Crystallization and preliminary X-ray diffraction study of 5 S rRNA from *Thermus thermophilus* HB8

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5 S rRNA *Crystals* *Vapour diffusion method* *X-ray diffraction* *Three-dimensional structure*

1. INTRODUCTION

5 S rRNA is a common ribosomal component which is present in various species from prokaryotes to eukaryotes. Many attempts were made to elucidate its structure and function [1]. A number of the models for its secondary structure including that by Nishikawa and Takemura have been proposed [1].

The best way for elucidating a definite three-dimensional structure of a macromolecule is to prepare its single crystal and analyze it by X-ray crystallography. Crystallization is thus a significant step of the analysis. 5 S rRNA from extremely thermophilic bacteria may be more suitable for crystallization, since it is supposed to have a more stable structure than those from usual non-thermophilic bacteria. We describe here that the RNA purified from *Thermus thermophilus* HB8 can be certainly crystallized. 5 S rRNA is the second natural nucleic acid that has been obtained as a single crystal.

The nucleotide sequence of the same RNA as used for the crystallization has been recently reported by Kumagai et al. [2].

2. MATERIALS AND METHODS

Thermus thermophilus HB8 cells harvested at the log phase of growth were kindly supplied by Dr Toshiyuki Sai (Mitsubishi Petrochemical Co., Ltd, Ibaragi, Japan). A low molecular weight RNA fraction containing tRNAs and 5 S rRNA was obtained according to the phenol extraction method by Holley [3]. 5 S rRNA was purified by two runs of chromatography on a Sephadex G-100 column (3.1 × 90 cm) pre-equilibrated with 0.02 M Tris–HCl buffer (pH 7.4) containing 0.1 M NaCl and 1 mM EDTA. The same buffer was used for elution. 5 S rRNA was precipitated with ethanol and dried with ether. The yield of the RNA was 60 mg from 300 g (wet weight) cells.

Crystallization was carried out using a vapour diffusion technique as described by Morikawa et al. [4]. Twenty to 60 μl solution on a siliconized depression slide, which contained the RNA, various salts and a buffer, was equilibrated with 10 to 15% 2-methylpentane-2,4-diol of 20 ml. Crystals usually appeared in 10 days at 5°C. They were also obtained at 25°C with the same shape but smaller sizes.

In X-ray diffraction experiments a precession camera (Enraf-Nonius) was used with a Rigaku 4012-K1 X-ray generator and also an oscillation camera (Enraf-Nonius) with a Rigaku FR-B rotating anode X-ray generator.

3. RESULTS AND DISCUSSION

3.1. *Crystallization*

Screening tests by the vapour diffusion method demonstrated that the crystallization sensitively depends upon the ratios of $MgCl_2$ and spermine to the RNA but not upon that of Na cacodylate, as observed in the crystallization of $tRNA_f^{Met}$ from *E.coli* [4]. It suggests that the binding of those divalent or polyvalent cations is stereochemically restricted by a conformation of the RNA which

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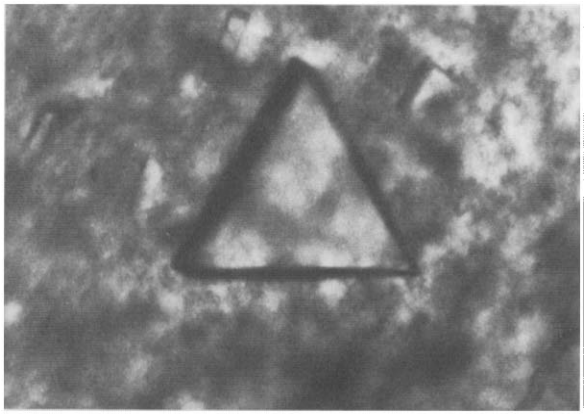

Fig.1. Crystals grown from *Thermus thermophilus* HB8 5 S rRNA. Maximum dimension 0.4 mm.

provides particular binding sites. Monovalent cations, on the other hand, bind to the RNA in a more non-specific manner where they play only a role for compensating the negative charges of polynucleotide phosphates. In order to increase the possibility of successful crystallization, two-dimensional phase diagrams [4,5] were constructed, changing continuously the ratios of $MgCl_2$ and spermine to 5 S rRNA. Different from *E. coli* $tRNA_f^{Met}$, the successful conditions so far found produced the same type of crystals, indicating fewer polymorphs of 5 S rRNA crystals.

The best crystals were grown, when 0.15% 5 S rRNA solution containing 5 mM $MgCl_2$, 0.7 mM spermine and 10 mM Na cacodylate–HCl (pH 7.0) was equilibrated against a reservoir of 10% 2-methylpentane-2,4-diol at about 5°C. As shown in fig.1, the crystals usually appeared with regular triangle plates of which dimensions are sometimes 400 μm × 150 μm. The crystals with a more irregular shape were occasionally grown, which looked like an hexagonal cylinder obliquely cut off. From characteristics of birefringence observed under a polarized microscope in addition to such crystal habits, it was probable that the crystals belonged to trigonal system.

The crystals were subjected to electrophoresis on 10% polyacrylamide gel [6] (120 × 120 × 0.6 mm) at 100 V for 4 h to give only a single band corresponding to 5 S rRNA (not shown). It was reported that *T. thermophilus* 5 S rRNA prepared by chromatography of Sephadex G-100 contains two components, major and minor [2]. At present it re-

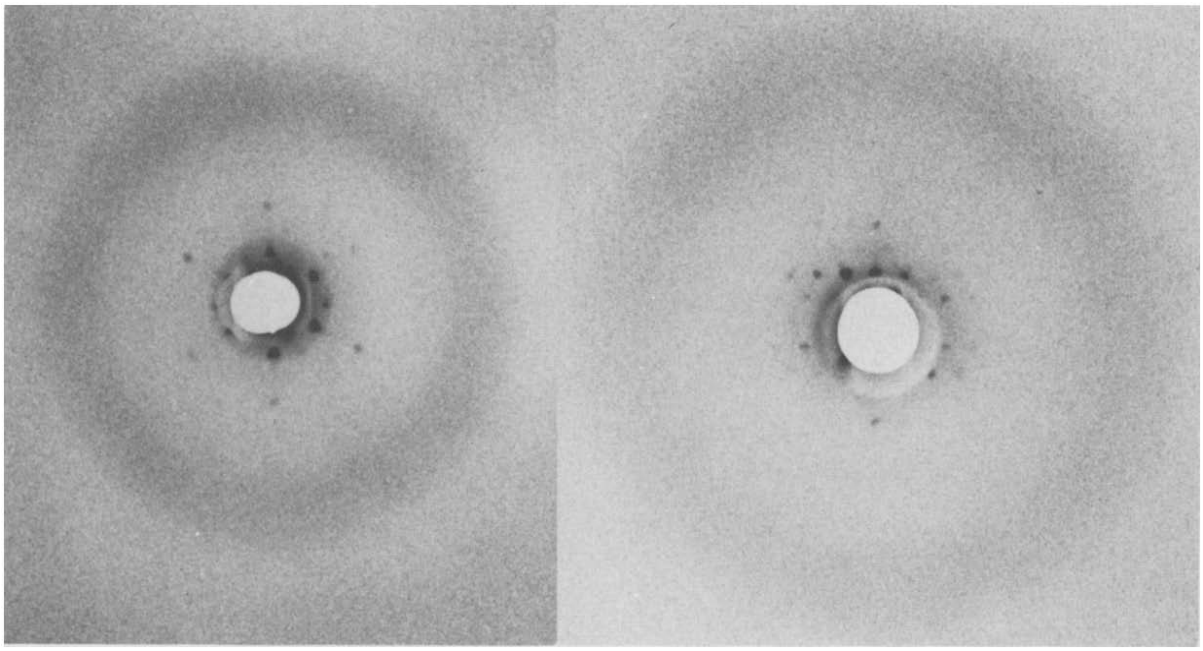

Fig.2. Left: An X-ray precession photograph of hk0 plane of 5 S rRNA crystals taken by using CuK_α radiation. The resolution is about 25 Å. Right: An X-ray precession photograph of 5 S rRNA crystals. The direction of incident beams was a little inclined to *c*-axis.

mains uncertain whether or not the crystallized RNA contains the minor component. In addition, the crystals disappeared by pancreatic RNAase digestion. These results confirmed that the crystals were grown from 5 S rRNA.

3.2. *X-ray diffraction*

Precession photographs were taken at 14°C to determine the space group and unit cell dimensions. They revealed that 5 S rRNA was crystallized in a trigonal lattice with cell dimensions of a = 99 Å, b = 99 Å and c = 360 Å, corresponding to a unit cell volume 3.06 × 10^6 Å^3. The space group is most likely to be $P3_121$ (or its enatiomorph $P3_221$), although the possibility of P321 could not be excluded because the extinction law of $l=3n$ for 001 reflections was not very certain due to low resolution of the diffraction patterns. According to the unit cell volume and the molecular weight of the RNA, it is most reasonable to conclude that 5 or 6 molecules are contained in an asymmetric unit; this corresponds to 30 or 36 molecules in a unit cell. The reflections were observable up to 25 Å using the largest crystal available. Oscillation photographs taken at 9°C showed no significant improvement of the molecular order within crystal lattice. It should be noted that on hhl plane the reflections with $l=3n$ exhibited markedly strong intensities. This may correspond to a particular arrangement of the molecules which makes 120 Å repeat along c-axis in the crystal lattice.

It is of interest, if possible, to observe the orientation of the reflection which arises from stacked bases in the double helix, since the secondary structures of 5 S rRNA so far proposed commonly indicate the presence of some double helical segments. To find their orientations to the crystal axes, one of the crystals was exposed to X-rays for a longer time upon the rotation of c-axis by every 15 degrees around a-axis. No particular oriented reflection was found. Only diffuse rings corresponding to roughly 3.5 Å, 7.8 Å and 13 Å spacings were observed, and their intensities did not greatly change depending upon the rotation angles (not shown). Their spacings and intensity distribution were consistent with the fibre diffraction pattern of double stranded reovirus RNA [7]. These results demonstrate that the orientation of the double helices are irregular in the crystal lattice. It remains unknown whether the irregularity results from the arrangement of the double helical segments within 5 S rRNA molecule itself, or the molecular arrangement within the crystal lattice.

We are aware that the present crystal does not diffract X-rays well enough to elucidate the detailed three dimensional structure. This may be related to considerable variability in conformation of 5 S rRNA molecules, presumably, because of longer loops. However, the first success in obtaining single crystals of 5 S rRNA raised the prospect that its model at atomic level will be ultimately built up by X-ray crystallographic analysis.

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