CRYSTALLIZATION OF YEAST PHENYLALANINE TRANSFER RNA

Barbara S. Vold
Department of Microbiology
Scripps Clinic and Research Foundation
La Jolla, Calif., 92037

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The crystallization of purified yeast $tRNA^{Phe}$ is described. The method involves the exposure of the magnesium salt of tRNA to chloroform at $3^{o}C$. The ability of chloroform to organize water structure may be important to crystallization.

The tertiary structure of tRNA molecules as inferred by indirect methods led to the proposal of a bent clover leaf model (Cramer et al., (1969). Direct X-ray crystallographic analysis, however, would provide definitive evidence. Crystalline tRNA preparations have been reported by several groups, for review see Nature 221, 131 (1969). The formation of microcrystalline E. coli formylmethionine tRNA from dioxane/water solutions was reported by Clark et al. (1968). Cramer et al. (1968) obtained large single crystals of yeast phenylalanine tRNA by this method and E. coli phenylalanine tRNA single crystals have been obtained from ethanol/water solutions by Hampel et al. (1968). We have prepared single crystals of yeast phenylalanine tRNA from chloroform/water mixtures. Although attempts to ship these to Dr. A. Rich for X-ray crystallography failed due to the instability of the crystals, Kim and Rich were able to apply the method to obtain single crystals of E. coli formylmethionine tRNA from which gave clearly discernible diffraction maxima (Kim and Rich, 1968).

METHODS

Purification of yeast $tRNA^{Phe}$

Commercial tRNA was further purified by the method of Lindahl and Fresco (1967) and the $tRNA^{Phe}$ isolated by chromatography on benzoylated DEAE-cellulose (Gillam et al., 1967). The B-DEAE column adsorbs uncharged yeast $tRNA^{Phe}$ whereas all other tRNA's are eluted in a saline buffer; $tRNA^{Phe}$ is then eluted in saline buffer containing 10% ethanol. The ethanol fraction contained a contaminant which did not accept amino acids and had low hypochromicity. Therefore, further chromatography on methylated albumin-silicic acid (MASA) was employed. Tubes with highest phenylalanine acceptor activity were recovered and rechromatographed on MASA, and the tubes with a constant specific activity combined, Figure 1.

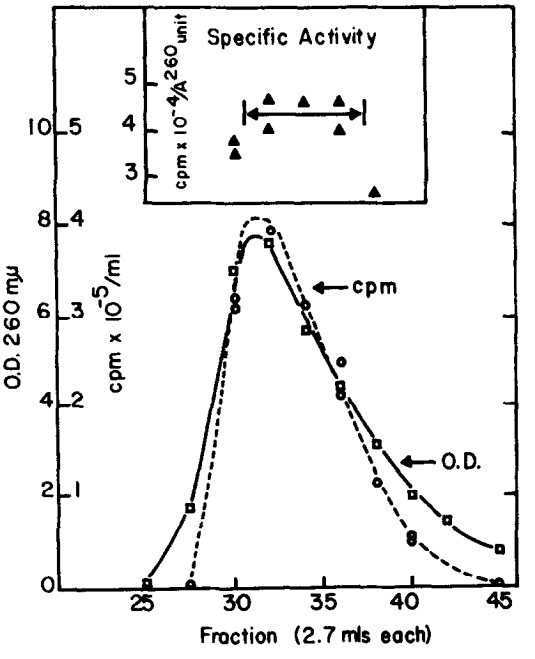

Figure 1. Rechromatography of $tRNA^{Phe}$ on MASA. A 2.4 x 6.6 cm column prepared according to Stern and Littauer (1968) was used with a gradient of 250 ml each of 0.6 and 2 M NaCl in 0.05 M NaAc buffer pH 5.5; flow of 40 ml/hr, 25°C.

The $tRNA^{Phe}$ was 95-100% pure as judged by (1) an acceptor activity for phenylalanine of 1.00 ± 0.05 mole per 1.00 mole RNA, (2) a constant specific activity on rechromatography on MASA, and (3) a typically biphasic melting curve, Figure 2.

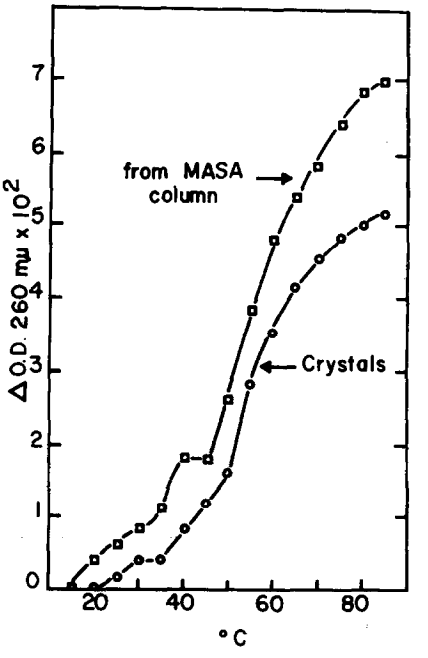

Figure 2. Melting Curves of $tRNA^{Phe}$ from MASA Column and Crystals. Initial O. D. was 0.197 and 0.373 respectively in 0.15 M NaCl, 0.015 sodium citrate, pH 7.0.

Crystallization

Purified $tRNA^{Phe}$, 100 A^{260} units, recovered from an MASA eluate by ethanol precipitation and dried in vacuo, was dissolved in 1 ml deionized water and dialysed extensively against 10 mM $MgCl_2$ at 3°C. The solution was then filtered through a 0.45 μ Millipore Swinnex Filter. One ml of chloroform (Mallinckrodt Analytical Reagent) was added and the mixture placed over anhydrous calcium sulfate (Drierite) in a closed container at 3°C. After two weeks, crystals were found at the interface.

RESULTS AND DISCUSSION

Photomicrographs of two typical crystals are shown in Figure 3. These crystals were transferred from the chloroform:water interface to a microscope slide with a pipette, air dried, and examined at room temperature. The crystals disintegrated slowly. We therefore worked at temperatures below 10°C with as little mechanical damage as possible.

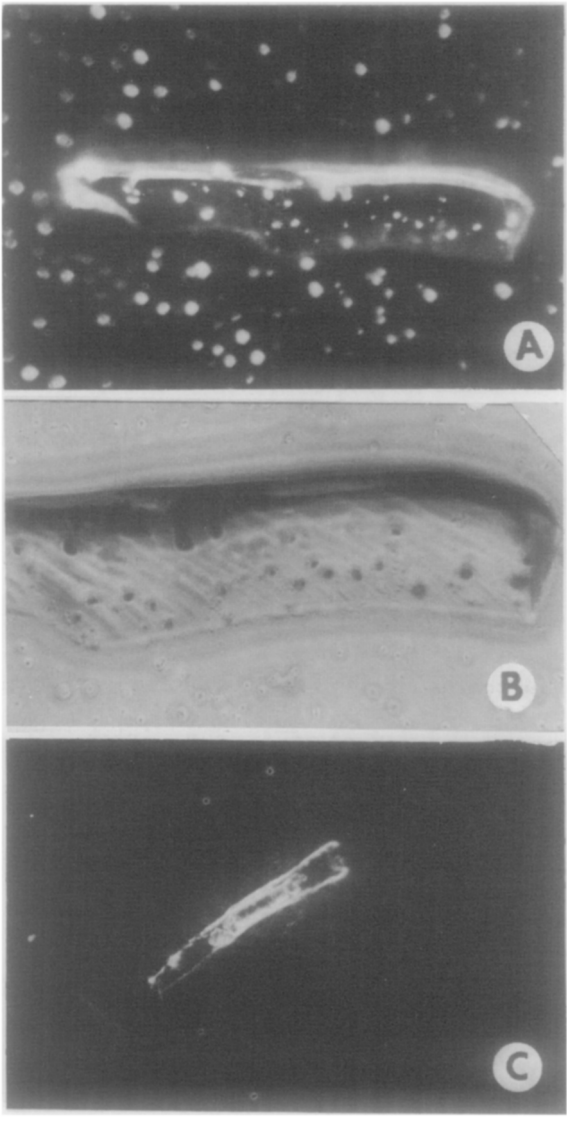

Figure 3. Polaroid Photomicrographs of Crystals.
Approx. dimensions 0.47 x 0.07 mm and 0.24 x 0.04 mm.

Smaller crystals recovered from the same preparation and dissolved in water had a 280/260 O. D. ratio of 0.48. The melting curves of the resolvated crystals and the $tRNA^{Phe}$ from the MASA columns were similar but the former showed reduced hypochromicity below 40°C. Fresco et al. (1966) suggested that the biphasic melting curve of purified yeast tRNA's results from two different kinds of structural changes, i.e. from 20-40°C a shape change occurs with relatively small loss of secondary structure, whereas above 50°C a change in secondary structure occurs which is paralleled by altered sedimentation coefficient and intrinsic viscosity. From the data in Figure 2, we infer that the secondary structure of the crystals was unchanged, although a shape change may have been caused by the crystallization or recovery and solvation process. Material from MASA columns which lacked amino acid acceptor activity and control vials with 10 mM $MgCl_2$ did not form crystals.

The central feature of this crystallization procedure is the use of a biphasic chloroform:aqueous tRNA-$MgCl_2$ solution. Chloroform was chosen for the ability to structure water molecules, as emphasized below. It seemed possible that such regions of organized water molecules might fill any irregular spaces in the surface of the tRNA molecule and make the molecules regular enough to organize into a crystal. It was also possible that an apolar association might occur which would stabilize the single stranded loops.

Chloroform can make clathrate hydrate crystals (van der Waals and Platteeuv, 1959). The chloroform-water clathrate is a Structure II hydrate, 17 Å cubic unit cell, $CHCl_3$-17 H_2O (Miller, 1961). Chloroform might exist as "iceberg" strucutes in solution surrounded by organized water molecules (Frank and Evans, 1945). Since molecules of this type are sometimes

anesthetics, Pauling (1961) and Miller (1961) independently suggested that the anesthetic effect is caused by the formation of organized water regions around macromolecules or membranes.

It has been experimentally demonstrated that Xenon, which forms gas hydrates and acts as an anesthetic, associated with haemoglobin in solution (Schoenborn et al., 1964). It was not determined, however, if this association was an apolar binding or a hydrate stabilization phenomenon. If chloroform is a part of the tRNA crystal, the study of the crystal organization may give insight into the nature of the association of gas hydrates with macromolecules.

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REFERENCES

Clark, B. F. C., Doctor, B. P., Holmes, K. C., Klug, A., Marcker, K. A., Morris, S. J., and Paradies, H. H., Nature 219, 1222 (1968).

Cramer, F., Doepner, H., Haar, F. v. d., Schlimme, E., and Seidel, H., Proc. Natl. Acad. Sci. U.S. 61, 1384 (1969).

Cramer, F., Haar, F. v. d., Saenger, W., and Schlimme, E., Angew. Chem. 80, 969 (1968).

Frank, H. S. and Evans, M. W., J. Chem. Phys. 13, 507 (1945).

Fresco, J. R., Adams, A., Ascione, R., Henley, D., and Lindahl, T., Cold Spring Harbor Symp. Quant. Biol. 31, 527 (1966).

Gillam, I., Millward, S., Blew, D., von Tigerstrom, M., Wimmer, E., and Tener, G. M., Biochemistry 6, 3043 (1967).
Hampel, A., Labanauskas, M., Connors, P. G., Kirkegard, L., RajBhandary, U. L., Sigler, P. B., and Bock, R., Science 162, 1384 (1968).
Kim, S. and Rich,A., Science 162, 1381 (1968).
Lindahl, T. and Fresco, J. R., Methods in Enzymology 12A, 601 (1967).
Miller, S., Proc. Natl. Acad. Scie. U.S. 47, 1515 (1961).
Pauling, L., Science 134, 15 (1961).
Schoenborn, B. P., Featherstone, R. M., Vogelhut, P. O., and Susskind, C., Nature 202, 695 (1964).
Stern, R. and Littauer, U. Z., Biochemistry 7, 3469 (1968).
van der Waals, J. H. and Platteeuv, J. C., Adv. Chem. Phys. 11, 1 (1959).