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Crystals of the chemically synthesized acceptor stem of tRNA[Ala] from *Escherichia coli* diffracting to high resolution

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Abstract

The acceptor stem of tRNA[Ala] from *E. coli* has been chemically synthesized and crystallized. This duplex contains a G·U base pair in position 3–70, which is the main identity element for alanyl-tRNA synthetase from *E. coli*. The crystals are stable in the X-ray beam for a long period of time and diffract to 1.7 Å resolution. The monoclinic crystals reveal a $C2$ space group with $a = 35.0$, $b = 47.5$, $c = 26.2$ Å, $\beta = 102.3^\circ$ and one acceptor stem per asymmetric unit.

1. Introduction

The translation of the genetic code into proteins is established by the recognition of transfer RNA's by their specific aminoacyl-tRNA synthetases and by the correct codon–anticodon interactions. The sites of the tRNA which are recognized by the synthetase can vary between the tRNA species. In the case of tRNA[Ala] from *E. coli*, a single G·U base pair in the acceptor helix is the major identity element for the interaction with the synthetase (Hou & Schimmel, 1988). Even the complete substitution of the anticodon of this tRNA has no influence on the aminoacylation with alanine (Hou & Schimmel, 1988). In addition it could be demonstrated that short hairpins, composed of just the seven base pairs of the acceptor stem (Francklyn & Schimmel, 1989) or the first four base pairs of this stem alone, stabilized by a tetra loop (Shi, Martinis & Schimmel, 1992), are substrates for the corresponding synthetase.

At present only several crystal structures of tRNA's (Moras, 1989) and three high-resolution structures of tRNA's, complexed with their specific synthetase, are known (Rould, Perona, Söll & Steitz, 1989; Ruff *et al.*, 1991; Biou, Yaremchuk, Tukalo & Cusack, 1994). Moreover, only a few short RNA molecules were analyzed by X-ray crystallography (Baeyens, De Bondt & Holbrook, 1995; Betzel *et al.*, 1994; Cruse *et al.*, 1994; Leonard *et al.*, 1994; Holbrook, Cheong, Tinoco & Kim, 1991; Dock-Bregeon *et al.*, 1988; Schindelin *et al.*, 1995; Portmann, Usman & Egli, 1995). One reason for few structural investigations of short RNA's was the lack of a suitable method to produce large amounts of RNA for a long time. This problem was solved by the introduction of the *tert*-butyldimethylsilyl protection group for the 2'-hydroxyl of the ribose (Ogilvie, Theriault & Sadana, 1977) in combination with either the phosphoramidite chemistry (Usman, Pon & Ogilvie, 1985) or the H-phosphonate chemistry (Garegg, Lindh, Regberg, Stawinski & Strömberg, 1986).

We focused our interest on crystallization and X-ray analysis of the acceptor stem of tRNA[Ala] from *E. coli*, which was recently characterized by NMR spectroscopy (Limmer, Hofmann, Ott & Sprinzl, 1993; Ott, Arnold & Limmer, 1993). This acceptor stem consists of a seven-base-paired double helix (Fig. 1) with a G·U base pair in position 3 of the helix. According to Hou & Schimmel (1988) this G·U base pair is the major identity element in tRNA[Ala] from *E. coli* and its replacement by other base pairs abolishes the aminoacylation with alanyl-tRNA synthetase from *E. coli*. Some of the RNA duplexes investigated by X-ray spectroscopy also contain one or more G·U base pairs. Often these G·U pairs were stabilized by a bridging water molecule, between the unpaired guanine amine and the ribose hydroxyl of uracil, in the minor groove of the RNA (Holbrook *et al.*, 1991; Cruse *et al.*, 1994), resulting in only a small distortion of the A-RNA sugar-phosphate backbone. The G·U base pair, as the major identity element of tRNA[Ala], in contrast is embedded in a certain base-pair context which is also important for the recognition of the synthetase. In addition the replacement of this G·U base pair with a G·A, C·A or U·U wobble pair had no influence on the identity of tRNA[Ala] (McClain, Chen, Foss & Schneider, 1988). Therefore, it seems that this G·U base pair leads to an irregularity in the helix of the acceptor stem of tRNA[Ala], which should be recognized in the three-dimensional structure of the chemically synthesized RNA.

2. Methods

Both strands of the acceptor stem were prepared in milligram quantities by automated chemical synthesis on a Gene Assembler Plus (Pharmacia) using 2'-*tert*-butyldimethylsilyl protected H-phosphonate monomers as described (Ott *et al.*, 1994). Purification was carried out by denaturing polyacrylamide gel electrophoresis. Crystals were grown at 298 K using the hanging-drop vapor-diffusion method. The 5–10 µl droplets contained 1 mM of the RNA duplex in 12.5 mM sodium cacodylate pH 6.5, 50 mM $MgSO_4$, 6%(v/v), 2-methyl-2,4-pentanediol (MPD) 1 mM of spermine and were equilibrated against 1 ml of 35–45%(v/v) MPD in the same buffer.

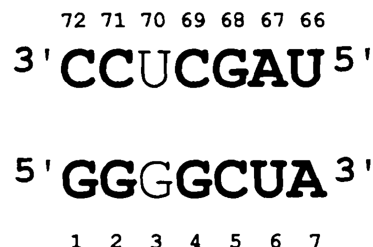

Fig. 1. Acceptor stem of tRNA[Ala] from *E. coli* with the G·U wobble base pair in position 3–70. The numbers of the bases are according to Steinberg, Misch & Sprinzl (1993).

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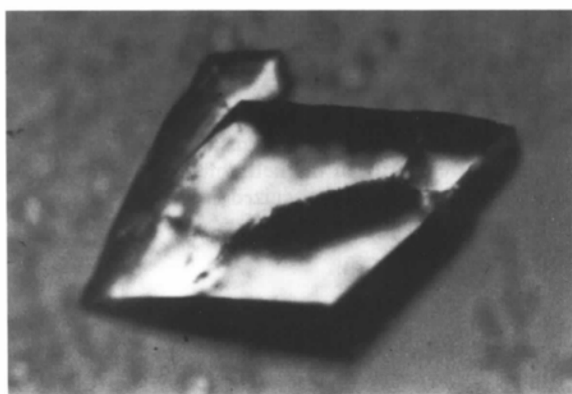
3. Results and concluding remarks

The octahedrally shaped crystals appeared within one week and achieved final dimensions of 0.2 × 0.2 × 0.1 mm (Fig. 2*a*). After extensive washing with 25 m*M* sodium cacodylate pH 6.5, 100 m*M* $MgSO_4$ and 2 m*M* spermine in 12%(*v*/*v*) MPD the crystals were dissolved in water and analysed by denaturing 20% polyacrylamide gel electrophoresis (PAGE) in the presence of 7 *M* urea. Only the bands corresponding to both strands of the intact acceptor stem were detected (Fig. 2*b*).

Diffraction data were collected on a MAR Research imaging-plate detector (180 mm diameter) which was mounted on a direct-drive Rigaku Denki (Japan) RU H2R X-ray generator and operated at 4.8 kW with 0.3 × 0.3 mm focus. Data were measured at 297 K with graphite-monochromated Cu $K\alpha$ radiation. Data processing, unit-cell and space-group determination were carried out with *DENZO* (Otwinowski, 1993). The space group *C*2 with $a = 35.0$, $b = 47.5$, $c = 26.2$ Å, $\beta = 102.3°$ was confirmed by precession photography. With one RNA duplex per asymmetric unit the packing parameter is $V_M = 2.2$ Å^3 Da^{-1}, calculated according to Matthews (1968). The diffraction data set to 1.7 Å contains 4571 independent reflections. The overall $R_{\rm sym}$ value is 5.8%, with $R_{\rm sym}$ values of 4% for the inner resolution shell up to 8 Å and 22% for the outer shell from 1.8 to 1.7 Å ($R_{\rm sym} = \sum |I_{i,j} - \langle I_i \rangle| / \sum I_{i,j}$, where $I_{i,j}$ are the measurements contributing to the mean reflection intensity, $\langle I_i \rangle$). 98% of the reflections expected at the maximal resolution are present in the data set. Symmetry-equivalent reflections with $I > \sigma(I)$ were measured 2.4 times on average over the entire resolution range. Molecular replacement will be used to determine the three-dimensional structure of the RNA heptamer. In this short communication we wanted to demonstrate that RNA synthesized from H-phosphonates can be used not only for NMR investigations, reported by Limmer *et al.* (1993) and Ott *et al.* (1993), but can also be analyzed by X-ray crystallography. The crystals of the acceptor stem of $tRNA^{Ala}$ from *E. coli* were extremely stable in the X-ray beam and diffracted to a resolution suitable for three-dimensional structure determination.



(*a*)

1 2 3

(*b*)

Fig. 2. (*a*) One of the crystals used for X-ray investigations; (*b*) analytical 20% PAGE in the presence of 7 *M* urea of the two chemically synthesized single strands of the acceptor stem of $tRNA^{Ala}$ from *E.coli* (lane 1: $^{5'}$GGGGCUA$^{3'}$ and lane 3: $^{5'}$UAGCUCC$^{3'}$) and of the duplex obtained from dissolved crystals (lane 2). The slower migration of the single strand in lane 1 is due to its high content of guanosines.

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