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Characterization of Crystals of Small Ribosomal Subunits

Crystals of intact small ribosomal subunits from *Thermus thermophilus* have been obtained from functionally active particles. The crystals ($P42_12$, 407 Å × 407 Å × 171 Å) are suitable for X-ray crystallography analysis to 9·9 Å using synchrotron radiation at cryo-temperature. Crystallographic data from native and a potential heavy-atom derivative have been collected.

During recent years we have crystallized intact ribosomal particles from several bacterial sources. Initially, well-shaped crystals were obtained from large (50 S) ribosomal subunits (Yonath *et al.*, 1980, 1988; Yonath & Wittmann, 1988) as well as from whole (70 S) ribosomal particles (Wittmann *et al.*, 1982; Glotz *et al.*, 1987). Of these only the crystals from the large subunits were found suitable for crystallographic analysis (Yonath *et al.*, 1984, 1986*a*,*b*, 1988; Makowski *et al.*, 1987; Yonath & Wittmann, 1988). Only very recently, could we (Glotz *et al.*, 1987; Yonath *et al.*, 1988) as well as others (Trakhanov *et al.*, 1987) grow microcrystals of the small (30 S) ribosomal subunits. Furthermore, as a result of an intensive systematic effort, we were able to refine the conditions for preparation of ribosomal particles suitable for crystallization. Consequently, we are now able to grow medium-sized crystals of 30 S ribosomal subunits from *Thermus thermophilus* that are suitable for X-ray crystallographic studies. These crystals were obtained from active particles similarly to the other crystals of ribosomal particles grown in our laboratory (Yonath *et al.*, 1988; Yonath & Wittmann, 1988), and the crystalline material retained its integrity and activity.

The crystals grew spontaneously or by seeding at 4 °C or at 19 °C from methylpentanediol (MPD†), in hanging drops. Like all other crystals of ribosomal particles they have at least one very thin edge. They appear as wide needles and reach a size of 0·3 mm × 0·07 mm × 0·07 mm (Fig. 1). Using the available, not-most-intense synchrotron beam, their diffraction patterns contained reflections with Bragg resolutions of 9·9 Å (1 Å = 0·1 nm).

Crystallographic data have been collected photographically using a collimated synchrotron beam at cryo-temperature, employing a procedure similar to that described recently (Hope, 1988) but with a few modifications (Hope *et al.*, 1988). Before cooling, the crystals were immersed in a solution containing all the components present in their mother liquor, but with a higher viscosity. This was obtained by raising the concentration of MPD from 15% (in which the crystals were kept) to 30%. After soaking for a few minutes the crystals were placed between the two glass films of a double-layer glass spatula (the "sandwich spatula"), which was soldered to a brass pin (Fig. 2) that could be mounted on a goniometer head. The spatula with the crystal was instantaneously cooled in liquid propane, and transferred to the X-ray rotation camera on which the crystals were kept surrounded by a stream of nitrogen gas at a temperature of around 90 K throughout the entire period of data collection.

As observed for other crystals of ribosomal particles, at this temperature there was hardly any radiation damage for days (Hope *et al.*, 1988). Consequently, a complete data set could be collected from a single crystal. To date we have collected two data sets from native crystals (to 20 and 12 Å resolution, Fig. 3) and one set (to 18 Å) of a potential heavy-atom derivative, from a crystal soaked in a 1 mM solution of a tetrairidium cluster (S. Weinstein & W. Jahn, unpublished results).

The 30 S subunits are packed in crystals with tetragonal ($P42_12$) symmetry, with unit cells of dimensions of 407 Å × 407 Å × 171 Å. The shape and size of the small ribosomal subunits from bacteria have been extensively studied by electron microscopy as well as by neutron and light scattering (for reviews, see Wittmann, 1983; Hardesty & Kramer, 1986). Furthermore, as a result of our three-dimensional image-reconstruction studies, we are now able to suggest an approximate "envelope" for the 30 S subunit (Z. Berkovitch-Yellin, D. Schuler, A. Yonath & H. G. Wittmann, unpublished results). This was obtained by subtracting the 30 Å reconstructed image of the 50 S subunit (Yonath *et al.*, 1987) from that of the 70 S ribosome reconstructed at 47 Å (Arad *et al.*, 1987; Yonath & Wittmann, 1988). On the basis of this information, which gives a reliable approximation of the size of the 30 S subunit, and assuming that about 50% of the volume of the crystals is occupied by the solvent, we conclude that each asymmetric unit contains two particles. For a molecular weight of 700,000 V_m is around 2·5 Å^3/dalton. This value is close to the values calculated for crystals of other large nucleoprotein structures, such as viruses (Hogle, 1982),

† Abbreviation used: MPD, methylpentanediol.

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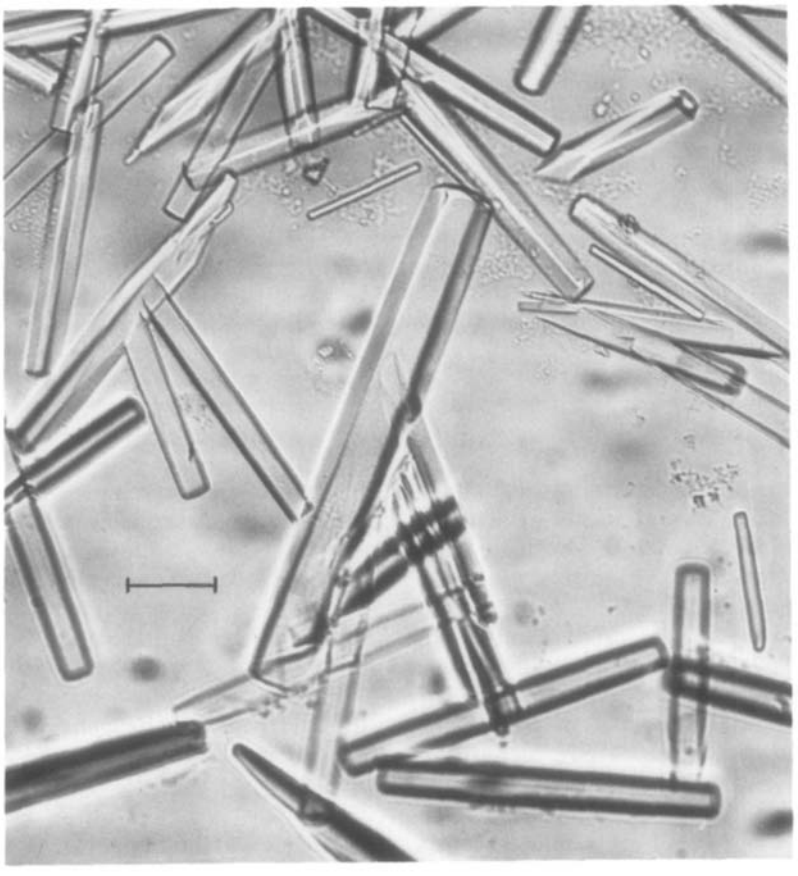

Figure 1. Crystals of 30 S ribosomal subunits from *T. thermophilus*, grown at 19 °C from 15% MPD in the presence of H-I buffer (Yonath *et al.*, 1980) at pH 6·6. The ribosomes were prepared according to Glotz *et al.* (1987). Bar represents 0·2 mm.

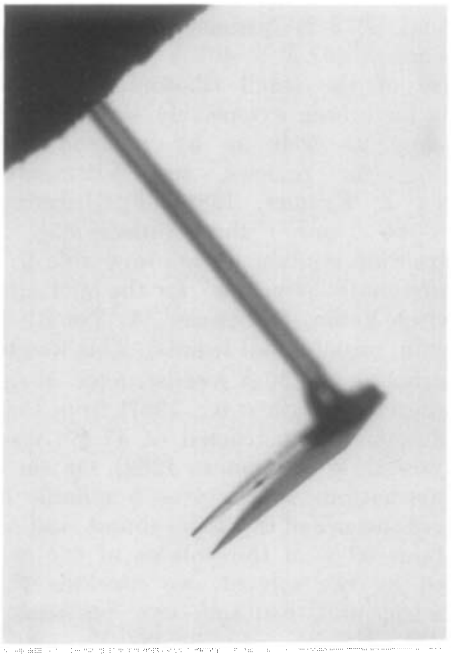

Figure 2. The tip of a double-layer (sandwich) spatula used for mounting crystals in cryo-temperature experiments.

nucleosomes (Richmond *et al.*, 1984) or the crystals of the 50 S ribosomal subunits from *Halobacterium marismortui* (Makowski *et al.*, 1987).

Since the 30 S particle has a characteristic elongated shape, we may be able to place it in the unit cell by using the rotation-translation function. In later stages we expect that, in solving the structure of the 30 S subunit, we shall be able to benefit from the vast knowledge that has accumulated about it. All the components of the 30 S subunit from a similar bacterium, *Escherichia coli*, have been characterized chemically (Wittmann, 1982, 1983), and their relative locations were oriented in space using biochemical (Brimacombe *et al.*, 1988) and neutron-diffraction methods (Moore, 1988). The major role that the 30 S particle plays in the process of protein biosynthesis has been established (for reviews, see Chambliss *et al.*, 1980; Hardesty & Kramer, 1986). It is known that this particle provides the binding sites for several components that take part in protein biosynthesis, such as the initiation factor, the messenger and the transfer RNA molecules. We hope to be able to crystallize complexes of these particles in distinct functional states, such as the initiation complex, using methods similar to those employed for obtaining crystals of complexes of the 50 S ribosomal subunits with tRNA and short

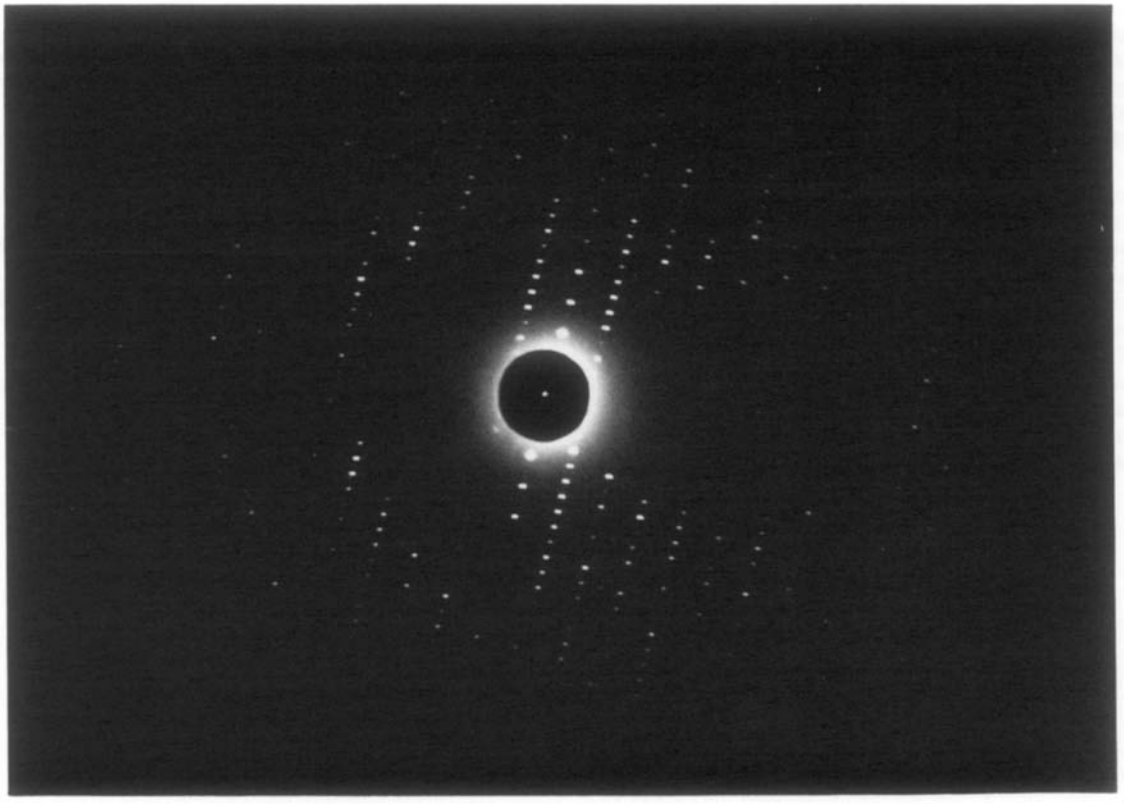

Figure 3. A 3° rotation photograph of a crystal similar to those shown in Fig. 1. The pattern was obtained at 90 K at Station A1/CHESS, operating at about 5·3 GeV and 30 to 60 mA. The crystal-to-film distance of 300 mm was partially filled with helium. Diameter of the collimator = 0·3 mm; wavelength = 1·558 Å.

segments of a nascent protein chain (Yonath & Wittmann, 1988; Gewitz *et al.*, 1988). We may be able to use the bound components for the determination of phase information. Specifically, we plan to use tRNA molecules to which we shall bind heavy-atom clusters. Thus, we expect that in the near future we shall be able not only to elucidate the overall structure but also to locate on it specific functional sites.

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