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CRYSTALLISATION OF $tRNA^{Leu}_{CUG}$ FROM *ESCHERICHIA COLI* AFTER PURIFICATION WITH HYDROXYAPATITE

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SUMMARY

$tRNA^{Leu}_{CUG}$ from *E.Coli* was purified by column chromatography on benzoylated DEAE-cellulose, followed by hydroxyapatite prepared by an improved method. Crystals obtained by vapour diffusion gave X-ray diffraction out to 7 Å in the *hk*O projection and 10 Å in *h*O*l*. The space group was $P4_22_12$ with $a = b = 133$ Å, $c = 66$ Å and 8 molecules in the unit cell. Birefringence showed preferred orientation of RNA helical regions in the *ab* plane.

INTRODUCTION

There are five leucine accepting sub-species of *E.Coli* tRNA (1) which differ in chromatographic behaviour and coding properties. One of these was among the first to be crystallised for study by X-ray diffraction (2). We have purified and crystallised a second sub-species by fractionation on BDC‡ followed by hydroxyapatite (3), the latter being prepared by a new method (4) to give a high flow rate combined with good resolution. Our methods had the advantage of being capable of large yields without chemical modification of tRNA.

The tRNA was identified as $tRNA^{Leu}_{CUG}$ because it was the most abundant leucine acceptor and the first to elute from BDC (1,5). The sequence of its 87 nucleotides has been determined by others, who purified

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‡ BDC = benzoylated DEAE-cellulose.

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it with BDC after prior extraction from a polyacrylamide gel (6) or BDC chromatography of derivatised material (5).

MATERIALS AND METHODS

Crystals were grown at 5°C by vapour diffusion (3) from 2.1 - 2.5 M $(NH_4)_2SO_4$ containing 5 - 10 mM $MgCl_2$, 5 - 10 mM sodium cacodylate (pH 6.0), 1 - 2 mg ml^{-1} tRNA and 2.5 - 10 mM $CoCl_2$ or $NiCl_2$. They were mounted with drops of mother liquor in thin-walled glass or quartz capillaries (Pantak, Windsor, U.K.). X-ray diffraction was carried out in a refrigerated cabinet containing a Supper precession camera fed by a toroidal mirror (7) from a Marconi-Elliott rotating anode generator. The mirror removed radiation other than CuKα, and also reduced the instrumental broadening of maxima.

Amino-acid acceptor assays and polyacrylamide gel electrophoresis were as previously described (3). Birefringence measurements were carried out at 5°C with a quartz wedge compensator.

RESULTS AND DISCUSSION

The crystals were truncated tetragonal prisms of size up to 0.5 x 0.2 x 0.2 mm (3). Fig. 1 shows two diffraction patterns. Resolution was best in the *hk*0 projection, where strong maxima extended to 10 Å and weak ones to 7 Å. In the *h*0*l* projection there were no maxima beyond 10 Å. Soaking trials with possible heavy-atom reagents such as samarium (III) and osmium tetroxide failed to produce significant intensity changes.

The space group was uniquely identified as $P4_22_12$ with cell dimensions $a = b = 133 \pm 1$ Å, $c = 66 \pm 1$ Å. There were differences comparable to the error quoted between crystals grown in $NiCl_2$ and $CoCl_2$. Crystal density measurements were consistent with 8 molecules per unit cell, giving a water content of about 83% and a volume per molecule of 1.45×10^5 Å^3. These conclusions were confirmed by dissolving a crystal in water and measuring the ultraviolet absorbance.

As expected from the symmetry, crystals showed no birefringence when viewed down the *c* axis, but there was a birefringence of about 8×10^{-3} when viewing down the *a* or *b* axis. The slow axis was parallel

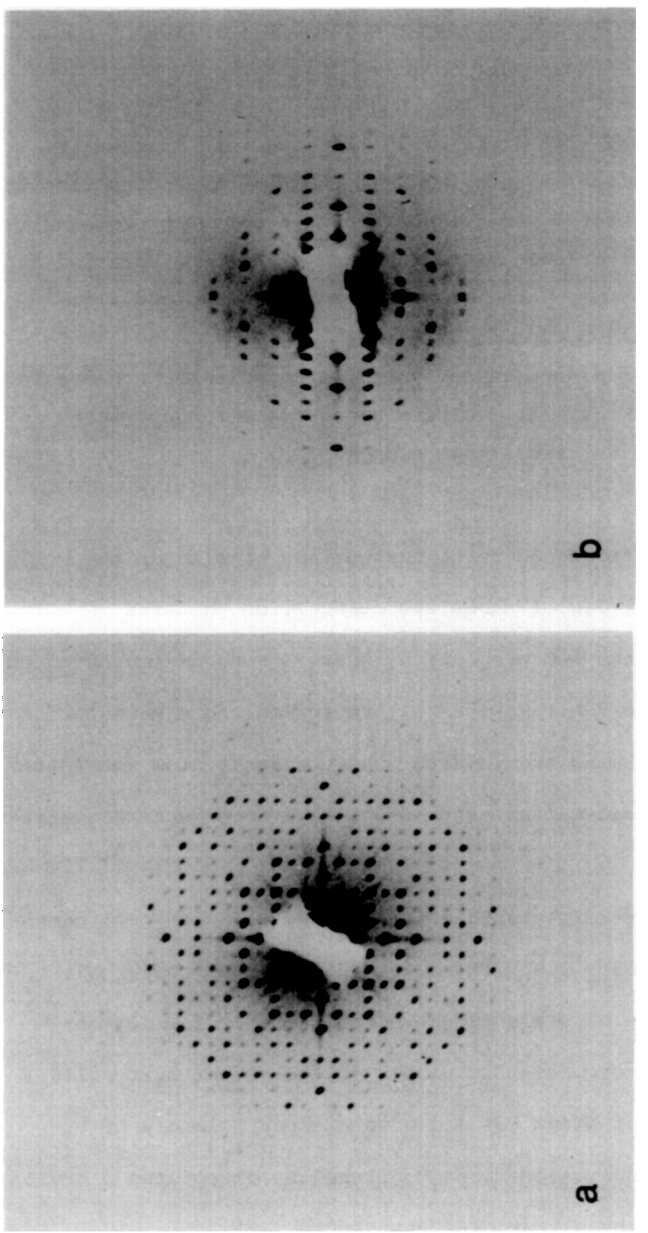

Fig. 1. 12^{o} precession photographs of $tRNA^{Leu}_{CUG}$ from *E.Coli*. Exposure times were about 24 h.

(a) *hkO* projection, crystal grown in presence of $NiCl_2$; a^* and b^* axes are parallel to sides of print.

(b) *hOl* projection, crystal grown in presence of $CoCl_2$; c^* axis vertical.

to the *c* axis, indicating preferred orientation of RNA helical regions in the *ab* plane. A plausible packing scheme would be two layers of four tRNA molecules, stacked above each other in the *c* axis direction.

The leucine-accepting activity of a dissolved crystal was 1700 picomoles per absorbance unit at 260 nm; this was close to the theoretical maximum for a tRNA of 87 nucleotides, and was significantly greater than the value of 1100 given by the purified starting material. Gel electrophoresis also showed a faster-moving minor component (probably $tRNA^{Lys}$) which was absent from the crystals.

The $tRNA^{Leu}$ studied by Young *et al* (2) was described as eluting from BDC late in the NaCl gradient but before the fractions requiring elution with ethanol. It was probably therefore the molecule specific for UUG (1). It crystallised, like $tRNA^{Leu}_{CUG}$, in a tetragonal cell but with different habit, space group and cell constants (8).

If any $tRNA^{Leu}$ could be induced to form better crystals the results of structural analysis would be of particular interest, because all those so far sequenced (5,6,8-11) have unusually large variable loops compared with $tRNA^{Phe}$ from yeast, whose structure is known (12,13). The present results suggest that, although the data are so far of insufficient quality to allow structural analysis to proceed, further efforts with $tRNA^{Leu}$ from *E.Coli* might be rewarding.

ACKNOWLEDGEMENT

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REFERENCES

1. Blank, H.-U., and Söll, D. (1971) J. Biol. Chem. 246, 4947-4950.
2. Young, J.D., Bock, R.M., Nishimura, S., Ishikura, H., Yamada, Y., RajBhandary, U.L., Labanauskas, M., and Connors, P.G. (1969) Science 166, 1527-1532.
3. Spencer, M., Neave, E.J., and Webb, N.L. (1978) J. Chromatogr. 166, 447-454.
4. Spencer, M. (1978) J. Chromatogr. 166, 435-446.

5. Blank, H.-U., and Söll, D. (1971) Biochem. Biophys. Res. Commun. 43, 1192-1197.
6. Dube, S.K., Marcker, K.A., and Yudelevich, A. (1970) FEBS Lett. 9, 168-170.
7. Elliott, A. (1965) J. Sci. Inst. 42, 312-316.
8. Labanauskas, M., Connors, P.G., Young, J.D., Bock, R.M., Anderegg, J.W., and Beeman, W.W. (1969) Science 166, 1530-1532.
9. Pinkerton, T.C., Paddock, G., and Abelson, J. (1973) J. Biol. Chem. 248, 6348-6365.
10. Chang, S.H., Kuo, S., Hawkins, E., and Miller, N.R. (1973) Biochem. Biophys. Res. Commun. 51, 951-955.
11. Randerath, K., Chia, L.S.Y., Gupta, R.C., Randerath, E., Hawkins, E.R., Brum, C.K., and Chang, S.H. (1975) Biochem. Biophys. Res. Commun. 63, 157-163.
12. Rich, A., and RajBhandary, U.L. (1976) Annu. Rev. Biochem. 45, 805-860.
13. Brennan, T., and Sundaralingham, M. (1976) Nucl. Acid Res. 3, 3235-3251.