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Crystallization of engineered *Thermus flavus* 5S rRNA under earth and microgravity conditions

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Thermus flavus 5S rRNA with a molecular weight of about 40 kDa was modified at the 5′ and 3′ ends. Crystals were obtained under earth and microgravity conditions. The best crystals were obtained during NASA space mission STS 94. For the first time, it was possible to collect a complete data set from 5S rRNA crystals to 7.8 Å resolution and to assign the space group as *R*32, with unit-cell parameters $a = b = 110.3$, $c = 387.6$ Å, $\alpha = \beta = 90$, $\gamma = 120^\circ$.

1. Introduction

Ribosomal 5S rRNA is an essential component of the ribosome. It is 120 nucleotides long. Ribosomal particles lacking 5S rRNA have a strongly reduced activity in protein synthesis (Nomura & Erdmann, 1970; Nierhaus & Dohme, 1974; Dohme & Nierhaus, 1976; Erdmann *et al.*, 1971; Hartmann *et al.*, 1988). In particular, the peptidyl-transferase activity is greatly reduced. Because of its functional importance and the fact that 5S rRNA interacts specifically with several ribosomal proteins (Horne & Erdmann, 1972), it is of great interest to determine the three-dimensional structure of this RNA. Nearly 1000 different prokaryotic and eukaryotic 5S rRNA sequences have been determined so far. The predicted secondary structure is shown in Fig. 1 (Specht *et al.*, 1990). The size of 5S rRNA limits the possibility of three-dimensional structure determination by NMR. Therefore, we have previously attempted to crystallize several 5S rRNA species for X-ray analysis (Lorenz *et al.*, 1991). Crystals of 5S rRNA isolated from the thermophilic bacterium *T. flavus* suitable for X-ray diffraction were obtained and analysed. These crystals diffract to about 8 Å resolution and are very sensitive to radiation. For this reason, the application of cryotechniques is mandatory. In parallel, we divided the *T. flavus* 5S rRNA into five domains, A to E, and synthesized these domains chemically for crystallization experiments (Lorenz *et al.*, 1993; Nolte *et al.*, 1995). The structures of domains A (Betzel *et al.*, 1994) and E (Perbandt *et al.*, 1998) were analysed to 2.4 and 3.0 Å resolution, respectively. Assuming that these fragments have the same conformation as in the native 5S rRNA molecule, their three-dimensional structures will help to construct a three-dimensional model of the complete 5S rRNA.

The intrinsic flexibility of the whole 5S rRNA molecule and small differences in the primary structure seem to influence significantly the ability to produce well ordered crystals. To overcome this, we now work with engineered variants of *T. flavus* 5S rRNA in order to improve the crystal quality. Here, we present our preliminary results on the crystallization of genetically expressed variants of *T. flavus* 5S rRNA. The variations in the primary sequence are in the 3′- and 5′-end regions of the RNA. We had evidence that these parts of the molecule are important for their ability to crystallize. With these variants, we performed crystallization experiments under laboratory and microgravity conditions. Previous experiments under microgravity indicated that crystals grown in space were vastly improved in size and diffracting power when compared with those grown under identical earth conditions. However, all crystals are very sensitive to mechanical stress during transport and crystal handling and mounting. Consequently, special freezing techniques are required and were applied for the experiments described. A crystal grown in space is shown in Fig. 2. In this communication, we present the expression and purification of *T. flavus* 5S RNA variants, their crystallization under terrestial and microgravity conditions and the results of the preliminary X-ray analysis.

2. Materials and methods

2.1. Cloning of *T. flavus* 5S rRNAs and their purification

A 3200 bp fragment of the genomic DNA from *T. flavus* AT 62 was ligated into the vector pT7T3 18U (2890 bp) and expressed in cells of *Escherichia coli* strain X90. PCRs with the isolated plasmids and different 3′- and 5′-end primers were performed. The PCR products

were ligated into the pUC 18 vector and expressed in cells of *E. coli* strain X90. The isolated plasmids were tested for their correct inserts by DNA sequencing. After linearization of the plasmids, we sythesized the 5S rRNA variants during run-off transcriptions. The 5S rRNA products were purified by Sephadex G150 gel chromatography and by hydrophobic affinity interaction chromatography.

2.2. Crystallization

Crystals were grown by the hanging-drop vapour-diffusion method in Linbro 24-well plates. Different precipitation agents, additives and buffers were screened. The best crystals were obtained from a 40–50% saturated ammonium sulfate solution with 300 m*M* caesium chloride, 5 m*M* cobalt chloride, 15 m*M* calcium chloride, 20 m*M* spermine and 50 m*M* sodium cacodylate at pH 7.0. The hanging drops contained 2 µl 0.1 m*M* 5S rRNA and 2 µl of the described crystallization solutions. The reservoir contained 300 µl of the same solution. Crystals grew in a few days to dimensions of about 0.2 × 0.2 × 0.4 mm. For X-ray characterization using synchrotron radiation, they were transferred to a cryosolvent containing the same components as the reservoir and additionally 28% of glycerine at a concentration of 58% saturated ammonium sulfate.

For crystallization experiments with the engineered 5S rRNA variants under microgravity conditions, we used two different systems: firstly, the APCF (Advanced Protein Crystallization Facility) hanging-drop reactors supplied by the ESA (European Space Agency) and secondly, a vapour-diffusion crystallization system, VDA-2 (vapour diffusion apparature), supplied by NASA (National Aerospace and Aeronautic Agency) with the support of the Center of Macromolecular Crystallography at the University of Alabama at Birmingham (UAB). Both hardware systems were adapted on board shuttle flights. The crystallization time was almost 16 d. The RNA drop volume in all experiments was 8 µl and the reservoir volume varied from 250 to 1000 µl. Parallel ground control experiments were performed under identical conditions.

3. Results

The best crystals, suitable for X-ray analysis, were grown in the vapour-diffusion apparatus during the shuttle flight STS 94. 2 d after arrival, selected crystals were collected, documented and stored in liquid nitrogen. This is very important, as the 5S rRNA crystals are known to be extremely fragile and sensitive to ageing and temperature changes. The best crystal diffracted to a moderate resolution of about 7.4 Å and 99% of all data in the resolution range 50–7.9 Å were collected using the synchrotron radiation at ELETTRA (beamline 5.2). The data were collected at a wavelength of 1.0 Å from a single crystal under cryo-conditions (100 K) using an Oxford Cryosystems Cryostream. The crystal and data-collection parameters are summarized in Table 1. The images were processed using the programs *DENZO* (Otwinowski, 1991) and *SCALEPACK* (Otwinowski, 1993). The $R_{\rm sym}$ over all data is 7.9%. The space group was now reassigned to be *R*32. Some years ago, Morikava *et al.* (1982) performed crystallization experiments on the ribosomal 5S rRNA using PEG as a precipitant and also obtained crystals assigned to the space group *R*32. It is remarkable that the space group is obviously independent of the precipitant used. Assuming an RNA density of 1.7 g cm^{-3} and in view of the moderate quality of diffraction, the asymmetric unit contains most probably one molecule, with a solvent content of 69%, resulting in a Matthews coefficient (Matthews, 1968) V_m of 5.6 Å^3 Da^{-1}. Assuming two molecules in the asymmetric unit, the corresponding solvent content would only be 39%. The limited resolution is also an indication of highly flexible regions in the 5S rRNA. This assumption is strongly supported by the results of Correll *et al.* (1997). Under mild nuclease digestion, they have obtained a 62 nt fragment of the whole 5S RNA lacking the complete B and C domains (see Fig. 1). It was possible to crystallize this truncated molecule and to analyse the structure at 3 Å resolution. These results in combination with our experiments are a strong indication of the flexibility of the B and C domains in the absence of stabilizing ribosomal binding proteins.

Table 1
Crystal parameters, data collection and processing.

Space group	*R*32
Unit-cell dimensions	
a, *b* (Å)	110.3
c (Å)	387.6
V_m (Å^3 Da^{-1})	5.6
Crystal size (mm)	0.2 × 0.2 × 0.4
X-ray source	ELETTRA, beamline 5.2
Wavelength (Å)	1.0
Temperature of data collection (K)	103
Resolution range (Å)	20–7.8
Total rotation range (°)	180
Number of reflections	34178
Number of unique reflections	1051
$R_{\rm sym}$ (%)	7.9
$R_{\rm sym}$, last resolution shell (%)	29.3
Number of reflections with $I > 2\sigma(I)$ in last resolution shell (%)	39.9
Completeness (%)	99
Completeness, last resolution shell (%)	93

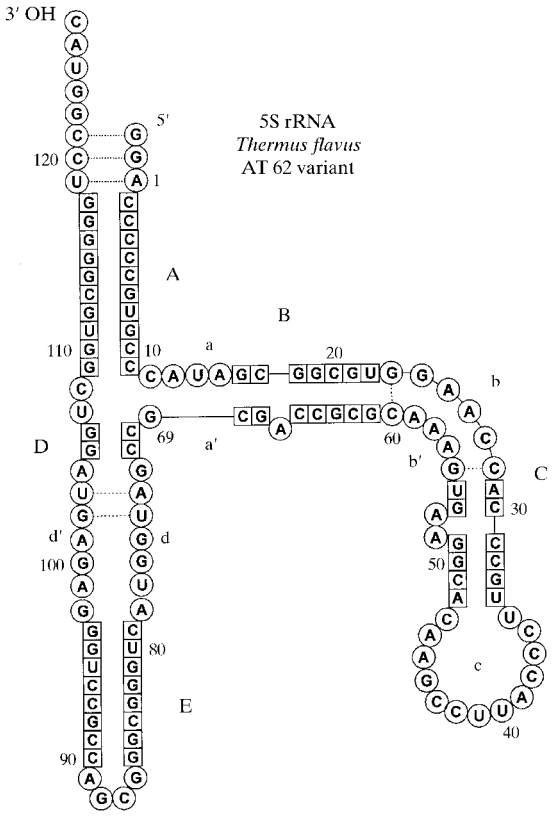

Figure 1
Secondary structure of the ribosomal 5S rRNA from *T. flavus*.

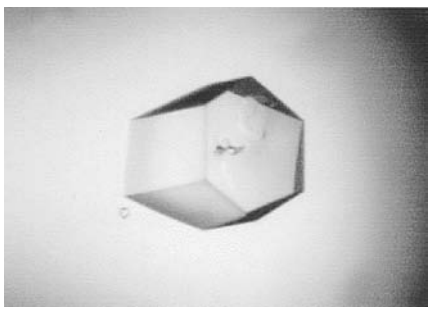

Figure 2
Crystal of a *T. flavus* 5S rRNA variant grown under microgravity conditions.

The results presented here indicate that it is possible, in principle, to grow crystals of 5S rRNA for X-ray analysis and to analyse the structure at moderate resolution. This structure should indicate the regions of high flexibility and engineering techniques can be applied to stabilize the three-dimensional structure or to remove disordered regions which may not be of functional interest. The redesigned structure will be used for crystallization and X-ray studies at a resolution high enough to reveal functional details. In parallel, we have characterized 5S rRNA binding proteins which will be used for co-crystallization studies and X-ray analysis.

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