Crystallization and Preliminary Diffraction Studies of 5 S rRNA from the Thermophilic Bacterium Thermus flavus

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Crystals of purified 5 S rRNA from Thermus flavus have been obtained. The crystals diffract up to 8 Å resolution, using synchrotron radiation, and have the monoclinic space-group C2. The unit cell has the dimensions a = 190 Å, b = 110 Å, c = 138 Å and β = 117°. The cell volume suggests the presence of four 5 S rRNA molecules per asymmetric unit.

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For the complete understanding of protein biosynthesis the structures of the ribosomes should be known at atomic resolution. Although good progress has been made in crystallizing bacterial ribosomes (Yonath et al., 1980; Yonath & Wittmann, 1989; Hansen et al., 1990), it is clear that their complete X-ray structural analysis will not be accomplished for a long time. It would therefore be of great value in the meantime to determine the structures of ribosomal components. With this goal in mind we have concentrated our efforts on the structure and function of ribosomal 5 S rRNAs, which have been shown by reconstitution techniques to be essential for ribosomal function (Nomura & Erdmann, 1970; Erdmann et al., 1971a,b; Nierhaus & Dohme, 1974; Dohme & Nierhaus, 1976; Hartmann et al., 1988). Solving the three-dimensional structure of the ribosomal 5 S rRNAs by X-ray diffraction techniques to the highest possible resolution would in addition increase our knowledge of RNA structures in general, since so far only the X-ray structures of several tRNAs (Moras, 1989; Ruff et al., 1988) and the RNA duplex (U(UA)9A)2 (Dock-Bregeon et al., 1989) have been determined.

Another advantage to studying the 5 S rRNAs is the fact that they interact with several ribosomal proteins to form specific RNA–protein complexes (Horne & Erdmann, 1972). Thus, the structural studies can later be extended to such RNA–protein complex systems in order to increase our knowledge of how proteins recognize, and interact with, ribonucleic acids.

On the basis of chemical and enzymic modification studies (Digweed et al., 1986; Erdmann et al., 1987; Lorentz et al., 1988, 1989) and the alignment of 667 5 S rRNA sequences (Specht et al., 1990), we conclude that the secondary structures of cubacterial, archaeabacterial and eukaryotic 5 S rRNAs are known in principle (Specht et al., 1990). The molecules contain five helical regions interrupted by several loops and single-stranded sequences. The 5 S rRNAs are approximately 120 nucleotides long and have a molecular weight of 40,000.

Several attempts have been made in the past to crystallize 5 S rRNAs. Morikawa et al. (1982a,b) reported the first successful crystallization of a 5 S rRNA from Thermus thermophilus. Unfortunately the crystals diffracted to a resolution of only 25 Å (1 Å = 0.1 nm). The other report is by Abdel-Meguid et al. (1983), who crystallized an Escherichia coli 5 S RNA fragment and its complex with the protein EL-25. Although a resolution of up to 5 Å was reported in the case of the RNA fragment, there has been until now no further detailed structural data available on these studies. From the experience of other laboratories, who have so far solved the structures of only five distinct tRNAs (for a review, see Moras, 1989), we decided to study the 5 S rRNA crystallization by testing the following 18 different 5 S rRNA species: Azobacter vinelandii, Bacillus licheniformis, Bacillus stearothermophilus, Bacillus subtilis, Caulobacter, E. coli, Halobacterium cutirubrum, Micrococcus luteus, Proteus vulgaris, Pseudomonas fluorescens, rat liver, Staphylococcus aureus, Streptococcus sanguis, and Thermus flavus.
**Figure 1.** Separation of the 25S rRNA forms A and B from *T. flavus* by (a) fast protein liquid chromatography/phenyl-Superose column chromatography and (b) and (c) denaturing gel electrophoresis. (a) Separation of *T. flavus* 5S rRNA obtained from Sephadex G-100 chromatography. Peak A corresponds to the 5S rRNA A form, peak B to the B form. The 5S rRNAs were eluted by a salt gradient ranging from 0.8 to 0.9 M ammonium sulphate in 10 mM-ammonium acetate at pH 6.5. (b) Denaturing 5S rRNA gel electrophoresis. Lane 1: peak A (5S rRNA A form) from phenyl-Superose column. Lane 2: peak B (5S rRNA B form) from phenyl-Superose column. Lane 3: 5S rRNA mixture applied to phenyl-Superose column. (c) Denaturing gel electrophoresis of different 5S rRNA materials before and after crystallizations. The denaturing gel electrophoresis conditions were as follows. Gel: 9:5% (w/v) acrylamide, 0.5% (w/v) N,N-methylenebisacrylamide, 7 M urea, 90 mM-tris(hydroxymethyl)-ammonium methane, 90 mM-boric acid, 25 mM-EDTA (pH 8.4). Buffer: 90 mM-Tris(hydroxymethyl)-ammonium methane, 90 mM-boric acid, 2.5 mM-EDTA (pH 8.4). Lane 1: 5S rRNA fraction obtained from Sephadex G-100 chromatography as used in crystallization experiments. Lane 2: electrophoresis of 1 dissolved crystal obtained from the material analysed in lane 1. Lane 3: a form of 5S rRNA after isolation on phenyl-Superose and prior to crystallization. Lane 4: 5S rRNA as isolated from a crystal obtained from material analysed in lane 3. Lane 5: B form of 5S rRNA after isolation on phenyl-Superose and prior to crystallization. The B form gave only microcrystals under the conditions studied.
evaluated using a modified version of the MOSFLM film integration package (Leslie et al., 1986).

The preliminary assignment of the *T. flava* 5S rRNA crystal lattice is monoclinic, space group C2, with unit cell dimensions $a = 190$ Å, $b = 110$ Å, $c = 138$ Å and $\beta = 117^\circ$. The unit cell volume is $2.69 \times 10^6$ Å$^3$. Assuming that the 5S rRNA molecules have a mol. mass of 160,000 per asymmetric unit, the value of the packing volume is 4.3 Å$^3$/dalton, which is higher than the range usually observed for protein crystals (1.7 to 3.5 Å$^3$/dalton; Matthews, 1968), but has been observed for crystals of tRNAs. This gives a solvent content for the crystals of 70%. All images used in characterizing the cell were recorded at room temperature from a single crystal, without indication of major radiation damage. From the results presented we conclude that the ribosomal 5S RNA can eventually be crystallized to determine its atomic structure. We are determined to continue these studies by trying other 5S rRNA species and different isolation procedures until this goal has been reached.

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References


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