Crystal structure of acceptor stem of tRNA^{Ala} from *Escherichia coli* shows unique G•U wobble base pair at 1.16 Å resolution

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ABSTRACT

The acceptor stem of *Escherichia coli* tRNA^{Ala}, rGGGGCUA•rUAGCUCC (ALA^{wt}), contains the main identity element for the correct aminoacylation by the alanyl tRNA synthetase. The presence of a G3•U70 wobble base pair is essential for the specificity of this reaction, but there is a debate whether direct minor-groove contact with the 2-amino group of G3 or a distortion of the acceptor stem induced by the wobble pair is the critical feature recognized by the synthetase. We here report the structure analysis of ALA^{wt} at near-atomic resolution using twinned crystals. The crystal lattice is stabilized by a novel strontium binding motif between two *cis*-diolic O3'-terminal riboses. The two independent molecules in the asymmetric unit of the crystal show overall A-RNA geometry. A comparison with the crystal structure of the G3-C70 mutant of the acceptor stem (ALA^{C70}) determined at 1.4 Å exhibits a modulation in ALA^{wt} of helical twist and slide due to the wobble base pair, but no recognizable distortion of the helix fragment distant from the wobble base pair. We suggest that a highly conserved hydration pattern in both grooves around the G3•U70 wobble base pair may be functionally significant.

Keywords: aminoacyl synthetase specificity; ion binding to RNA; non-Watson–Crick base pair; protein–RNA recognition; RNA hydration; transfer RNA; X-ray crystallography

INTRODUCTION

Every tRNA carries the code for its amino acid as a trinucleotide sequence within the anticodon loop. For correct aminoacylation of their cognate tRNA, many aminoacyl-tRNA synthetases do not rely on this code for recognition but require identity elements within the acceptor stem of the tRNA (Arnez & Moras, 1997). The main identity element in tRNA^{Ala} for recognition by *Escherichia coli* alanyl-tRNA synthetase (AlaRS) is the G3•U70 base pair (Hou & Schimmel, 1988; McClain & Foss, 1988), in particular the free N2 amino function facing the shallow minor groove of the helix (Musier-Forsyth et al., 1991, 1995; Beuning et al., 1997b). If G3•U70 is changed to G3-C70 or A3-U70 or isosterically to I3•U70, the tRNA is no longer a substrate for AlaRS (Hou & Schimmel, 1988; McClain & Foss, 1988;

Musier-Forsyth et al., 1991). The specificity of AlaRS is independent of the anticodon loop (Park & Schimmel, 1988). Instead, chemically synthesized RNA fragments mimicking the acceptor stem of tRNA^{Ala} can be specifically aminoacylated by AlaRS in vitro (Francklyn & Schimmel, 1989). Specific recognition of tRNA acceptor stem-derived oligonucleotides is not restricted to AlaRS, but has been demonstrated for a number of synthetases from both structural classes (Schimmel, 1993; Saks & Sampson, 1996). The correspondence of a specific amino acid to an acceptor stem oligonucleotide has been proposed to be a remnant of a primordial operational RNA code for amino acids (Schimmel et al., 1993).

The replacement of the G3•U70 wobble base pair by some base–base mismatches or its shift to an adjacent position in the tRNA acceptor stem yields substrates that can be aminoacylated by AlaRS to some extent (McClain et al., 1988). This prompted the proposal that, in addition to the “direct” recognition of the G3•U70 within a regular A-type helix, a helical distortion induced by G3•U70 but distant from the wobble base pair is a further element of “indirect” recognition (Gabriel

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This article is dedicated to Prof. W. Saenger on the occasion of his 60th birthday.

et al., 1996; McClain et al., 1996). Considerable effort has been invested to establish a suitable model for the recognition process both in vitro and in vivo (McClain et al., 1988, 1996; Gabriel et al., 1996; Beuning et al., 1997b).

To establish the determinants of tRNA^{Ala} identity, two independent NMR studies of tRNA^{Ala} microhelices were performed. One proposed distorted models of A-form RNA with a partial C2'-endo sugar pucker at U70 (Limmer et al., 1996). In the other study, a distortion of the phosphodiester backbone at position G3 together with a distinct G1•C72:A73 stacking interaction was reported (Ramos & Varani, 1997). Taken together, the NMR work favors the model of indirect recognition of the tRNA acceptor helix by AlaRS.

Here we present the crystal structure at near-atomic resolution of the 7-bp acceptor stem of *E. coli* tRNA^{Ala} (rGGGGCUA•rUAGC UCC, ALA^{wt}), which provides a detailed look at the geometry of this unique fragment (Fig. 1). The direct comparison with the 1.4-Å crystal structure of the C70 mutant (rGGGGCUA•rUAGCCCC, ALA^{C70}) allows us to analyze the contribution of the G•U wobble base pair in terms of helical distortion of the A-form RNA double helix.

RESULTS

Overall RNA structures and crystal packing

Both ALA^{wt} and ALA^{C70} crystallize with two independent molecules (A and B) per asymmetric unit. All four individual RNA molecules exhibit overall A-form geometry as judged from the number of base pairs per helical turn (10.9–11.3), the mean rise (2.81–2.91 Å), inclination (6.6–10.6°), and displacement (3.86–4.23 Å)

FIGURE 1. Acceptor stem of *E. coli* tRNA^{Ala}. The G3•U70 wobble base pair highlighted in red is the main recognition element for alanyl-tRNA synthetase. The insert shows the numbering scheme adopted in this paper.

of the base pairs with respect to a curved helix axis (Lavery & Sklenar, 1989). All sugar puckers are in C3'-endo conformation except for one C2'-exo conformer at G1 of the ALA^{wt}-A molecule.

The two RNA heptamer helices of ALA^{wt} that constitute the asymmetric unit of the triclinic unit cell form continuous double helices within the crystal, with an end-to-end arrangement of duplexes (Fig. 2). At the junction, the helices stack onto each other with a negative helical twist of -17.2° . Because of this arrangement, the continuous helices produced by crystal packing display a wide-open major groove uncharacteristic of A-type duplexes. They are oriented along the short body diagonal of the P1 unit cell. ALA^{C70} is packed similarly in its crystals.

In both ALA^{wt} and ALA^{C70} the phosphate backbone torsion angles are distributed around the A-form average and exhibit no significant deviation from it. This holds true also for nucleotides G3 of ALA^{wt}, whose α/γ torsion angles remain within the common (–)gauche/(+)gauche regime, unmodulated by wobble base pairing that has been reported to stabilize an extended backbone with α/γ trans/trans (Biswas et al., 1997; Ramos & Varani, 1997). The P–P distances along the strands remain between 5.60 and 6.16 Å in all four duplexes. Neither the average displacement nor the average slide of base pairs are affected by the presence or absence of G3•U70.

The least-squares superposition of all possible pairs of RNA duplexes indicates a structural polymorphism of the RNA helices (Table 1) that is probably related to crystal packing. Judging from root-mean-square deviations of equivalent atoms, the two copies of ALA^{wt} are no more similar to each other than is ALA^{wt} to ALA^{C70}. The maxima of structural difference between ALA^{wt} and ALA^{C70} are located in the region of the mutation site where the wobble arrangement influences the local geometry of the helix. In other words, the overall geometry of all compared molecules shows no sequence-dependent deviation except for the C70 mutation and the resulting base pairing itself.

TABLE 1. Least-squares superposition of RNA molecules^a

	ALA ^{wt} -B	ALA ^{C70} -A	ALA ^{C70} -B	NMR model ^b
ALA ^{wt} -A	0.883	0.629	0.653	1.298
ALA ^{wt} -B		0.817	0.695	1.342
ALA ^{C70} -A			0.458	1.395
ALA ^{C70} -B				1.349

^aRoot-mean-square deviation (Å) after least-squares superposition of all equivalent RNA atoms.

^bRepresentative conformer from the ensemble of structures described by Ramos & Varani (1997) selected with LSQMAN (Kleywegt, 1996). In the NMR model, base pair A7-U67 of tRNA^{Ala} is replaced by a C-G base pair sealed with a UUCG tetraloop and the ACCA end is present.

FIGURE 2. Overall structure and crystal packing in the triclinic unit cell of ALA^{wt}. The two independent copies of the heptamer helix stacking atop each other with retrograde twist are shown in red and green (left). Unit cell translation generates continuous double helices. The resultant double helical columns along the short body diagonal of the unit cell (right) are stabilized by the interhelical binding of Sr²⁺ ions (purple). All molecular drawings prepared with BobScript (Esnouf, 1997) and Raster3D (Merritt & Murphy, 1994).

The G•U wobble base pair

The G3•U70 pair of ALA^{wt}-A is depicted with the nearest water coordination shell in Figure 3 (top). It exhibits the expected wobble arrangement, pointing the free N2 amino group into the minor groove and displaying a shear value of 2.8 Å. The N2 forms a hydrogen bond to a water molecule that is also involved in hydrogen bonding to O2 and O2' of U70. An intermolecular contact to an O2' of the backbone of a symmetry-related RNA molecule saturates the second donor function of the free amino group. The angle between the two acceptor oxygens and the N2 of G3 is 74°. Coming from the major-groove side, two water molecules saturate the hydrogen bonding potential of the wobble base pair. They bind to O4 of U70 and O6 of G3, and N7 of G3, respectively, and are hydrogen bonded to each other. ALA^{wt}-B displays a nearly identical wobble geometry and hydration pattern with perfect conservation of all hydrogen bonds of the G•U pair. Again, the free amino group of G3 is further engaged in an intermolecular hydrogen bonding contact to an O2' at an angle between the nitrogen and the two acceptor oxygens of 93°. The water molecule filling the minor groove cavity created by the wobble arrangement has also been found in tRNA and in other high resolution X-ray structures of G•U pairs (Westhof, 1987; Biswas et al., 1997). Therefore it must be regarded as an integral part of the double helical structure. Based on the present

high-resolution structure, we suggest that the same holds true for the two major-groove water sites. The structure of the G3-C70 base pairs from the ALA^{C70} mutant (Fig. 3, bottom) does not differ from the other G-C base pairs present in the molecules. In the two independent copies of ALA^{C70}, the hydration patterns show a smaller degree of conservation than the G3•U70 pairs in ALA^{wt}.

An analysis of helical parameters (Table 2) was performed to investigate to what extent the structural perturbation afforded by G3•U70 propagates along the Watson–Crick duplex. The wobble arrangement is directly reflected in the shear values at G3•U70, but other base–base parameters remain unaffected. A significant modulation of the helical twist is observed (Fig. 4). Both ALA^{wt} duplexes are underwound with a twist of 23.5° (26.9°) at the step G2-C71/G3•U70 and overwound with 41.5° (35.8°) for the base-pair step G3•U70/G4-C69. The slide value shows the same modulation because it is correlated with the change in twist. ALA^{C70} does not show this modulation. The minor groove does not change its width of approximately 9.9 Å when the G3•U70 base pair is replaced with G3-C70. All other helical parameters vary around typical A-form RNA values and show no sequence-dependent differences between the structures. Therefore, this X-ray analysis fails to provide evidence for a structural perturbation imprinted by the unique G•U wobble base pair on the tRNA^{Ala} acceptor helix.

FIGURE 3. G3•U70 wobble base pair of ALA^{wt}-A with $2dF_o - mF_c$ difference electron density contoured at 1.2σ (top). Interbase hydrogen bonds are shown as heavy broken lines (green); all other hydrogen bonds are shown as thin broken lines (red). H-bond donor-acceptor distances are given in angstrom units. The hydration pattern of the G•U base pair with one water molecule hydrogen bonded to three functional groups in the minor groove and two water molecules hydrogen bonding to groups in the major groove is identical in both independent molecules of the asymmetric unit. Bottom: G3-C70 base pair of ALA^{C70}-A with $2dF_o - mF_c$ difference electron density contoured at 1.5σ . The G3-C70 base pair of ALA^{C70}-B is hydrogen bonded to three water molecules in the major groove.

Metal ion binding

In ALA^{wt} we observe a unique cation binding site where the O3'-terminal riboses of two RNA duplexes chelate a Sr²⁺ ion. The coordination sphere of the cation is completed by five water molecules (Fig. 5). The cation sits at the center of a slightly distorted trigonal prism

TABLE 2. Conformational parameters^a

		X Disp dx (Å)	Inclination η (°)	Twist Ω (°)	Rise Dz (Å)	Slide Dy (Å)
ALA ^{wt} -A	mean	-3.95	8.96	32.99	2.83	-0.19
	σ	0.20	1.01	5.75	0.20	0.45
ALA ^{wt} -B	mean	-3.92	7.63	31.74	2.88	-0.12
	σ	0.29	1.36	2.94	0.24	0.31
ALA ^{C70} -A	mean	-3.86	6.60	32.29	2.91	-0.14
	σ	0.23	0.71	2.35	0.11	0.21
ALA ^{C70} -B	mean	-4.23	10.58	32.59	2.81	-0.10
	σ	0.22	0.87	1.33	0.24	0.26
NMR model ^b	mean	-3.77	16.43	31.97	2.84	0.28
	σ	0.34	5.13	4.45	0.43	0.36
Fiber model ^c	mean	-5.14	22.66	32.70	2.55	0.00

^aCalculated with CURVES (Lavery & Sklenar, 1989). Mean values and their standard deviations are shown for selected parameters in the two independent molecules of both ALA^{wt} and ALA^{C70}.

^bRepresentative conformer from the ensemble of NMR structures described by Ramos & Varani (1997) selected with LSQMAN (Kleywegt, 1996).

^cCoordinates for calf thymus DNA in A conformation (Chandrasekaran & Arnott, 1989)

formed by the 3'OH groups and four water oxygens. A fifth water and the 2'OH functions are located above the rectangular faces of the prism. All Sr²⁺-oxygen distances are between 2.5 and 2.7 Å. With 47 Å², the *B* value of the unique water oxygen that defines a corner of the prism is about twice as large as those of the other coordinating oxygens.

The interhelical mode of Sr²⁺ binding (see also Fig. 2) is thought to afford a stabilization of the RNA crystal and thus to permit acquisition of near-atomic resolution X-ray diffraction data. As of now, very little is known about strontium-RNA interactions. Sr²⁺ has been shown to play a role in folding of and RNA cleavage by the group I intron-derived *Tetrahymena* ribozyme if it is added in millimolar concentrations (Celander & Cech, 1991; Chowrira et al., 1993; Streicher et al., 1996), but its binding to RNA has never been demonstrated directly in a structural study. In addition to the Sr²⁺ site, several Na⁺ ions were identified in the crystal structures of ALA^{wt} and ALA^{C70} on the basis of short contacts of about 2.3 Å to oxygen atoms and unusually low *B* factors of about 5–7 Å² when treated as water sites.

DISCUSSION

Here we describe the crystal structure analysis at near-atomic resolution of a 7-bp RNA duplex corresponding to the acceptor stem of *E. coli* tRNA^{Ala}. This small RNA fragment carries the main determinants for specific recognition by AlaRS (Hou & Schimmel, 1988; McClain & Foss, 1988). It was hoped that a high-resolution crystal structure could contribute to resolving a controversy about the role of the unique G3•U70 wobble base pair in tRNA^{Ala} that might either be recognized directly by

FIGURE 4. **A:** Selected helical parameters of ALA^{wt} and ALA^{C70} according to CURVES (Lavery & Sklenar, 1989). Shear, twist and slide are given for both copies of both RNA heptamers. The prominent shear of G3•U70 is a direct consequence of the wobble arrangement and the clearest discriminator between ALA^{wt} and ALA^{C70}. **B:** Structural superposition of ALA^{wt}-A (red) and ALA^{C70}-A (green) after least-squares fit.

AlaRS (Musier-Forsyth et al., 1991, 1995; Beuning et al., 1997b) or indirectly through introducing a structural perturbation into the acceptor stem (McClain et al., 1988, 1996; Gabriel et al., 1996). The structure may be of interest also in a more general sense, because for the first time it shows a single G•U base pair at near-atomic resolution in a synthetic RNA double helix that is undisturbed by end effects or neighboring mismatches.

An important feature of the ALA^{wt} crystal structure is the geometry of the G•U base pair and its hydration. In the two independent molecules of the unit cell, both the wobble geometry and the hydration pattern with one water firmly bound in the minor groove and two major-groove water sites are perfectly conserved. These three waters, therefore, must be considered integral parts of the tRNA^{Ala} acceptor stem structure. They will influence the energetics of specific acceptor stem binding by AlaRS either by forming part of the protein–RNA

interface or by being displaced from their binding sites and released into the bulk solvent. Direct biochemical evidence for the involvement of water molecules in immediate vicinity to G3•U70 in minor-groove interactions has been presented (Henderson et al., 1998).

The presence of two independent RNA duplexes in the crystal structures of both ALA^{wt} and ALA^{C70} provides a welcome check as to whether structural variations along the double helix are imprinted by the nucleotide sequence or result from other effects such as crystal contacts. This is crucial with respect to the direct versus indirect recognition controversy surrounding tRNA^{Ala} binding by AlaRS. A careful examination of the crystal structures along these lines reveals a clear structural difference between the duplexes at sequence position 3/70, but hardly any features outside the wobble position by which ALA^{wt} would systematically and consistently differ from ALA^{C70}. In particular, the wild-type helix and the C70 mutant show an indistinguish-

FIGURE 5. **A:** Strontium coordination between two *cis*-diolic groups of 3'-terminal ribose units. $2dF_o - mF_c$ difference electron density is shown at 15σ around the Sr^{2+} ion (yellow) and at 2.5σ for the rest of the structure (blue). At this level, one water oxygen with elevated atomic displacement factor remains without density. **B:** The nine coordinating oxygen atoms from four hydroxyl groups and five waters (blue spheres) sit at the corners of a slightly distorted trigonal prism or reside above the rectangular faces of the prism.

able global minor groove geometry and a standard A-form backbone throughout. This latter observation is at variance with earlier NMR evidence (Limmer et al., 1996; Ramos & Varani, 1997) of a more variable and distorted conformation. Taken together, the high-resolution crystal structures appear to agree better with models emphasizing a direct interaction of AlaRS with the G3•U70 wobble base pair of microhelices (Hou & Schimmel, 1988; Musier-Forsyth et al., 1991, 1995; Beuning et al., 1997b) than with a line of thought implicating an (additional) structural distortion of the acceptor stem. In view of the distinct water structure around G3•U70, it seems likely that the wobble base pair is recognized in its specifically hydrated form. In the case of the complete tRNA, we cannot exclude, of course, that the presence of the ACCA end might influence the conformation of the acceptor stem. The notion of a

central importance of the G•U wobble base pair is, however, reinforced by studies demonstrating its significance for AlaRS binding over a wide range of organisms (Shiba et al., 1995; Chihade et al., 1998).

An apparent paradox rests in the significance of the G3 amino group for binding of AlaRS to tRNA^{Ala} and to microhelices derived from it (Musier-Forsyth et al., 1991, 1995; Beuning et al., 1997b), as it is widely accepted (Arnez & Moras, 1997) that class II tRNA synthetases approach the acceptor stem from the major-groove side. The importance of the minor groove for this interaction is emphasized by the observation in the present study of a water molecule firmly bound by three hydrogen bonds next to the exposed amino group. It must be concluded that, in addition to the dominant minor-groove discrimination, AlaRS makes use of major-groove contacts as was shown recently by atomic group

replacements at the first base pair of the tRNA^{Ala} acceptor stem (Beuning et al., 1997a).

MATERIALS AND METHODS

Crystallization and X-ray diffraction data

The synthesis and crystallization of ALA^{wt} was reported earlier (Ott et al., 1996). The heptamer rUAGCCCC was synthesized accordingly, hybridized to rGGGGCUA to yield ALA^{C70}, and crystallized by hanging-drop vapor diffusion at 21 °C after initial screening (Berger et al., 1996) from 40 mM sodium cacodylate, pH 6.0, 12 mM spermine tetra-HCl, 100 mM KCl, 10% (v/v) 2-methyl 2,4-pentanediol (MPD), mixed 1:1 with 1 μ L of 0.5 mM RNA duplex. The reservoir consisted of 35% (v/v) MPD. Because of the high MPD concentration in the droplet after equilibration, the solution was suitable for cryo-cooling experiments.

The diffraction experiment yielding the best X-ray data was performed at a temperature of 100 K at beamline X11, EMBL Outstation at DESY, Hamburg, using a MAR Research imaging plate detector (300 mm diameter) on a 200 $\times$ 100 $\times$ 50 μ m³ crystal of ALA^{wt}. Data processing, unit cell, and space group determination were done with DENZO/SCALEPACK (Otwinowski & Minor, 1997). Initially, the data were indexed in space group C2 with $a = 33.0$ Å, $b = 47.6$ Å, $c = 26.2$ Å, $\beta = 101.9^\circ$. After identification of pseudomerohedral twinning (see below) the diffraction pattern was reindexed in space group P1 with $a = 26.3$ Å, $b = c = 28.9$ Å, $\alpha = 110.5^\circ$, $\beta = \gamma = 96.9^\circ$. The reindexed and merged data had an overall R_{sym} of 9.9% for 23,758 unique reflections and 54% for the highest resolution shell between 1.17 and 1.16 Å with an overall completeness of 85.5% between 15 and 1.16 Å. Diffraction data from an ALA^{C70} crystal were collected on a 180-mm MAR Research imaging plate detector. Again, the reflections could be indexed in space group C2, but were reindexed in P1 (Table 3) after detection of pseudomerohedral crystal twinning.

Structure solution and refinement

The structure of ALA^{wt} was solved by a SIRAS phasing approach through the analysis of a derivative RNA duplex containing 5-iodouridine at sequence position 6 of ALA^{wt}. Details of the structure analysis including detection and treatment of crystal twinning will be published elsewhere (U. Mueller, Y.A. Muller, R. Herbst-Irmer, M. Sprinzl & U. Heinemann, submitted). Briefly, a SIRAS map could be interpreted in terms of a disordered structure where heptameric RNA duplexes were superimposed onto each other after a 180° rotation about the molecular pseudodyad traversing the central G-C base pair. The refinement of the disordered model in C2 was possible, but a better description of the structure could be obtained through twin refinement of two duplexes in the P1 cell using the SHELXL-97 (Sheldrick & Schneider, 1997) refinement program, which is capable of handling this kind of twinning (Herbst-Irmer & Sheldrick, 1998).

A few first cycles of rigid body, positional, and isotropic displacement factor refinement with data up to 1.8 Å gave R/R_{free} values of 24.9%/30.8% with application of similarity restraints for the riboses and the phosphodiester backbone and standard bond length and angle restraints for the aro-

TABLE 3. Diffraction data and refinement statistics

	ALA ^{wt}	ALA ^{C70}
Space group	P1	P1
Cell edges (Å)	26.2/28.9/28.9	26.7/26.7/30.5
Cell angles (°)	110.5/96.9/96.9	104.3/104.3/91.7
Radiation source	DESY X11	DESY BW7A
Wavelength (Å)	0.907	1.021
Resolution range (Å)	15–1.16	15–1.4
Crystal mosaicity (°)	0.6	1.2
Unique observations	23,758	13,834
Completeness ^a (%)	88.4/75.7	89.5/80.7
Multiplicity	2.7	1.6
$\langle I/\sigma(I) \rangle^{a,b}$	12.6/1.5	11.4/2.3
$R_{\text{sym}}^{a,c}$ (%)	9.9/54.0	5.7/28.8
Molecules per asymmetric unit	2 duplexes	2 duplexes
Final R/R_{free}^d (%)	12.4/18.0	15.8/21.8
RNA atoms	586	586
Water oxygen atoms	149	134
rmsd ^e bond lengths (Å)	0.007	0.028
rmsd ^e bond angle distances (Å)	0.019	0.010
rmsd ^e base planes (Å)	0.032	0.020
Positional esd ^f from least-squares matrix (Å)	0.041	0.053

^aResolution shells 15–1.16 Å/1.17–1.16 Å for ALA^{wt} and 15–1.40 Å/1.45–1.40 Å for ALA^{C70}.

^b I : reflection intensity.

^c $R_{\text{sym}} = 100 \times (\sum_{h,i} |I_{h,i} - I_h| / \sum_{h,i} I_{h,i})$ where the summation is over all observations $I_{h,i}$ contributing to the reflection intensities I_h .

^d R_{free} (Brünger, 1992) based on 5% of the data selected from thin resolution shells with DATAMAN (Kleywegt & Jones, 1996).

^ermsd: root-mean-square deviation, here with respect to RNA library obtained from <http://ndb-mirror-2.rutgers.edu/NDB/archives/proj/standards.html>.

^fesd: estimated standard deviation.

matic bases. Base planarity and hydrogen bonding of the base pairs were restrained over the whole period of refinement. During the refinement a bulk solvent correction according to Babinet's principle (Moews & Kretsinger, 1975) was applied to the data and low resolution data below 15 Å were omitted. A very prominent 20 $\sigma F_o - F_c$ peak could be assigned as a Sr²⁺ cation that improved the model to R/R_{free} of 21.4%/27.8%. Extending the data stepwise to 1.16 Å and using restrained anisotropic displacement factors for all model atoms led to the final structure of 586 RNA atoms in two duplexes, one strontium and one sodium cation, and 143 fully occupied water oxygen atoms plus 11 half occupied waters that yielded an R value of 12.4% and an R_{free} value of 18.0%.

The structure of ALA^{C70} was first solved in space group C2 with AMORE (Navaza, 1994) using a canonical A-form RNA model with the appropriate sequence. At this stage, twinning was not anticipated, since the cell dimensions differ from those of ALA^{wt}. Refinement in space group C2 up to high resolution converged at R/R_{free} of 15.9%/22.1%, but showed considerable $F_o - F_c$ density within the base-pair planes of G1-C68, G4-C71, and A7-U74, where a pseudo twofold symmetry perpendicular to the central base pair changes the purine-pyrimidine pattern. This indicated the presence of twinning in this crystal form, too. By analogy to the protocol followed for ALA^{wt}, the data were reindexed in P1 and the structure was refined anisotropically, making use of the twin matrix. After subsequent addition of 133 fully occupied water

oxygen positions and two sodium cations, the final R/R_{free} was 15.8%/21.8%. The last round of refinement was performed using all 13,695 reflections between 15 and 1.4 Å, which led to $R = 16.0\%$. Coordinates and structure factor amplitudes for both ALA^{wt} and ALA^{C70} were deposited with the Nucleic Acid Data Bank (NDB), accession codes AR0009 and AR0010, respectively.

ACKNOWLEDGMENTS

We are grateful to A. Gonzalez and V. Lamzin for help with diffraction data collection at DESY, Hamburg, and to P. Schimmel (Scripps Research Institute, La Jolla, California), H. Sklenar, and Y.A. Muller (MDC, Berlin) for helpful comments on the manuscript. Supported by the Deutsche Forschungsgemeinschaft within the Schwerpunktprogramm RNA-Biochemie under He 1318/12 (U.H.) and Sp 243/5-3 (M.S.) and the Fonds der Chemischen Industrie.

Received December 23, 1998; returned for revision January 27, 1999; revised manuscript received February 2, 1999

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