A METHOD FOR CRYSTALLIZATION OF SERINE-TRANSFER-RNA. CO-CRYSTALLIZATION OF t-RNA WITH CADMIUM- AND COPPER ION IN WATER-DIOXANE

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Transfer ribonucleic acid has been the subject of a number of structural investigations [1,2]. Our information of its three-dimensional conformation is incomplete. The only way to obtain this information is by means of X-ray crystallographic investigation of single crystals. The determination of the primary sequence of yeast-t-RNA and the suggestion of a cloverleaf base-pairing arrangement by Holley et al. in 1965 [3,4] was a major advance. Since then the sequences of four yeast t-RNA's have been determined [5–8]. Although numerous base changes are observed all five t-RNA's can be arranged in a cloverleaf pattern very similar to that proposed by Holley. Nevertheless it has not been possible to deduce a detailed tertiary structure of transfer-RNA because suitable single crystals for X-ray work have not been available.

In the following the production of crystals of serine-t-RNA and the investigations of the crystals with X-ray powder techniques is reported.

The starting material was brewer's yeast. The serine-t-RNA was purified by using counter current distribution [9,10] and hydroxylapatit column chromatography. Further purification was done using ion-exchange DEAE Sephadex columns. The details of the purification are reported elsewhere. The t-RNA could be charged fully under standard conditions. According to the pattern of counter current distribution which was discussed by Zachau [11] serine-t-RNA I and II were the major serine-t-RNA's.

The uncharged t-RNA was dialyzed against 0.002 M EDTA (pH 5.6) for twelve hours and then against an aqueous solution of $CdSO_4$ (10^{-6} M) and K_2SO_4 in phosphate buffer, pH 7.4. The same procedure was done with $CuCl_2$ and KCl. After further extensive dialysis against deionized water the excess of inorganic ions was removed and then the t-RNA solution was lyophilized. A 2.2% aqueous solution of serine t-RNA was prepared and was placed at one end of a quartz capillary (see fig. 1).

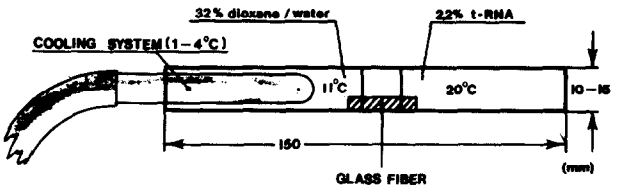
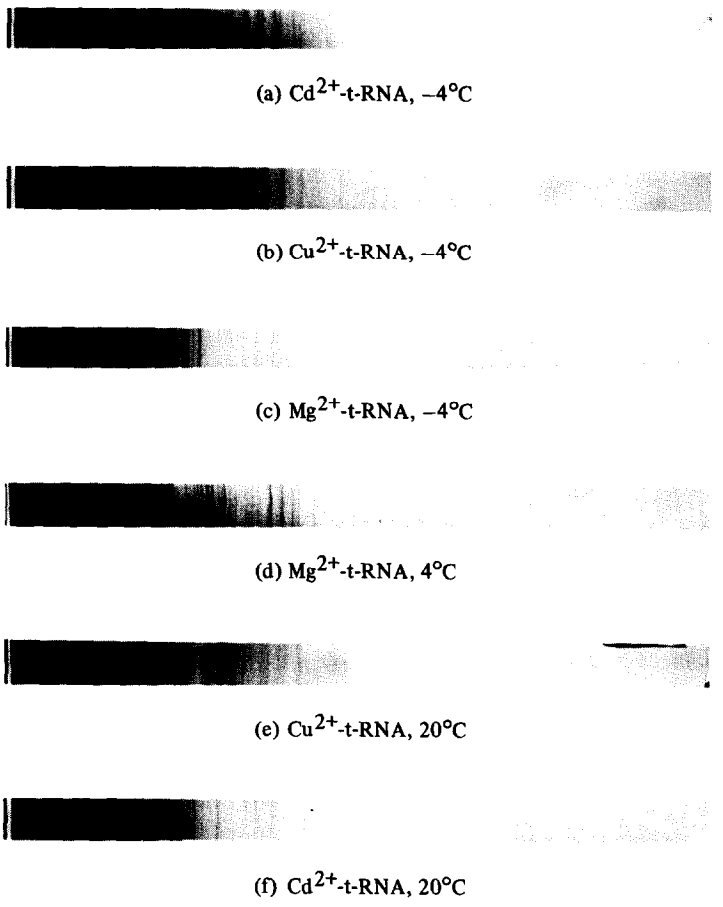


Fig. 1

On the other end of the capillary a 32% dioxane/water solution was placed and cooled down to −4°C. The two liquids were connected by a little glass fiber. Exchange of liquid between the two droplets (compartments) occurred via creeping of a thin layer film on the glass capillary. In order to get a temperature gradient a small cooling system was worked out at the left side of the capillary in fig. 1. All manipulations were done in red light to avoid peroxidation of dioxane. After one week the liquids were sufficiently equilibrated to start crystallization on the glass fiber. In order to get bigger crystals the crystallization was done in the cold at −4°C. After fourteen days, small needles with clear shape were obtained. The crystals showed extinction under the polarizing microscope.

After centrifugation X-ray powder photographs of the needles were taken in a Guinier camera (11.46 cm diameter). The pictures were taken at −4°C and copper radiation was used. Typical powder patterns are seen in figs. 2 and 3 (1.7-fold magnification). Tables 1 and 2 show the spacing of the cadmium and copper-



(a) Cd^{2+}-t-RNA, –4°C

(b) Cu^{2+}-t-RNA, –4°C

(c) Mg^{2+}-t-RNA, –4°C

(d) Mg^{2+}-t-RNA, 4°C

(e) Cu^{2+}-t-RNA, 20°C

(f) Cd^{2+}-t-RNA, 20°C

Fig. 2

t-RNA derivatives, suggesting that these are isomorphic.

The X-ray pictures of figs. 2d and 2e show the product of the same crystallization procedure with magnesium as counter ion at different temperatures. The concentration of t-RNA was 1.5% and it was dialyzed against 10^{-3} M $MgSO_4$ solution. The broadening of the lower angle lines makes calculation of the spacings impossible.

The X-ray pictures of figs. 2f and 2g show crystallization of copper and cadmium t-RNA at room temperature at a dioxane concentration of about 36%. Unfortunately, the micro crystals became damaged after two hours exposure. The Mg-salt was fully chargeable before and after the X-ray exposure (1450 cpm per $O.D._{260}$ unit of t-RNA) whereas the Cd-salt showed a chargeability of 64% before and after (930 cpm per $O.D._{260}$ unit of t-RNA). The copper salt could be charged to 54% (773 cpm per $O.D._{260}$ unit of t-RNA) before exposure and had lost biological activity after exposure.

That the crystals really contain t-RNA is further suggested by their disappearance when treated with ribonuclease.

Further work is in progress with the aim to produce single crystals of sufficient size and quality for single crystal X-ray crystallography which should make a complete structure determination possible.

Table 1
Cd^2 + t-RNA

No.	$1/d$ (A^{-1})	d (A)
1	0.01015	99.01
2	0.01405	71.14
3	0.02032	49.41
4	0.02271	44.04
5	0.02318	43.14
6	0.02695	37.10
7	0.02752	36.34
8	0.02928	34.15
9	0.03387	29.53
10	0.03548	28.18
11	0.03615	27.66
12	0.03868	25.85
13	0.03944	25.35
14	0.04241	23.57
(15	0.04468	22.38)
16	0.04684	21.35
17	0.04928	20.29

Table 2
$CuSO_4$ + t-RNA

$1/d$ (A^{-1})	d (A)
0.0115	89.68
0.0160	62.38
0.0182	55.03
0.0243	41.13
0.0258	38.74
0.0291	34.37
0.0303	32.95
0.0319	31.34
0.0331	30.19
0.0338	29.57
0.0343	29.16
0.0347	28.76
0.0366	27.30
0.0375	26.65
0.0380	26.25
0.0387	25.78

The X-ray pictures 4 and 5 show the product of the same crystallization procedure with magnesium as counter ion at different temperatures. The concentration of t-RNA was 1.5% and it was dialyzed against 10^{-3} M $MgSO_4$ solution. The broadening of the lower angle lines makes calculation of the spacings impossible.

The X-ray pictures 6 and 7 show crystallization of copper and cadmium t-RNA at room temperature at a dioxane concentration about 36%. Unfortunately, the micro crystals became damaged after two hours exposure. The Mg-salt was fully chargeable before and after the X-ray exposure (1450 cpm per O.D.$_{260}$ unit of t-RNA) whereas the Cd-salt showed a chargeability of 64% before and after (930 cpm per O.D.$_{260}$ unit of t-RNA). The copper salt could be charged to 54% (773 cpm per O.D.$_{260}$ unit of t-RNA) before exposure and had lost biological activity after exposure.

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