CRYSTALLIZATION AND MOLECULAR PACKING OF E. COLI ARGININE TRANSFER RNA[†]

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SUMMARY

Reproducible conditions for the crystallization of *E. coli* arginine tRNA are described. The crystals belong to the trigonal space group $P3_121$ or $P3_221$ with two independent molecules in the asymmetric unit and twelve molecules in the unit cell. The unit cell dimensions are $a = b = 99.4$ Å, $c = 92.9$ Å. It appears that the long axes of the molecules are parallel to the c axis of the crystal. A packing of the molecular projections in the base plane is proposed which is consistent with the experimental Patterson function.

INTRODUCTION

Single crystal diffraction studies of tRNA by a number of different investigators are currently in progress (1-9). These efforts have not yet unraveled the conformation of the molecule but the crystallographic results and other evidence favor a prolate molecule with an axial ratio of roughly 3 to 1. The molecule is assumed to be 80 or 90 Å long and to have a maximum transverse dimension of about 35 Å. It is usually assumed that the molecule has an extended double helical core parallel to its long axis. This results from the stacking of two or more of the double helical arms along the same helix axis.

In this report we describe conditions for growing single crystals of argining tRNA from *E. coli*. A two dimensional molecular packing scheme has been deduced from an analysis of the hk0 X-ray diffraction data.

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EXPERIMENTAL

Using the vapor equilibration system previously described (4) arginine tRNA from E. coli[1] has been crystallized. The crystals were grown in 10 µl droplets that initially contained 178 OD_{260}/ml E. coli arginine tRNA, 0.05 M NH_4Cl, 0.005 M Na-cacodylate (optional), pH 6.0, 0.01 M $MgCl_2$, 0.01 M $HgCl_2$, 0.01 M spermidine (optional), and 10% saturated $(NH_4)SO_4$.[2] For growth at 8° C the sample droplet was equilibrated against a reservoir containing 34% saturated $(NH_4)_2SO_4$ or for growth at room temperature against a reservoir of 40% saturated $(NH_4)_2SO_4$. Crystals grown at these two temperatures differ morphologically but give indistinguishable X-ray diffraction patterns. Most of the X-ray diffraction data were from crystals grown at room temperature.

Conditions identical to those described above but lacking the $HgCl_2$ only occasionally lead to crystallization. The optimum ratio of Hg^{++} to tRNA is between 2.5 and 25 mercury ions per tRNA molecule. Omilianowski (10) has shown that in the pH range 7 to 9, the first molecule of Hg^{++} bound by $tRNA^{Arg}$ interacts with the 4-thiouracil at the eighth nucleotide from the 5' terminus. If there are additional binding sites, they bind Hg^{++} much less strongly than this interaction. Several pure tRNA species from E. coli are known to contain 4-thiouracil at this locus.

We have grown crystals of phenoxyacetylarginyl-$tRNA^{Arg}$ under the same conditions as the uncharged tRNA species. These crystals are generally smaller and less well defined than the arginine tRNA crystals but they show the same growth habit. In addition, these crystals show the same strong but not absolute requirement for Hg^{++}

We have also grown crystals of arginyl-$tRNA^{Arg}$ but due to the instability at room temperature and at neutral or alkaline pH, experiments were conducted

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[1] The $tRNA^{Arg}$ used in this work was prepared at the Biology and Chemical Technology Division of the Oak Ridge National Laboratories and supported in part by the National Institutes of General Medical Sciences. The tRNA samples were 70% and 100% pure and were from lots 15-141 and 15-143 respectively.

[2] Ammonium sulfate concentrations are expressed in terms of percent saturation at 8°C regardless of the temperature of the crystallization experiment.

at pH 4.0 and 8°C. The sample, identical to that described above but without the $HgCl_2$, crystallized after equilibration against a reservoir containing 40% saturated $(NH_4)_2SO_4$. These crystals were hexagonal like the others but were much shorter and did not grow reproducibly.

Neither crystals of arginyl-$tRNA^{Arg}$ nor its phenoxyacetyl derivative were large enough to permit collection of X-ray diffraction data.

Preliminary crystallographic data were recorded on film using the precession method (Fig. 1). The Elliott rotating anode X-ray unit and the precession camera were housed at 8°C. Three dimensional diffractometer intensity data up to 6 Å resolution have been measured at room temperature by the θ-2θ scan technique. In the analysis to be described a total of 31 independent hk0 reflections (up to 9.5 Å resolution) were used. These data were corrected for Lorentz and polarization factors. The density of the crystals was measured in a calibrated density gradient of carbon tetrachloride and cyclohexane.

RESULTS AND ANALYSIS

Table I gives the principal crystallographic results. Fig. 2 shows the *c* axis projection of the experimental Patterson map both with (Fig. 2a) and without (Fig. 2b) the strong 110 reflection.

The analysis which locates the molecular projections in the base plane is similar to that carried out by Labanauskas *et al.* (3) who determined the base plane packing for *E. coli* leucine tRNA and yeast formylmethionine tRNA. The tRNA molecule is represented by an eleven base pair RNA double helix (11). Effective scattering charges are assigned to the base and phosphate group of each nucleotide. The ribose moiety is not expected to scatter significantly since its average electron density is close to that of the ammonium sulfate solution. With the axis of the double helix parallel to the *c* axis of the unit cell we computed values of the hk0 structure factors (the 110 reflection was omitted) and of the residual $R = \Sigma||F_{obs}| - |F_{calc}||/\Sigma|F_{obs}|$ where F_{obs} and F_{calc} are the experimental and the computer generated structure factors. The residual R is a function of where the helix axis intersects the base plane.

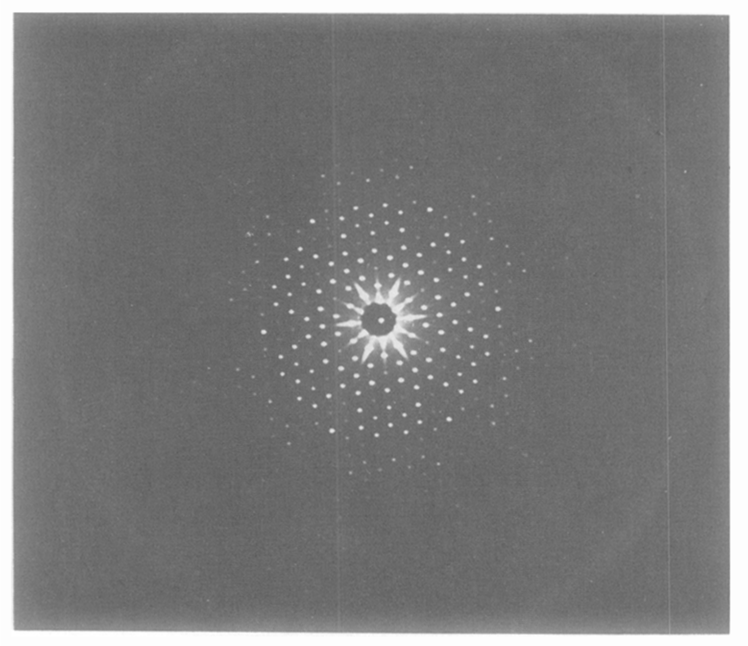

Fig. 1. A 12° precession photograph of the hk0 reflections from a crystal of E. coli arginine tRNA.

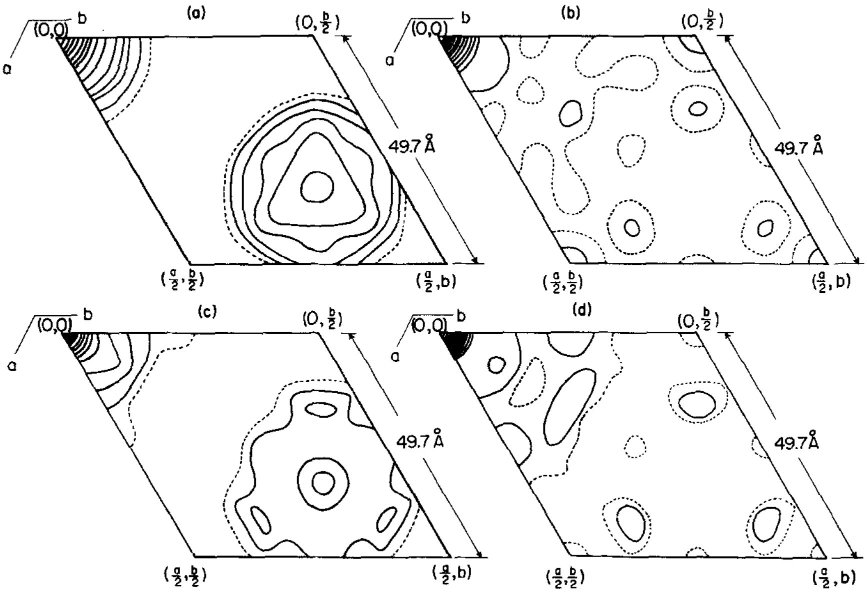

Fig. 2.

TABLE I

CRYSTAL DATA FOR *E. COLI* ARGININE tRNA

Space Group:	$P3_121$ or $P3_221$
Unit Cell:	*a* = *b* = 99.4Å , *c* = 92.9Å
Crystal Density:	1.40 ± 0.02 gm/ml (46% tRNA by mass). Two independent molecules (A and B) in the asymmetric unit; twelve molecules in the unit cell.
Molecular Coördinates:	The helical axes of the two molecules of the asymmetric unit intersect the base plane at x = 0.36*a*, y = 0.10*b* (A) and x = 0.58*a*, y = 0.12*b* (B). See Fig. 3.

Computations were made for a regularly spaced set of intersections which covered the base plane at a 2Å grid interval. Three large minima in R and several small minima were found. Because only one double helix was used this type of search identifies only A-A or B-B vectors. However it is a useful guide for the placing of pairs of double helices.

We next computed residuals and Patterson syntheses for pairs of double helices each one placed at or near a minimum of R found in the first search. Over 100 combinations of positions were considered but only the coördinates given in Table I gave a computed Patterson (Fig. 2d) in good agreement with the experimental Patterson. The 110 structure factor was computed for the coördinates of Table I and found to be very strong in agreement with experiment. The residual is 0.44 both with and without the 110 reflection. The computed Patterson including the 110 reflection is shown in Fig. 2c.

Fig. 2 (a) The *c* axis projection of the Patterson function using the experimental hk0 intensities from a crystal of *E. coli* arginine tRNA. (b) Same as (a) but with the 110 reflection excluded. (c) The *c* axis projection of the Patterson function using the $h\bar{k}0$ structure factors calculated for two 11 base pair RNA double helices placed at the positions in table I. (d) Same as (c) but with the 110 reflection excluded. Contour separations are 7.5% of the maximum at the origin. The zero contours are dashed.

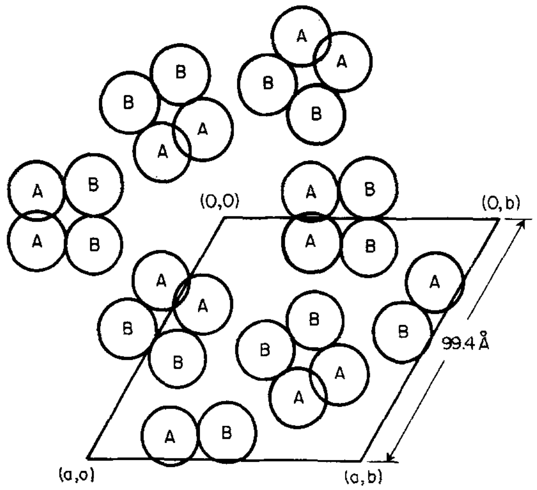

Fig. 3. Packing arrangement in the E. coli arginine tRNA unit cell. The two independent molecules A and B are represented in projection by 20 Å diameter circles.

The packing projected in the base plane is given in Fig. 3. The circles are 20Å in diameter, about the diameter of the double helix, and their centers are the intersections of the helix axes with the base plane.

Some Pattersons were calculated for a model of the entire molecule (12) which of course lacks circular symmetry in projection. In this model three of the four base paired arms are arranged along the same helical axis. When this axis is placed at the positions shown in Fig. 3 the residual (with the 110 reflection included) varies from 0.32 to 0.46 depending on the rotation of the two molecules about the helical axis. Other coordinates for the helix axes gave poorer residuals, never less than 0.48, under similar rotations.

The purpose of these tests was simply to show that our suggested packing is probably correct even if a more realistic model is used. We have not attempted any refinement of the model using the present data.

DISCUSSION

It should be remembered that each circle in the base plane (Fig. 3) represents the projection of a long column of molecules parallel to the c axis. The separation of successive molecules in the column is the c translation (92.9 Å). Adjacent columns can be displaced relative to one another along the c axis so that the thicker parts of the molecules of one column fit between the molecules of the other column.

We see from Fig. 3 that the column projections cluster in groups of four. Within a cluster the axial separation of adjacent columns is about 20 Å, approximately the diameter of the RNA double helix. One may speculate that this separation is imposed by contacts between double helical cores of the molecules of adjacent columns. If each molecule makes such contacts with the molecules above and below it in the adjacent columns the length of the double helical core must be greater than half the c translation, about 46.5 Å. The clustering of the molecular projections into groups of four is a quite definite result of the analysis. More uniform packings gave obviously poorer agreement of the observed and computed Patterson functions.

For *E. coli* leucine tRNA and yeast formylmethionine tRNA it was found (3) that the molecular projections cluster in pairs. The axial separation of the columns in the pair is 12 Å. For these crystals the c translation is 137 Å. Because of the large c translation much weaker restrictions are placed on the fitting together of adjacent columns.

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