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# Crystals of Wild-type, Mutated, Derivatized and Complexed 50 S Ribosomal Subunits from *Bacillus stearothermophilus* Suitable for X-ray Analysis

Three-dimensional single crystals of wild-type and mutated 50 S ribosomal subunits from *Bacillus stearothermophilus*, as well as crystals of reconstituted subunits containing heavy-atom clusters and complexes of these subunits with tRNA and a short nascent polypeptide chain, were grown from polyethylene glycol in the presence of salts at low concentrations. Within experimental error, all these crystals are isomorphous, packed with monoclinic symmetry ($C2$) in unit cells of $a=300$ Å, $b=546$ Å, $c=377$ ($\pm 1\%$) Å and $\beta=112°$. Using synchrotron radiation at 85 to 100 K they diffract to 11 Å resolution and can be irradiated for hours without disintegrating, so that a complete data set could be collected from a single crystal.

A few years ago we reported the growth of microcrystals of the large ribosomal subunits from *Bacillus stearothermophilus* (Yonath *et al.*, 1980). These were too small for X-ray crystallography, but "powder" diffraction data from samples containing large numbers of microcrystals showed fairly sharp rings and oriented arcs with spacings up to 3·5 Å (1 Å = 0·1 nm), similar to those of gels of ribosomes and extracted rRNA (Zubay & Wilkins, 1960; Klug *et al.*, 1961; Langridge & Holmes, 1962). These patterns encouraged us to pursue subsequent crystallographic studies.

Because ribosomes from eubacteria disintegrate at high salt concentrations, at the early stages of our studies we used low molecular weight alcohols for crystallization. As a result, even the large crystals that grew from alcohols were only marginally suitable for crystallographic studies, since any handling of the crystals or replacement of the growth medium with a different solution was virtually impossible. Nevertheless, since at that time these were the only available three-dimensional crystals of intact ribosomal particles, we have invested much effort in obtaining some meaningful diffraction data from these crystals (Yonath *et al.*, 1984, 1986*a*,*b*).

We also obtained crystals from the large ribosomal subunits from *Halobacterium marismortui* (Makowski *et al.*, 1987), which diffract to better than 6 Å and are currently being studied crystallographically.

In parallel, we have continued our efforts to grow suitable crystals from ribosomal particles of *B. stearothermophilus*. Ribosomes from this source are attractive not only because they are obtained from eubacteria, but also because they may yield an interpretable model, since they are well-characterized chemically and we are able to modify them in a controlled fashion using genetic as well as biochemical procedures. It was possible therefore to mutate the bacteria so that one ribosomal protein (BL11) is omitted, as well as to detach a few selected proteins from the 50 S subunits (Gewitz *et al.*, 1987). These proteins can, in turn, be reconstituted together with the depleted core particles. Moreover, some of the detached proteins can be chemically modified prior to the reconstitution, and we are using them for covalent binding of heavy-atom clusters (S. Weinstein *et al.*, unpublished results).

As a result of an intensive search, we have succeeded in growing crystals the of large ribosomal particle from *B. stearothermophilus* using non-volatile materials. Currently we can obtain well-shaped crystals from solutions containing low concentrations of salts, resembling the natural environment of the ribosomes within the living cells, using small amounts (up to 2·5%) of polyethylene glycol (PEG†) as a crystallizing agent.

Like all other crystals of ribosomal particles grown in our laboratory, these crystals were obtained from functionally active particles, and the crystalline material retained its integrity and biological activity. Furthermore, it was observed that the ribosome preparations from which good alcohol-grown crystals were obtained also yielded crystals of high quality when PEG is used. Thus, it appears that the basic requirements for crystallization depend more on the procedures for the preparation of the ribosomal particles than on the choice of the crystallization agents.

Large crystals, diffracting to 11 Å, grow by vapour diffusion at 19 °C (Glotz *et al.*, 1987) as flat needles (0·4 mm × 0·15 mm × 0·05 mm) with a unit cell of $a=300$ Å, $b=546$ Å and $c=377(\pm 1\%)$ Å, $\beta=112°$, and monoclinic ($C2$) symmetry. As in the case of the alcohol-grown crystals of these subunits, the

† Abbreviation used: PEG, polyethylene glycol.



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diffraction patterns of the PEG-grown crystals occasionally include oriented arcs with distinct spots, with spacings extending to 3·5 Å, similar to those seen on the above mentioned "powder" samples (Yonath *et al.*, 1980). Hence it seems that the internal order of these crystals is better than can be measured by us at present. Although currently the crystals of the 50 S subunits from *B. stearothermophilus* diffract to lower resolution that those of 50 S subunits from *H. marismortui*, it is conceivable that in the future they will prove to be more suitable for crystallographic studies. This is partly due to the more amenable mosaic nature of these crystals (0·5° to 1°, compared to 3° for the crystals of halophilic 50 S subunits), which should facilitate data evaluation.

A common feature in the diffraction patterns of crystals of ribosomal particles is that the reflections of Bragg spacings smaller than about 20 Å decay within a few minutes of irradiation at 0 to 19 °C. The present crystals show no exception from this rule. Therefore we have attempted to employ our newly developed procedures for collecting data at cryo-temperature (H. Hope *et al.*, unpublished results; Yonath *et al.*, 1988).

In our initial experiments in cryo-crystallography we immersed crystals of 50 S subunits from *H. marismortui* in an inert hydrocarbon (Hope, *et al.*, 1988). It was found that this procedure could be used neither for the crystals from *B. stearothermophilus* nor for those of 30 S particles from *Thermus thermophilus* (Yonath *et al.*, 1988). Upon cooling after immersion in a hydrocarbon, the crystals of the 50 S ribosomal subunits from *B. stearothermophilus* produced smeared reflection spots to only 35 to 40 Å. A new procedure for collecting data from these crystals was therefore developed (Hope *et al.*, 1988). The basic idea was to replace the hydrophobic hydrocarbon by viscous hydrophilic solutions that mimic the growth medium of the crystals. The potential quality of the diffraction patterns, i.e. 11 Å resolution of sharp diffraction spots, was recovered as follows. Crystals were transferred into solutions containing all the compounds required to maintain their stability, and 20 to 30% of ethylene glycol, which served as an anti-freeze leading to improvement in the spot-shape. To increase the viscosity of these solutions, the original content of 6 to 8% PEG of $M_r$ 6000, was replaced by 25 to 45% of polyethylene glycol of $M_r$ 20,000 to 40,000.

After immersion, the crystals were positioned between two layers of thin glass films, glued to a thin glass rod, the "sandwich spatula" (Hope *et al.*, 1988; Yonath *et al.*, 1988), and "shock cooled" in liquid propane at about 85 K. Usually the cooled droplet turned "glassy" upon cooling. The specimen was then brought directly from the liquid propane into a stream of nitrogen, at about 85 to 100 K, which was kept blowing constantly around the crystal on the X-ray camera until the end of data collection. In all experiments carried out at cryo-temperature there was hardly any radiation damage even after hours in the synchrotron X-ray beam. In this way a complete set of diffraction data could be collected from a single crystal.

Using this method, crystallographic data were collected from crystals of 50 S subunits from *B. stearothermophilus*, of their mutant, missing protein BL11, and of reconstituted subunits, formed from the mutated core particles and protein BL11 to which either an undecagold or a tetrairidium cluster was bound (Weinstein *et al.*, unpublished results). We are currently using conventional film-processing programs for evaluation of these data. Preliminary crystallographic information was also obtained from a complex of these subunits with $tRNA^{Lys}$ + a short chain of poly(Lys) (Gewitz *et al.*, 1988), and from particles to which a heavy-atom cluster (undecagold or tetrairidium) was covalently attached prior to crystallization (S. Weinstein & W. Jahn, unpublished results).

The shape and the size of the 50 S subunit have been determined at medium resolution (around 30 Å) by three-dimensional image reconstruction from two-dimensional crystalline sheets (Yonath *et al.*, 1987). Using this information as well as that obtained by other biophysical techniques, such as scattering of light, X-rays or neutron beams (for a review, see Wittmann, 1983) and electron microscopy (Radermacher *et al.*, 1987; for reviews, see Wittmann, 1983; Hardesty & Kramer, 1986), we can conclude that there are four subunits in each asymmetric unit, and that about 50% of the volume of the unit cell is composed of solution.

This letter demonstrates that crystals of 50 S ribosomal subunits from *B. stearothermophilus*, which were grown under close to physiological conditions, are suitable for data collection at 11 Å resolution. We hope to be able to increase the resolution limit by growing larger and more ordered crystals. Once we elucidate the three-dimensional structure, we will compare it with that of the halophilic ribosomal subunit. We expect that these studies will not only lead to a better understanding of protein biosynthesis but also reveal basic differences between eubacteria and halobacteria.

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