02.8-1 CRYSTALLOGRAPHY OF INTACT RIBOSOMAL PARTICLES.

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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.

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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

those obtained for interlayer and interchain distances and for the width of the main phase transition calorimetric peak. At the highest dose (~80 kGy) molecules of cross linked adjacent radicals and other molecular species are also formed. Appreciable differencies, with some similarities, were observed in the behaviour of DSPC and DPPC liposomes under γ-irradiation.

02.10-1 CALCULATION OF THE VIBRATIONS OF HELICAL STRUCTURES IN POLYPEPTIDES.

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In the last few years there has appeared a number of experimental reports and theoretical analyses concerning the low-frequency motions in biomacromolecules. Brown et al. /1/ observed that low-frequency Raman bands exist in certain proteins, and these vibrations appeared to be sensitive to the conformation of a protein. Among the component elements of protein molecules helices occupy a prominent position.

For the theoretical investigation of vibrations of helical structures in proteins, we have used a molecular mechanics procedure to calculate the total energy including the energy contributions of the $C_i{=}O_i \ldots H_{i+n}{-}N_{i+n}$ (n = 3, 4) H-bonds of a polyglycine helix model depending on the dihedral angles φ, ψ forming regular helical structures. We have additionally calculated the following geometrical parameters of this helix model depending on φ-, ψ-angles:

(i) the length per residue of the helix - 2SH (Å),

(ii) the angle of winding of the helix - WDG (deg.),

(iii) the H-bond distances $d_n{=}O_i \ldots H_{i+n}$ (n = 3, 4),

(iv) the H-bond angles $\omega_n{=}C_i{=}O_i \ldots H_{i+n}$ (n = 3, 4) and

(v) the H-bond angles $\eta_n{=}O_i \ldots H_{i+n}{-}N_{i+n}$ (n = 3, 4).

The calculated energy surface allows to define a "low energy pathway" (LEP) in the φ,ψ-plane along which the vibrations of the regular polyglycine helix can take place. The changes of all the geometrical helical parameters (i)-(v) along the LEP enable us to give the following interpretation of the vibration of this helix model. The vibration of the helix can be divided into two regions along the LEP.

In the first region from A (φ_A = -72.0°, ψ_A = -17.0°) up to B (φ_B = -71.5°, ψ_B = -32.5°) on the LEP the helix performs a longitudinal and a torsional vibration.

In the second region from B up to C (φ_C = -33.5°, ψ_C = -71.5°) on the LEP the helix changes only the steric orientation of the peptides relative to the helix axis depending on the alteration of φ-, ψ-angles (and in this connection, changes of the H-bond geometry). But there is no longitudinal and only a minor torsional vibration in this second region.

/1/ Brown, K.G., Erfurth, S.C., Small, E.W. & Perticolas, W.L.L. (1972) Proc. Natl. Acad. Sci. U.S.A. 69, 1467-1469.

02.10-2 CATALYSIS IN THE CRYSTAL: SYNCHROTRON RADIATION STUDIES WITH GLYCOGEN PHOSPHORYLASE b.

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Direct observation of the progress of a catalysed reaction in crystals of glycogen phosphorylase b has been made possible through fast crystallographic data collection achieved at the Synchrotron Radiation source at Daresbury. In the best experiments, data to 2.7Å resolution (some 108,300 measurements; 21,200 unique reflections) were measured in 25 mins. In a series of time resolved studies in which the control properties of the enzyme were exploited in order to slow down the reaction, the conversion of heptenitol to heptulose-2-phosphate, the phosphorylysis of maltoheptaose to yield glucose-1-phosphate and the oligosaccharide synthesis reaction involving maltotriose and glucose-1-phosphate have been monitored in the crystal. Changes in electron density in the difference Fourier maps are observed as the reaction proceeds not only at the catalytic site but also the allosteric and glycogen storage sites. Phosphorylase b is present in the crystals in the T state and under these conditions exhibits low affinity for both phosphate and oligosaccharide substrates. However there are pronounced conformational changes associated with the formation and binding of the high affinity deadend product, heptulose-2-phosphate, which show that movement of an arginine residue, Arg569, is critical for formation of the substrate-phosphate recognition site. Recent results from the refinement of the ligand complexes will be discussed.