the absence of lipid and ions, it crystallizes in a monoclinic form ($P2_1$, a=15.2, b=26.7, c=31.7) from methanol and an orthorhombic form ($P2_12_12_1$, a=24.8, b=32.4, c=32.7) from ethanol. In the presence of cesium it crystallizes in a twinned form ($C222_1$) and in an orthorhombic single crystal form ($P2_12_12_1$, a=32.1, b=52.1, c=31.2) (Kimball and Wallace, 1984). Using single wavelength anomalous scattering from the cesium ions for phasing, we have calculated a 1.8 Å resolution map for the latter form, built a preliminary model, and are in the process of refining the structure (Wallace and Hendrickson, in prep). The molecule in these crystals forms a cylinder with a column of cesium and chloride ions complexing with the peptide backbone that lines the 4.4 Å diameter pore. The structure is of a left-handed antiparallel intertwined double helix with beta-sheet-like hydrogen bonding and a superhelical twist with 6.3 residues per turn. A different crystal form ($P2_122$, a=32.8, b=27.5, c=26.8) is prepared in the presence of lipid molecules, and appears to contain a 2:1 lipid-to-gramicidin monomer complex (Wallace, 1986). The solvent content of these crystals is very low, and the lipid molecules appear to be highly ordered in the crystals. The space group is compatible with a bilayer motif for the lipids. A 1.5 Å native data set have been collected on this crystal form and attempts to phase the data by anomalous scattering from the lipid phosphates MIR, and molecular replacement with calculated models are underway. (Supported by NSF Grants DMB85-17866 and DMB 87-96205.

02.9-2 STUDIES OF STRUCTURAL MODIFICATIONS INDUCED γ-IRRADIATION ON DISTEAROYLPHOSPHATIDYLCHOLINE LIPOSOMES.

G.Albertini°, E.Fanelli°, L.Guidoni*, F.Ianzini*, P.Mariani°, R.Masella+, F.Rustichelli°, V.Viti*.

° Istituto di Fisica Medica, Facoltà di Medicina e Chirurgia, Università Ancona, Italy.
o Dipartimento di Scienze Chimiche, Università di Camerino, Italy.
* Laboratorio di Fisica, Istituto Superiore di Sanità, Roma, Italy.
+ Laboratorio di Metabolismo e Biochimica Patologica, Istituto Superiore di Sanità, Roma, Italy.

This paper reports an investigation of the structural and thermodynamical modifications induced by γ-irradiation on model membranes. Differential scanning calorimetry and X-ray diffraction were used to study the different phases and associated transitions of distearoylphosphatidylcholine multilamellar liposomes after ^{60}Co γ-irradiation. Changes were observed in the shape of calorimetric peaks and in the corresponding phase transition temperatures. In particular the appearing of a shoulder was observed at about 20 kGy. The three phases characteristic of lecithins with identical acyl chains were detected also for the highest value of irradiation dose. The formation of lysolecithin and stearic acid upon phospolipid degradation was observed. The lysolecithin concentration increases as a function of irradiation dose, until a saturation value is reached for a dose of 40 kGy. These results correlate quite well with