SHORT COMMUNICATIONS

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Acta Cryst. (1993). D**49**, 418-420

Crystallization and preliminary diffraction studies of the chemically synthesized domain *A* of *Thermus flavus* 5S rRNA: an RNA dodecamer double helix.

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(*Received* 4 *February* 1993; *accepted* 10 *February* 1993)

Abstract

Crystals of domain *A* of *Thermus flavus* 5S rRNA have been obtained. The space group was found to be $P4_3$ with unit-cell dimensions $a = b = 30.10$ and $c = 86.80$ Å. Data to 2.3 Å have been recorded and solution of the structure is currently underway by means of molecular-replacement techniques.

Introduction

The ribosomal 5S RNA is an essential component of the ribosome and it is, therefore, of interest to know its structure to eventually understand its biological function during protein biosynthesis at the atomic level (Erdmann, Fahnestock, Higo & Nomura, 1971; Hartmann, Vogel, Walker & Erdman, 1988). Since crystal structure analysis of whole 5S rRNA molecules could provide only low-resolution data at 8 Å until now (Lorenz *et al.*, 1991), we have turned to the chemical synthesis of the individual 5S rRNA domains *A* to *E* in order to determine their structure by X-ray crystallographic methods (Bald *et al.*, 1992; Erdmann *et al.*, 1993). The results presented in this communication, which show that the chemically synthesized domain *A* from *Thermus flavus* 5S rRNA can be crystallized at a resolution high enough to warrant its X-ray analysis, suggest that this approach may be feasible.

Methods

The chemical synthesis of domain *A* of *Thermus flavus* 5S rRNA was accomplished by employing phosphoramidite reagents developed in our laboratory (Bald *et al.*, 1992). The domain *A* synthesized consisted of the following two antiparallel strands:

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5' A U C C C C C G U G C C 3'
3' U A G G G G G C G U G G 5'
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For hanging-drop crystallization experiments, 2 µl RNA solution [5 mg RNA ml^{-1}, 5% 2-methyl-2,4-pentanediol (MPD), 200 m*M* $MgCl_2$, 20 m*M* Na cacodylate pH 6.5] was equilibrated against 1 ml buffer consisting of 30% MPD, 400 m*M* $MgCl_2$ and 40 m*M* Na cacodylate at pH 6.5. Best results were obtained at a crystallization temperature of 45 °C. As shown in Fig. 1, it was necessary to employ the seeding technique four times in order to obtain crystals large enough for X-ray analysis (up to 0.5 mm in length).

Results and concluding remarks

A number of crystals suitable for X-ray analysis of domain *A* of *Thermus flavus* 5S rRNA were obtained by the vapour-diffusion method. So far two data sets have been collected from these crystals. The crystals were mounted in thin-walled glass capillaries with some mother liquor. From one crystal a data set was collected up to 3.0 Å on a conventional sealed-tube X-ray source with Mo $K\alpha$ radiation and a graphite monochromator using an MAR 180 mm image-plate detector. The space group of the observed crystal was found to be $P4_3$ or $P4_1$ with unit-cell parameters of $a = b = 30.10$ and $c = 86.80$ Å. The packing parameter V_M was 2.6 $Å^3$ Da^{-1} (Matthews, 1968) for one helical fragment per asymmetric unit. This data set was used for the molecular-replacement calculations.

A second data set was collected to 2.3 Å resolution with synchrotron radiation using an MAR 300 mm image-plate detector at the EMBL beamline X11. The storage ring was operated in main-user mode at 4.7 GeV and 20-40 mA. The wavelength was 0.92 Å. The images of the first data set were processed using the program *DENZO* (Otwinowski, 1991). The reduced data set contains 1477 reflections and shows a completeness of 94%. The R_{merge}, defined as $R(I) = \Sigma|I-\langle I\rangle|/\Sigma I$, is 6.6%. The images collected using synchrotron radiation were processed using a modified version of the *XDS* program package (Kabsch, 1988). The unique data up to 2.3 Å contain 2170 reflections with R_{merge} of 3.7%. Finally the two data sets were scaled together. The resulting completeness for all data up to 2.4 Å is 83.5% and for all data up to 2.3 Å is 77.3% because of the limited completeness of only 50% in the resolution shell between 2.3 and 2.4 Å caused by radiation damage. The structure solution is currently being attempted by molecular replacement using the coordinates of the synthetic RNA helix: $[U(UA)_6A]_2$ (Dock-Bregeon *et al.*, 1989) as a starting model, and a new rotation and translation function program *AMORE* (Navaza, 1992). The rotation function gave a clear solution for

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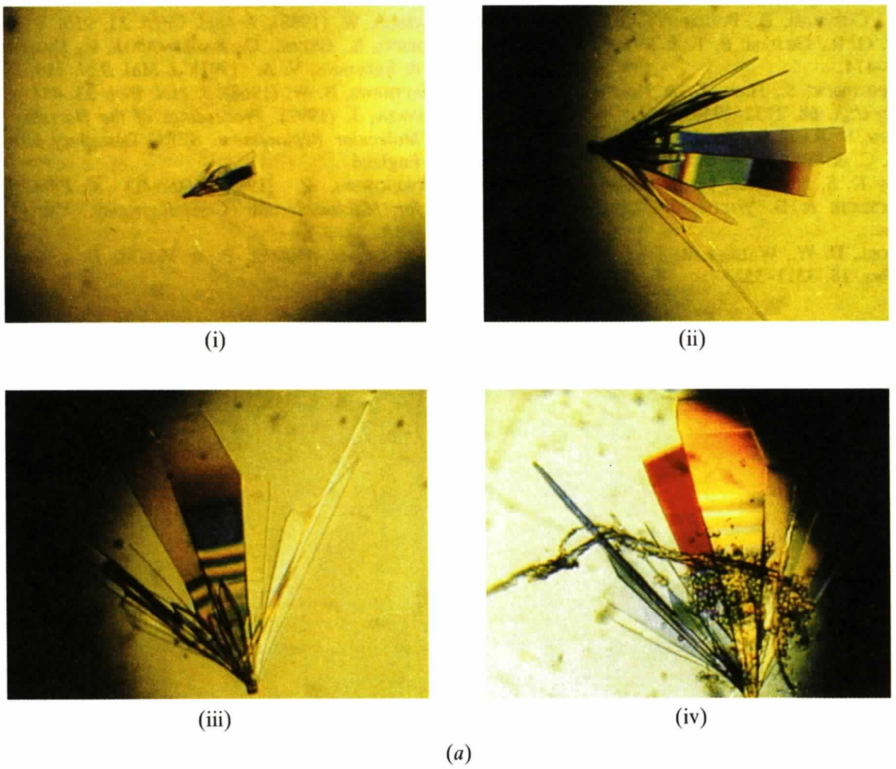
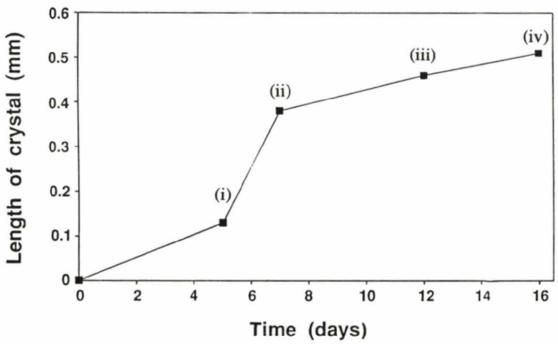


(*a*)



(*b*)

Fig. 1. (*a*) Crystals obtained from crystallization experiments of the chemically synthesized domain *A* from *Thermus flavus* 5S rRNA after repeated seeding: (i) original crystal after 5 days; (ii) crystal from (i) after seeding (2 days); (iii) crystal from (ii) after seeding (5 days); (iv) crystal from (iii) after seeding (4 days). (*b*) Crystal growth of domain *A* after each seeding step.

the orientation of the molecule. In the following translation search the space group was assigned to be $P4_3$ using all data in the resolution range of 8.0–3.0 Å and giving an *R* value of 41%. Preliminary refinement confirmed the correctness of this solution by applying restrained least-squares (*NUCLSQ*; Westhof, Dumas & Moras, 1985) and molecular-dynamics refinement (*X-PLOR*, Brünger, Karplus & Petsko, 1989).

Thus, the results shown demonstrate that chemically synthesized RNA domains can be crystallized and their structure can be determined by X-ray analysis. It is anticipated that the chemical synthesis of RNA molecules will eventually have the same impact on RNA structural information as previously seen for the DNA structures.

The authors thank the Deutsche Forschungsgemeinschaft, the Bundesministerium für Forschung und Technologie and the Fonds der chemischen Industrie eV for financial support.

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