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Characterization of Single Crystals of the Large Ribosomal Particles from a Mutant of *Bacillus stearothermophilus*

Single, three-dimensional crystals of 50 S ribosomal subunits, from a mutant of *Bacillus stearothermophilus* that lacks the protein L11, have been characterized using a synchrotron X-ray source. The crystals of the mutated particles grow under the same conditions and are isomorphous to those of the wild type of the same bacteria. They are orthorhombic, contain at least one 2-fold screw axis, and have unit cell dimensions of $a = 350(\pm 10)$ Å, $b = 670(\pm 10)$ Å, and $c = 910(\pm 10)$ Å. They diffract to 15 to 18 Å resolution at 4°C and are stable in the synchrotron beam for several hours.

For a better understanding of the structure of ribosomes, a molecular model is essential. To this end we have grown three-dimensional crystals and two-dimensional sheets of intact ribosomal particles from several bacteria (Wittmann *et al.*, 1982; Arad *et al.*, 1984; Yonath *et al.*, 1984, 1986*a*,*b* and unpublished results; Shevack *et al.*, 1985; I. Makowski *et al.*, unpublished results; J. Piefke *et al.*, unpublished results).

X-ray diffraction data have been collected from single crystals of the 50 S ribosomal subunits from *Bacillus stearothermophilus* (Yonath *et al.*, 1984, 1986*a*,*b*). Towards the next step in structure determination, the elucidation of phases, we have obtained a mutant of *B. stearothermophilus* in which one ribosomal protein, L11, is missing. The mutant (named TST) was isolated from strains 799 and NCA by growing the cells in the presence of thiostrepton at 60°C (Schnier *et al.*, unpublished results).

From the large ribosomal subunits of this mutant we were able to obtain well-ordered three-dimensional crystals and two-dimensional sheets. The mutated 50 S subunits crystallize under the same conditions as do those of the wild type (Yonath *et al.*, 1984, 1986*a*). They grow at 4°C by vapor diffusion from a mixture of methanol and ethylene glycol directly in X-ray capillaries, and reach their final size (0·7 mm × 0·2 mm × 0·1 mm) within two to three weeks.

For crystallographic studies we used well-formed single crystals, in capillaries containing all of the original mother liquor that were sealed with wax at 4°C immediately prior to the exposure. Still and rotation photographs of crystals aligned with their long axes parallel or perpendicular to the incident beam have been recorded. Using synchrotron radiation these crystals diffract to 15 to 18 Å. They last in the X-ray beam for two to six hours. Cell dimensions of an orthorhombic form, with at least one 2-fold screw axis, of $a = 350(\pm 10)$ Å, $b = 670(\pm 10)$ Å, and $c = 910(\pm 10)$ Å have been determined. These are isomorphous with those of the wild type. Loose packing is reflected both in the X-ray diffraction patterns and in electron micrographs of positively stained thin sections of embedded crystals.

Ribosomal particles of dissolved crystals comigrate in sucrose gradients together with the uncrystallized 50 S subunits from mutated ribosomes that were used as control. They have approximately the biological activity (in a poly(U) system) of control particles and, as shown by two-dimensional electrophoresis, contained all their proteins, except for L11 (data not presented).

Suitable heavy-atom derivatives are essential for structure determination. For an object as large, asymmetric and complex as the 50 S ribosomal subunit, it is necessary that these derivatives should be extremely dense and compact. Heavy-atom clusters are most suitable for this purpose. An appropriate compound is a gold cluster, $Au_{11}(CN)_3[P(C_6H_4CH_2NH_2)_3]_7$, in which the gold core has a diameter of 8·5 Å and has been prepared as a mono-functional labeling compound (Yang *et al.*, 1984; W. Jahn, unpublished results). To obtain usable heavy-atom derivatives for structure determination of a particle as large as the 50 S subunits, such compounds should be covalently bound to specific sites on the ribosomal particles. This can be achieved by direct interaction of the cluster with the intact particles. Alternatively, these clusters may be attached to single sites on isolated ribosomal proteins, which will then be incorporated into particles that lack this protein.

The TST mutant provides a suitable system for such studies, and L11, the protein missing in this mutant, is an appropriate candidate for this aim. Since, as mentioned above, the mutated particles crystallize in a form isomorphous with that of the wild type, it follows that protein L11 is not involved in the crystallographic forces of the wild type. In addition, we could show that the protein L11 from the wild type can be incorporated into the mutated subunits. These reconstituted particles also have been crystallized in a form isomorphous with the native and mutated crystals. It has further been shown (S. Weinstein, unpublished results) that

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binding of *N*-ethylmaleimide to the −SH group of protein L11 (M. Kimura, unpublished results) does not reduce the activity and crystallizability of the modified particles. Thus, modifying L11 with heavy-atom clusters is not expected to interfere with crystal packing and isomorphism.

It should be mentioned that, when protein L12 was removed from the wild-type particles using the method of Hamel *et al.* (1972), it was observed that 50 S subunits depleted of L12 do not crystallize under the same conditions as do native 50 S subunits, in contrast to those of the mutant lacking L11.

Since protein L11 is nearly globular (Giri *et al.*, 1978), its location may be determined in a Patterson electron density map with coefficients of $F(\text{wild}) - F(\text{mutant})$ and may serve, by itself, as a large heavy-atom derivative at about 20 Å resolution. Elucidation of the three-dimensional structure of the mutated particles by X-ray crystallography or three-dimensional image reconstruction followed by comparison with that of the wild type may reveal the location of the missing protein. In view of the labor and time involved in crystallographic analysis and the resolution of the expected results, three-dimensional image reconstruction seems to be the suitable technique for this aim. As mentioned above, we have already obtained two-dimensional sheets from the mutated particles. These are very well ordered (diffract to 25 to 30 Å) but are still too small for reconstruction studies.

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A. Yonath
M. A. Saper
F. Frolow

Department of Structural Chemistry
Weizmann Institute of Science
Rehovot, Israel

I. Makowski
H. G. Wittmann

Max-Planck-Institut für Molekulare Genetik
Berlin 33, West Germany

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