15. Towards a molecular model for the large ribosome particle

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Abstract

Large crystals of 50S ribosomal subunits, diffracting to 6Å have been grown. Crystal of the same particles from a mutant which lacks protein L11 are isomorphous to those of the wild type. An undecagold cluster was prepared and bound to a -SH group on isolated protein L11. This cluster was also used for soaking experiments. Two-dimensional sheets from 50S ribosomal subunits have been subjected to three-dimensional image reconstruction studies at 30Å resolution. The resulting model contains several projecting arms, arranged radially around a cleft which turns into a tunnel which may be the path taken by nascent protein.

Crystallographic Studies

To shed light on the role of ribosomes in protein biosynthesis, we undertook crystallographic studies. As an object for such studies, ribosomal particles are of enormous size, with no internal symmetry. Nevertheless, procedures for *in vitro* growth of crystals of intact ribosomal particles have been developed. These led to the production of crystals and sheets of whole ribosomes (Wittmann et al, 1982, Piefke et al, 1986) as well as of 50S ribosomal subunits (Yonath et al, 1984, 1986a,b, Yonath and Wittmann, 1987, Arad et al, 1984,

Makowski et al, 1987). Only active particles could be crystallized and the crystalline material retains its biological activity for long periods, in contrast to the short life time of isolated ribosomes. The best crystals are of 50S ribosomal subunits from Halobacterium marismortui and Bacillus stearothermophilus. The 50S particles are of molecular weight of 1,600,000 daltons with approximate size of 150x170x180 $Å^3$. They are composed of 30-35 different proteins and 2 RNA chains (Wittmann, 1983). Synchrotron radiation is essential for crystallographic studies of these crystals due to their fragility, their short life time and the large unit cell dimensions.

Halophilic ribosomal particles are stable and active at high salt concentrations. Taking advantage of the fact that these ribosomes maintain their activity over a wide range of salt concentrations, as well as of the major role played by the Mg^{++} in crystallization of ribosomal particles (Arad et al, 1984), we could obtain large, well ordered crystals from the 50S ribosomal particles from H. Marismortui. These (0.6x-0.6x0.2mm^3) are monoclinic ($P2_1$), diffract to 6Å (Fig. 1), and have relatively small, compactly packed unit cells of 182x-186x584 Å, $\gamma = 109^o$ (Makowski et al, 1987). Although up to 15 rotation photographs can be taken from an individual crystal, the high resolution terms appear only on the first diffraction patterns. Hence over 260 crystals were irradiated in order to obtain a complete data set.

Because ribosomes from eubacteria fall apart at high salt concentrations, they were crystallized from organic solvents. Currently crystals of the 50S subunits of B. stearothermophilus (2.0x0.3x0.3 mm) are obtained directly in X-ray capillaries, at 4^oC, from mixtures of methanol and ethylene glycol (Yonath et al, 1984, 1986a,b). The crystals of space group $P2_12_12$ are loosely packed in a unit cell of 360x680x921 Å. Since most of the crystals adhere to the walls of the capillaries in different directions, it was possible to obtain

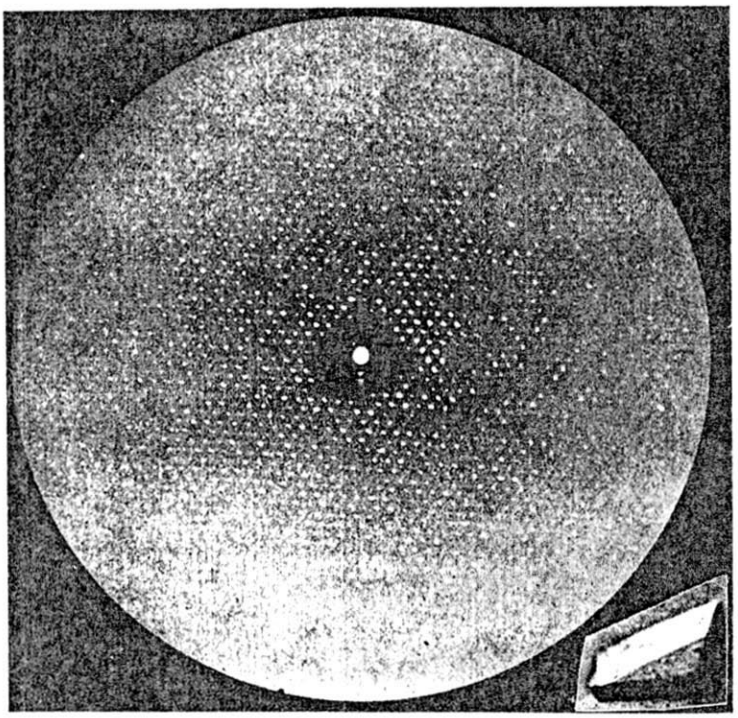

Figure 1. A 1° rotation pattern from the crystal in the insert obtained in 2 min. at 4°C with synchrotron radiation {(XII/EMBL/DESY) operating at 5 GeV, 34 mA. λ=1.51Å; slit collimation (0.3x0.3mm^2), distance=235mm}. Insert: a crystal of the 50S subunits of H. marismortui (Makowski et al, 1987).

diffraction patterns from all zones without manipulation. For an object as large as the 50S ribosomal subunit, it is necessary to use extremely heavy and dense compounds for derivation. An example is a gold cluster:

$$Au_{11}(CN)_3[P(C_6H_4CH_2NH_2)_3]_7$$

with a diameter of 8.5Å (Yang and Frey, 1984). Preliminary experiments show that crystals of H. marismortui soaked in this cluster are isomorphous to the native crystals and their X-ray patterns show intensity differences.

Because the surface of the particles has a variety of

potential binding sites, in parallel to soaking experiments attempts to bind the cluster covalently to the particles are in progress. A model compound radioactive N-ethylmaleimide was interacted with exposed -SH groups on the entire 50S ribosomal subunit. For both bacteria only two ribosomal proteins participted in the reactions (Weinstein, unpublished). A mutant of B. stearothermophilus which lacks protein L11 was obtained. Crystals of the 50S mutated ribosomal subunits are isomorphous to those of the wild-type (Yonath et al, 1986b). Protein L11 has only one sulfhydryl group to which the cluster, perpared as a monofunctional reagent, was bound.

Three Dimensional Image Reconstruction

The large size of ribosomal particles, which is an obstacle for crystallographic studies, permits direct investigation by electron microscopy. Two-dimensional sheets from 50S ribosomal subunits from B. stearothermophilus (148x370 Å γ = 109°) have been subjected to reconstruction studies at 30 Å resolution (Yonath et al, 1987). The resulting model (Fig. 2) shows several projecting arms arranged radially around the presumed interface with the 30S subunit. A cleft is formed between the projecting arms. This turns into a tunnel of a diameter of up to 25 Å and a length of 120 Å. The tunnel branches, forming a Y shape. An elongated region of low sensitivity was also detected in ribosomes from lizard (Milligam and Unwin, 1986).

The functional role of the tunnel is still to be determined. However, originating at the presumed site for actual protein biosynthesis, terminating on the other end of the particle, (Bernabeau and Lake, 1982), and being of a diameter large enough to accomodate every amino acid and of a length which could provide protection to a 40mer peptide in an extended conformation (Malkin and Rich, 1967, Blobel and Sabatine, 1970, Smith et al, 1978), this tunnel appears to be the path

taken by nascent protein. The reconstructed model shows higher detail than the image seen by electron microscopy due to the inherently more objective character of the analysis by diffraction methods. However, it can be positioned so that its projected view resembles the latter (Fig. 2).

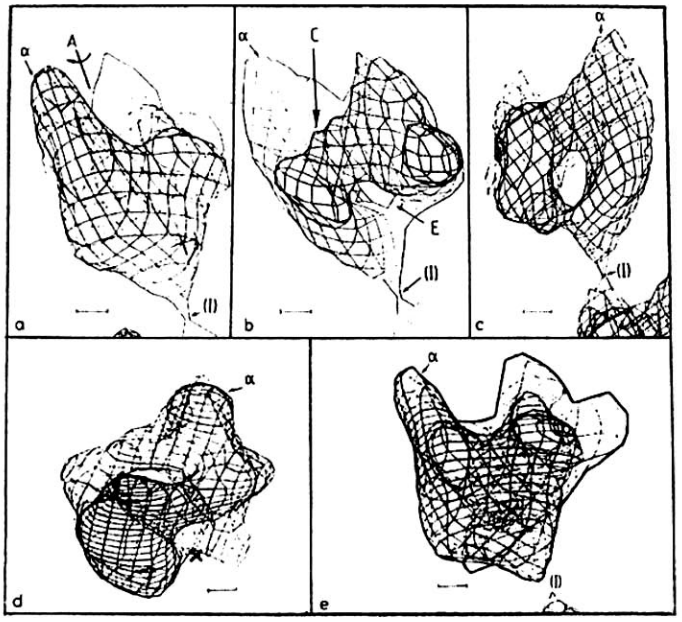

Figure 2. Computer graphics display of the outline of the reconstructed model of the 50S ribosomal subunit at 30 Å resolution. Heavy lines are in front, lighter ones in back. Image (a): A side view of the model, (I) is the contact between the two particles; (A) the approximate axis around which the model was turned to obtain the view in (b). Image (b): (C) is the cleft between the projecting arms, at the site it turns into the tunnel. The exit point of the tunnel is marked (E). Image (c) is a view into the branch of the tunnel from the exit point, (d) a view into the tunnel from the cleft. In image (e) the model is viewed in a projection which resembles images vizualized by electron microscopy.

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