the dibasic amino acid system is suggested in these studies. This conclusion can be made from human and rat kidney experiments (*13*), as well as from the cystinuria without lysinuria (*12*) and dibasic amino aciduria without cystinuria in humans (*14*). The dog may be a good model for the study of the etiology of excessive urinary cystine excretion. The mechanism of cystinuria which is enigmatic in humans may be studied more easily in the isolated cystinuric situation as it exists in the canine disorder.

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10. Virtually all of the intracellular form of the radioactivity of lysine and glycine is the same as that of the initial substrate. Cystine-S^{35} when accumulated intracellularly is present as cysteine and glutathione in the kidney and as cysteine in intestinal mucosa [B. States and S. Segal, *Anal. Biochem.* **27**, 373 (1969); I. Smith and S. Segal, *Proc. Nat. Acad. Sci. U.S.*, **63**, 926 (1969)]. The reduction of cystine which occurs rapidly is not associated with the brush-border fraction of intestinal cells [B. States and S. Segal, *Biochem. J.* **113**, 443 (1969)]. The distribution ratio therefore does not represent a concentration gradient for cystine, but the notation is used to represent uptake.
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Structural Studies on Transfer RNA: Crystallization of Formylmethionine and Leucine Transfer RNA's

Abstract. *Improved solvent systems were used to crystallize two different transfer RNA species. These crystals show increased mechanical and thermal stability over crystals obtained previously from a similar system. They have sufficient stability and crystalline order to be used in x-ray crystallographic studies.*

With the vapor equilibration technique (*1*), single crystals of transfer RNA (tRNA) with improved thermal and mechanical stability and higher crystalline order were grown. With aqueous ammonium sulfate as the precipitating agent, two different tRNA's were crystallized. These are leucine (Leu) tRNA from *Escherichia coli* and formylmethionine (fMet) tRNA from yeast. The *E. coli* $tRNA^{Leu}$ and the yeast $tRNA^{fMet}$ crystals were used for x-ray diffraction studies (*2*). Both of these tRNA's have been crystallized from eight solvent systems differing only in the nature of the polyvalent cation present.

The initial conditions for crystallization were 0.05*M* NH_4Cl, 0.005*M* sodium cacodylate *p*H 6.0, 0.005 to 0.0075*M* $MgCl_2$, 0.001 to 0.002*M* polyvalent cation, and 30 percent saturated ammonium sulfate. The initial tRNA concentration was between 4 and 10 mg/ml. The polyvalent cations used were spermine, spermidine, Hg^{2+}, Co^{2+}, Mn^{2+}, Cr^{3+}, Cu^{2+}, and Mg^{2+}. In all cases the chloride salts were used. The above sample (final volume, 10 μl) was then equilibrated in a sealed chamber containing a large and precisely controlled solvent reservoir of aqueous ammonium sulfate between 35 and 65 percent saturation, depending on the species of tRNA to be crystallized and on the temperature of the experiment. All concentrations of ammonium sulfate are expressed in terms of percent saturation at 8°C.

Crystals of *E. coli* $tRNA^{Leu}$ were obtained at 8°C from samples equilibrated against 35 to 40 percent saturated ammonium sulfate (*3*). The initial tRNA concentration was 10 mg/ml. A light precipitate forms in this system within 24 hours and crystals appear within 3 days to 4 weeks. The crystals are tetragonal bipyramids (Fig. 1, a and b). The type of polyvalent cation in no way affects the morphology of the crystals. The density of these crystals was 1.48 ± 0.02 mg/cm^3, as determined by use of a density gradient with carbon tetrachloride and cyclohexane. As judged by x-ray data (*2*), the unit cell is tetragonal with dimensions 46 by 46 by 137 Å. If the partial specific volume for tRNA is 0.53 ml/gm (*4*), the crystals are about 43 percent RNA by volume and contain four molecules per unit cell. These crystals show a strong extinction when viewed between crossed polaroids only when observed across the unique axis (Fig. 1c). The extinction is virtually absent when viewed down the unique axis.

X-ray diffraction indicates that there is a twinning in the crystal structure. Although the crystal quality needs to be improved, the diffraction patterns now available are of sufficient quality to provide information about the structure (*2*).

Crystals of yeast $tRNA^{fMet}$ were grown at 8°C and at room temperature. The sample was prepared as described (*5*) and was 90 to 95 percent pure as judged by amino acid acceptor activity. The crystals appear to be identical under both of the growth condi-

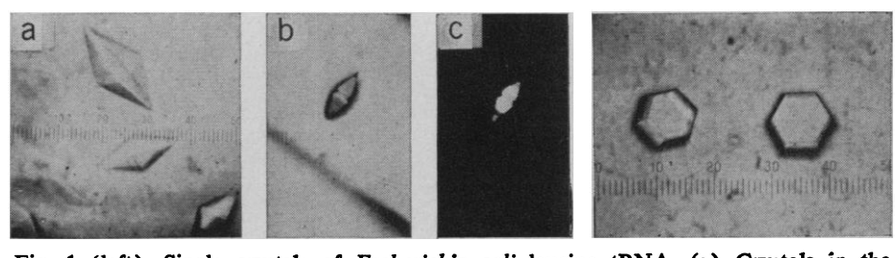

Fig. 1 (left). Single crystals of *Escherichia coli* leucine tRNA. (a) Crystals in the form of tetragonal bipyramids grown with 0.002*M* Hg^{+2} as the polyvalent cation. (b) Single crystals mounted in a quartz capillary ready for x-ray diffraction. (c) Same crystals as in (b) as seen between crossed polaroids. Scale, 0.02 mm per division in all photomicrographs. Fig. 2 (right). Single crystals of yeast methionine tRNA (formylatable) in the hexagonal prism form. These crystals were grown in the presence of 0.0015*M* spermine as the polyvalent cation. Scale, 0.02 mm per division.

tions. When grown at 8°C the sample droplets are equilibrated against a reservoir of 50 percent saturated ammonium sulfate, while those grown at room temperature are equilibrated against 65 percent saturated ammonium sulfate.

The yeast $tRNA^{fMet}$ crystals grow in three main forms: flat diamond-shaped sheets, tetragonal bipyramids, and hexagonal prisms. Only this last form (Fig. 2) has given useful x-ray diffraction patterns. These crystals form within 1 to 2 weeks with no prior formation of a precipitate as in the case of *E. coli* $tRNA^{Leu}$. Since only the hexagonal-prism form has been useful for x-ray diffraction work, conditions were sought to grow this form preferentially. This form can be grown almost to the complete exclusion of the others by slowly (over a 4- to 5-day period) approaching the final concentration of ammonium sulfate in the solvent reservoir with spermine or spermidine as the polyvalent cation in the sample droplet. With the density gradient technique, the density of the hexagonal-prism form of these crystals was 1.29 ± 0.02 g/cm^3. The x-ray data indicate that the unit cell is hexagonal with dimensions 115 by 115 by 137 Å (*2*). Therefore the crystals are about 15 percent tRNA by volume and contain 12 molecules per unit cell. These crystals show the same birefringence as that described for the *E. coli* $tRNA^{Leu}$ crystals.

Crystals were mounted for x-ray diffraction studies by drawing the crystal along with some of the mother liquor into a 0.7-mm quartz capillary. The mother liquor was removed, and the crystal was blotted dry with a filter paper strip. A small volume of solution, identical in composition to the mother liquor but with no tRNA, was added to the end of the capillary which is then sealed with vacuum grease. Crystals of both tRNA's mounted this way have lasted over 100 hours in the x-ray beam with no significant decrease in resolution.

Since this technique consumes only about 0.1 mg of tRNA per experiment, it is possible to survey a large number of crystal growth conditions with a minimum consumption of material. By a survey of the variables described here it has already been possible to significantly improve the thermal and mechanical stability as well as the crystalline order of the tRNA crystals. Efforts are now under way to further improve these crystal characteristics, and when the x-ray data warrant it, this same technique can be used to survey for satisfactory isomorphous replacements.

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Structural Studies on Transfer RNA: The Molecular Conformation in Solution

Abstract. *Small-angle x-ray scattering curves from four different species of transfer RNA in solution indicate that the molecules are of very similar size and shape. A model which has regions of the molecule folded tightly around a long helical core gives good agreement with the observed scattering curves.*

We performed small-angle x-ray scattering studies on dilute solutions of three purified species of tRNA (*1*): $tRNA^{fMet}$ (yeast), $tRNA^{Tyr}$ (yeast), and $tRNA^{Phe}$ (*Escherichia coli*). All samples were studied in 0.1*M* KCl, 0.005*M* $MgCl_2$, and 0.02*M* tris-HCl buffer (*p*H 7.2) at 20° to 25°C. Data were taken on a symmetrical four-slit diffractometer (*2*) with slit separations of 50 cm. Scattered intensities were measured for angles between 5.5 and 180 milliradians (mr), corresponding to Bragg spacings between 280 and 8.6 Å. The experimental curves after correction for slit height smearing effects are shown in Fig. 1a, together with the curve obtained under similar conditions from a fraction of yeast tRNA's rich in $tRNA^{Ala}$ (*3*). The $tRNA^{Ala}$ was dissolved in a buffer containing no $MgCl_2$, but without dialysis. It is probable that Mg^{2+} was present on the tRNA. All four species show very similar scattering curves, supporting the idea that most tRNA's have a similar three-dimensional conformation. We find radii of gyration between 23.5 and 25 Å, in agreement with other values (*3, 4*).

Assuming the general validity of the cloverleaf configuration for tRNA, we constructed Corey-Pauling-Koltun space-filling models; coordinates were assigned, and the theoretical, spherically averaged x-ray scattering curves were calculated and compared with the experimental curves. Coordinates used for the helical regions were those for the 11-fold helix model of reovirus double-stranded RNA (*5*). To reduce the computing time, coordinates were assigned only to the scattering centers of base, sugar, and phosphate groups; scattering factors for the groups were determined by summation of the atomic scattering factors for atoms in water (*6*). We have compared scattering curves calculated for segments of double-helical RNA using atomic coordinates with curves calculated from the group approximation, and we found that the group approximation gives curves which are qualitatively correct out to 160 mr. Bound K^+ ions were assigned to phosphate groups, but scattering due to a water and ion cloud shell was ignored. Since the sequence of nucleotides in the tRNA is unimportant for calculating the small-angle scattering curve, models were built to the form of an average tRNA of 78 nucleotides. Helical regions were poly G·poly C, and unpaired bases were about half purines and half pyrimidines.

In a similar model-building study, Lake and Beeman (*3*) had found that the inner region of the scattering curve, to 50 mr, corresponding to radius of