The Extraction and Purification of a Cysteine Transfer Ribonucleic Acid from Baker's Yeast

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(Received 26 September 1975)

1. A modification of the RPC 1 system of A. D. Kelmers, G. D. Novelli & M. P. Stulberg (1965) (*J. Biol. Chem.* **240**, 3979–3983) is described in which the support medium is a Celite of narrow range particle size treated with dichlorodimethylsilane. 2. By using this system an apparently pure preparation of tRNA^{Cys} was isolated from baker's yeast tRNA. 3. This preparation accepted at least 60% of the theoretical quantity of [3-¹⁴C]cysteine in a conventional assay and failed to accept isoleucine, phenylalanine, proline, serine or tyrosine. 4. A theoretical countercurrent-distribution curve calculated by assuming a distribution coefficient *K* of 2.03 was in excellent agreement with the profiles of *E*₂₆₀ and cysteine-acceptor ability after 537 transfers in the 1.85 M-phosphate/formamide/propan-2-ol system of C. M. Connelly & B. P. Doctor (1965) (*J. Biol. Chem.* **241**, 715–719). 5. Chromatography of tRNA^{Cys} on Bio-Gel P100 polyacrylamide beads afforded two components one of which was far less efficient than the other in accepting cysteine. The base compositions of the two were similar.

Since the first complete sequence of a transfer ribonucleic acid was deduced by Holley *et al.* (1965) about 60 tRNA sequences have been published, but to date no detailed reports of sequence studies of tRNA^{Cys} have appeared.

The interest in cytokinin-active substances prompted the preparation by Armstrong *et al.* (1969) of a partially purified tRNA^{Cys} by chromatographing brewer's yeast tRNA on benzoylated DEAE-cellulose. No investigations of the nucleotide sequence were reported but the presence of the cytokinin-active nucleoside $\gamma\gamma$ -dimethylallyl-adenosine (isopentenyl-adenosine) was confirmed.

This was followed by a report by Zekavat *et al.* (1972) of a method for purifying tRNA^{Cys} from baker's yeast by taking advantage of the ability of cysteinyl-tRNA^{Cys} to react with mercurial compounds to form an isolatable complex which was then decomposed to give pure tRNA^{Cys}.

We have examined many procedures for fractionating tRNA species from baker's yeast. The present paper describes the isolation of tRNA^{Cys} by the use of a modification of the RPC 1 system of Kelmers *et al.* (1965) which enabled us to prepare substantial quantities of tRNA^{Cys} in an apparently pure state. Conventional assays of our material at first suggested that it was about 60% pure, but further investigations, the inability of our preparations to accept other amino acids and the failure to detect heterogeneity by several methods, convinced us that our product was substantially pure.

This work has been briefly reported previously (Holness & Atfield, 1970, 1973).

Experimental

Materials

Transfer ribonucleic acid prepared in this laboratory from baker's yeast (*Saccharomyces cerevisiae*) by the method of Holley (1967) was used as starting material for the reversed phase chromatography.

Celite 545 (Johns-Manville Products Corp., London S.E.1, U.K.) (500 g) was freed from traces of iron and other substances absorbing in the u.v. by boiling a stirred suspension in 1 M-HCl for 1 h. This procedure was repeated twice more with fresh acid. In some preliminary experiments the Celite was freed from very fine particles by suspending it in water and decanting the cloudy supernatant after 5 min. After resuspension, the Celite fraction to be used could be poured as a thin slurry from the heavy coarse particles. This preparation is described as 'ungraded' Celite. Most experiments were performed by using a more carefully 'graded' Celite.

Batches (250 g) of Celite were graded according to particle size hydraulically with an apparatus similar to that devised by Hamilton (1958). Fines and coarse particles were removed leaving a fraction of relatively uniform particle size amounting to about 20% of the total.

Both 'graded' and 'ungraded' Celite batches were dried at 140°C for 18 h and treated with dichlorodimethylsilane (Hopkin and Williams, Chadwell Heath, Essex, U.K.) by a method similar to that described by Howard & Martin (1950). The Celite in small batches (25 g) was spread as a layer 1 cm deep in a large Petri dish and placed in a large desiccator

containing the vapour of dichlorodimethylsilane for about 6h. Combined batches were washed by stirring with methanol (5ml/g). The yellow-coloured methanol was decanted and the procedure repeated three times. The product was then dried at 100°C for 24h.

Chromosorb W (mesh size 100–120) from Johns-Manville Products Corp. was used without further treatment. Bio-Gel P100 polyacrylamide beads were supplied by Bio-Rad Laboratories, Bromley, Kent, U.K. DEAE-cellulose (Whatman DE32) was treated as described previously (Holness & Atfield, 1971).

Calcium phosphate was prepared as described by Main *et al.* (1959). Dilauryldimethylammonium chloride was a gift from General Mills, Kankakee, IL, U.S.A. ATP (disodium salt) was obtained from Hopkin and Williams, and CTP (trisodium salt) from Boehringer and Soehne, Mannheim, Germany.

DL-[³⁵S]Cysteine hydrochloride (37Ci/mol), DL-[3-¹⁴C]cysteine hydrochloride (56.7Ci/mol) and the uniformly ¹⁴C-labelled amino acids, alanine (7.9 Ci/mol), isoleucine (8.7Ci/mol), phenylalanine (15.3 Ci/mol), proline (8.2Ci/mol), serine (17.0Ci/mol), tyrosine (7.8Ci/mol) and the amino acid mixture derived from *Chlorella* hydrolysate were supplied by The Radiochemical Centre, Amersham, Bucks., U.K. Other chemicals were of A.R. grade.

Preparation of a cysteine synthetase

The simplified procedure of Holley *et al.* (1961) for the preparation of an aminoacyl synthetase from rat liver was followed up to the stage before the chromatography on DEAE-cellulose. Endogenous RNA was then removed by the addition of 0.1 vol. of aq. 10% (w/v) streptomycin sulphate and after being left for 10min at 0°C the precipitate was removed by centrifugation for 15min at 20000g. Solid (NH₄)₂SO₄ was then added to the supernatant to give a final concentration equal to 70% saturation. After being left for a further 10min at 0°C the protein precipitate was centrifuged down for 10min at 20000g, dissolved in about 30ml of 0.1M-Tris/HCl buffer, pH7.5, containing 1mM-thioethanol and dialysed overnight against 2 litres of the same buffer. Finally 0.25vol. of glycerol was added to the solution of activating enzymes which was stored in ampoules at -20°C.

Assay for cysteine-acceptor ability

The ability of tRNA preparations and of tRNA in column eluate and countercurrent-distribution fractions to accept cysteine was determined by the procedure described by Holley *et al.* (1961). The composition of the assay medium was modified, most significantly by the addition of CTP. Other changes brought about only marginal improvements in the acceptance of cysteine in the assay and the preferred composition is shown in Table 1. Assays of tRNA^{Cys} obtained by fractionation of crude tRNA on 'ungraded' Celite or in early countercurrent-distribution

Table 1. *Composition of the medium used for assay of the cysteine-acceptor ability of solutions of tRNA^{Cys}*

To each sample of the tRNA solution (0.05ml or 0.10ml) were added 0.15ml or 0.3ml of the assay medium, 0.1ml or 0.2ml of the solution of activating enzymes and water to make a total volume of 0.5ml. The procedure of Holley *et al.* (1961) was then followed. Radioactivity present on the planchettes containing the residues resulting from evaporation of the final assay solutions was measured by using a thin end-window Geiger-Müller counter (Isotope Developments).

Component	Amount in 5ml
ATP (dipotassium salt) in water (1 ml) and adjusted to pH7.0 with 1M-KOH	20mg
CTP (trisodium salt)	3 mg
Bovine serum albumin	3mg
Dithiothreitol	7.5mg
1M-MgCl ₂	0.25ml
2M-KCl	0.05ml
1M-potassium cacodylate buffer, pH7.5	1.0ml
[¹⁴ C]Cysteine	2Ci
Water added to make a total volume of 5.0ml	

experiments were performed with DL-[³⁵S]cysteine. Later DL-[3-¹⁴C]cysteine was used when it became available.

Preparation of the reversed-phase chromatographic column

The method used here is similar to that described by Kelmers *et al.* (1965) and by Kelmers (1966) but differs in the support medium and size of the column. Three support media were used at different times during these investigations; Chromosorb W, 'ungraded' Celite and 'graded' Celite. The stationary phase which was 4% (w/v) dilauryldimethylammonium chloride in amyl acetate, was added slowly to the stirred support medium. Samples amounting to about 5% of the volume of the stationary-phase solution were added at a time and stirring was continued until thoroughly mixed. The proportion of stationary phase solution to support medium was 0.5ml/g.

The coated support medium was then suspended in 0.35M-NaCl in buffer A [0.05M-Tris/HCl (pH7.4)/0.01M-MgCl₂]. All chromatographic solutions were kept saturated with amyl acetate to minimize leaching of the stationary phase.

In preliminary experiments with Chromosorb W or 'ungraded' Celite the column size was about 50cm × 1.5cm and the maximum load of tRNA was about 1g. Under ideal conditions with 'graded' celite the column size could be increased to 85cm × 3.5cm and the maximum load of tRNA to 10g.

Optimum conditions for the isolation of tRNA^{Cys}

The mixed tRNA (10g) was dissolved in 0.35M-NaCl in buffer A (200ml) and allowed to percolate on

to the column. The tRNA was then eluted by using a linear gradient of 0.35–0.55M-NaCl in buffer A (10 litres). The flow rate through the column was 3ml/min and the column effluent was monitored by using an LKB Uvicord spectrophotometer fitted with a flow cell with an optical path-length of 0.1 cm. The first 5 litres of effluent containing one unresolved tRNA peak was collected as one fraction. The remainder was collected in small fractions (9ml). Those fractions containing tRNA capable of accepting [¹⁴C]cysteine in the conventional assay were combined and the tRNA^{Cys} was precipitated by the addition of 2vol. of ice-cold ethanol. The product was centrifuged for 5min at 2000g, resuspended in fresh ice-cold ethanol, centrifuged as before and dried over H₂SO₄ in a vacuum desiccator.

Chromatography on Bio-Gel P100 polyacrylamide beads

Polyacrylamide beads were equilibrated with 0.05M-Tris/HCl (pH7.0)/0.01M-MgCl₂/0.45M-NaCl as described by Kelmers (1966) and packed into a column (90cm × 1 cm or 150cm × 1.5 cm). Liquid flow was briefly interrupted and the tRNA sample (5–30 mg) dissolved in the column buffer (0.5–1.0ml) was added carefully to the top of the packed bed but below the surface of the supernatant buffer. Liquid flow was resumed and fractions (1–2ml) were collected at a rate of 3 or 6/h. Fractions were assayed and those containing tRNA^{Cys} were combined, and the tRNA^{Cys} was precipitated and dried as described above.

Chromatography on DEAE-cellulose

DEAE-cellulose was equilibrated with 0.02M-Tris/HCl buffer (pH7.5)/7M-urea and packed into a column (240cm × 1 cm) as described by Holness & Atfield (1971). The column was washed with 0.25M-NaCl in the urea/buffer, and the tRNA^{Cys} (about 30mg in 1 ml of the buffer) was allowed to percolate on to the column. Elution was continued with a linear gradient of 0.25–0.55M-NaCl in the same buffer (800ml). tRNA^{Cys} was recovered from active fractions by precipitation with ethanol as above.

Chromatography on calcium phosphate

Calcium phosphate suspended in 0.005M-sodium phosphate buffer, pH6.8, was poured into a column to form a packed bed (67cm × 1.8cm). tRNA^{Cys} (46mg) dissolved in column buffer (2ml), was applied to the column and development continued with a linear gradient of 0.1–0.2M-NaCl in the same buffer (2 litres). Fractions (5ml) were collected at a rate of 4/h. tRNA^{Cys} was recovered by ethanol precipitation.

Countercurrent distribution

A 60 unit Craig-type apparatus adapted for continuous automatic operation was used. Two distribution systems were used at different stages in the work

and the method will be described in detail for the first system.

(a) *Phosphate/formamide/propan-2-ol* (Connelly & Doctor, 1965). The apparatus was set in the recycling mode and 10ml of lower (stationary) phase and 10ml of upper (mobile) phase were added to each of units number 5–60. tRNA^{Cys} (up to 100mg) was dissolved in 1.85M-sodium/potassium phosphate buffer, pH6.0 (20ml), and to this was added formamide (2ml) and propan-2-ol (8.8ml). After gentle shaking for 5min the phases were allowed to separate and the upper and lower phases removed leaving some interfacial tRNA. This was redissolved in phosphate buffer (20ml) and the procedure repeated. The volumes of the combined upper phases and the combined lower phases were each adjusted to 40ml by the addition of upper or lower phase and introduced into units 1–4. The phases were then equilibrated (10 cycles/min) for 3min and allowed to separate for 12min before transfer of the upper phase. When the required number of transfers had taken place the concentration of tRNA in each upper phase was determined by temporarily removing samples and measuring the *E*₂₆₀.

(b) *Tributylamine/butanol/dibutyl ether* (Zachau *et al.*, 1961). tRNA was converted into the tri-*n*-butylamine salt and subjected to countercurrent distribution by the procedure of Karau & Zachau (1964) by using a concentration of di-*n*-butyl ether equal to 22% of the volume of butan-1-ol in the system.

Nucleotide analyses

The base composition of tRNA preparations was determined as described by Holness & Atfield (1971). During preliminary stages of this work polynucleotide chains were degraded to mononucleotides by alkaline hydrolysis at 37°C, but because of our own observations that some deamination of cytosine could occur and those of other investigators on rearrangements of 1-methyladenine and 7-methyl-guanine under strongly alkaline conditions our more recent work has relied on complete hydrolysis with ribonuclease T2.

Spectrophotometric measurements

In addition to the routine monitoring of column eluates with an LKB Uvicord the absorbance of selected column and countercurrent-distribution samples was measured on a Unicam SP. 500 series 2 spectrophotometer.

Results and Discussion

While studying methods of fractionating tRNA from baker's yeast we investigated the reversed-phase systems described by Kelmers *et al.* (1965). Initially an 'ungraded' Celite was silanized and used as the support medium. Fig. 1 shows the latter part of the

Fig. 1. Chromatography by the RPC 1 system of Kelmers *et al.* (1965) of crude tRNA on 'ungraded' Celite treated with dichlorodimethylsilane

tRNA (0.4 g) dissolved in 5 ml of buffer A [0.05 M-Tris/HCl (pH 7.4)/0.01 M-MgCl₂ saturated with amyl acetate] was applied to the column (50 cm × 1.5 cm) of reversed phase 'ungraded' Celite packed in buffer A/0.3 M-NaCl. tRNA was eluted by using a linear gradient (1.6 litres) of 0.3–0.8 M-NaCl in buffer A. Fractions (10 ml) were collected at the rate of 2/h and assayed for the ability to accept cysteine (○), proline (□) and isoleucine (△) as described in the Experimental section. The values for cysteine incorporation are plotted on one-tenth the scale of that used for proline and isoleucine. ----, NaCl gradient; —, E_{260} .

Fig. 2. Chromatography by the RPC 1 system of Kelmers *et al.* (1965) of crude tRNA by using the optimum conditions for the isolation of tRNA^{Cys} on 'graded' Celite

Details of this procedure for the chromatography of the maximum load (10 g) of tRNA are given in the Experimental section. Fractions were combined and assayed for the incorporation of serine, tyrosine, isoleucine, phenylalanine and cysteine and the results are shown in Table 2. ●, E_{260} ; —, NaCl gradient.

elution profile of tRNA chromatographed on such a column. tRNA^{Ile} was found close to fraction 20 and tRNA^{Pro} in the centre of the profile. Some incipient resolution became visible towards the end of the elution profile with a substantial enrichment of cysteine-acceptor ability. The most active fractions appeared to contain at least 20% tRNA^{Cys}. After rewashing with starting buffer to restore the initial NaCl concentration the column could be reused three or four times before there was any significant fall in resolution. An 'enriched tRNA^{Cys}' fraction was obtained by combining the products of a number of such runs.

The use of Chromosorb W as the support phase gave a decreased resolution in the later stages of the elution and the column performance decreased even further after rewashing with starting buffer. It appeared likely that the larger particle size of the Chromosorb W compared with that of the silane-treated Celite might be an important factor in the observed decrease in resolving power and we therefore investigated the use of 'graded' silanized Celite as the supporting medium.

Fig. 2 shows the latter part of the elution profile obtained with 'graded' Celite. The earlier part of the profile showing little resolution into individual peaks of u.v.-absorbing material has been omitted. The tRNA species in fractions 55–78, 79–94, and 95–118 were combined into three fractions and each was assayed for its ability to accept serine, tyrosine, isoleucine, proline, phenylalanine and cysteine compared with that of the unfractionated tRNA used for the chromatography. These results are shown in Table 2 and demonstrate that fractions 95–118 contain mainly tRNA^{Cys} and this is confirmed by the fact that this fraction was unable to accept any labelled

Table 2. Ability of tRNA samples from the reversed-phase chromatography of total tRNA (Fig. 2) to accept amino acids in the conventional assay

tRNA samples from peak fractions 55–78, 79–94 and 95–118 were precipitated with alcohol as described in the text. Aqueous solutions of these samples and of starting tRNA were prepared and had E_{260} values respectively: 9.5, 15.2, 16.8 and 47.4. For each assay 0.05 ml of each solution was used with 0.2 ml of enzyme and 0.3 ml of medium.

Amino acid	Starting tRNA	$10^{-3} \times$ Observed incorporation (c.p.m./mg)		
		tRNA obtained from fractions:		
		55–78	79–94	95–118
Ser	2.4	10.9	7.8	1.3
Tyr	1.8	0.6	0.3	0.4
Ile	2.6	0.6	0.3	0.2
Pro	0.9	0.6	0.3	0.2
Phe	2.2	1.0	1.2	0.1
Cys	2.9	0.8	4.1	41.3

Fig. 3. Fractionation of the tri-*n*-butylamine salt of crude tRNA by countercurrent distribution

tRNA (1 g) was converted into the tri-*n*-butylamine salt and distributed for 60 transfers by using the solvent system butan-1-ol/water/tri-*n*-butylamine/acetic acid/di-*n*-butyl ether (100:130:10:2.5:22, by vol.) (Karau & Zachau, 1964). Fractions were assayed for the incorporation of [³⁵S]cysteine (○) as described in the Experimental section. ●, tRNA concentration.

amino acids when an amino acid mixture derived from ¹⁴C-labelled *Chlorella* was used in an assay. Such a mixture does not contain cysteine.

The striking improvement in resolution illustrated in Figs. 1 and 2 probably arises mainly from the vastly increased surface area per unit weight of the 'graded' Celite in comparison with Chromosorb W. A further factor may be the use of a gas-phase silanizing procedure, instead of the commercial liquid-phase procedure, which maximizes the area of silane coating. Phase-contrast microscopic examination of these two support media showed that the 'graded' Celite retained its original form of whole and broken diatoms, the maximum particle dimension of the majority being about 30 μm and the minimum about 5 μm. A few rod-like structures not exceeding 100 μm × 10 μm were also present. The structures appeared apparently unchanged after treatment with dichlorodimethylsilane. Chromosorb W, however, appeared under the microscope as spherical clumps about 150 μm in diameter.

Removal of very fine particles from the 'ungraded' Celite was also essential, for the presence of fines in the column packing has been shown to produce a marked deterioration in many chromatographic systems (e.g. Thompson, 1967).

Fig. 4. Countercurrent distribution of purified tRNA^{Cys}

tRNA^{Cys} (100 mg) was distributed by using the 1.85M-phosphate/formamide/propan-2-ol solvent system of Connelly & Doctor (1965) as described in the Experimental section. After 537 transfers the upper phase in every second tube of the apparatus was assayed for acceptor ability for [¹⁴C]cysteine (○) and for tRNA concentration (●). A theoretical curve (▲) was calculated assuming a distribution coefficient derived from absorbance values in successive pairs of tubes as described in the legend to Table 3.

Table 3. Calculation of the distribution coefficient *K* for tRNA^{Cys} in the countercurrent-distribution system of Connelly & Doctor (1965)

Values of the distribution coefficient *K* were calculated from successive pairs of readings up to and through the zone maximum by using the expression suggested by Karlson & Hecker (1950) namely

$$2 \log K = \log T_{n,r} - \log T_{n,r-2} - \log [(n+2-r)/r]$$

where *T* is the quantity of substance in tube *r* after *n* transfers.

Tubes from which absorbance values taken to calculate <i>K</i>	Distribution coefficient <i>K</i>
346 and 348	2.02
348 and 350	2.03
350 and 352	1.98
352 and 354	1.93
354 and 356	2.19
Mean	2.03

In countercurrent-distribution systems such as that described by Appgar *et al.* (1962) and also that of Karau & Zachau (1964) tRNA^{Cys} has a distribution coefficient in favour of the upper phase, resembling tRNA^{Ser} and tRNA^{Phe}. This is shown in Fig. 3 where

Fig. 5. Chromatography of purified $tRNA^{Cys}$ on a column of calcium phosphate

$tRNA^{Cys}$ (46mg) was applied to the column (67cm $\times$ 1.8 cm) and eluted with a linear gradient (2 litres) of 0.1–0.2M-NaCl in 0.005M-sodium phosphate buffer, pH6.8. Fractions (5ml) were collected at a rate of 4/h and assayed for cysteine acceptor ability (○) as described in the Experimental section. —, E_{260} ; ----, sodium phosphate gradient.

Fig. 6. Chromatography of purified $tRNA^{Cys}$ on a column of DEAE-cellulose in 7M-urea at pH7.9

$tRNA^{Cys}$ (30mg) was applied to the column (240cm $\times$ 1cm) and eluted with a linear gradient (800ml) of 0.25–0.55M-NaCl in 0.02M-Tris/HCl buffer, pH7.9, in 7M-urea. The ability of the fractions to accept cysteine (○) was assayed as described in the Experimental section. —, E_{260} .

after 60 transfers of an 'enriched' $tRNA^{Cys}$ in the latter type of system $tRNA^{Cys}$ moved at the front of the bulk of the tRNA.

Figure 4 shows the countercurrent distribution of a

pure $tRNA^{Cys}$ prepared on the RPC 1-type column. After 268 transfers in the 1.85M-phosphate system of Connelly & Doctor (1965) some asymmetry in the absorbance curve suggested that the preparation contained traces of other components. After a further 269 transfers a minor inactive component separated. The E_{260} and the incorporation of [^{14}C]cysteine by the tRNA of the main band were in excellent agreement with the theoretical curve calculated for a distribution coefficient K of 2.03 and a variance of 4.8. Calculations of K from successive pairs of readings up to and through the zone maximum gave the values presented in Table 3 and indicated that the material in this region of the curve was substantially homogeneous.

$tRNA^{Cys}$ could not be further purified by chromatography on hydroxyapatite (Fig. 5) nor by chromatography on DEAE-cellulose in 7M-urea (Fig. 6).

The incorporation of [^{14}C]cysteine by these preparations of $tRNA^{Cys}$ indicated a purity of less than 50%. The addition of dithiothreitol to the system to minimize the oxidation of cysteine to cystine led to only a small improvement in the uptake of [^{14}C]cysteine.

With unfractionated tRNA the incorporation of cysteine was linear with tRNA concentration up to only 100 μ g of tRNA/ml, but the addition of CTP (0.2mg/ml of assay medium) overcame this defect; the uptake was then linear to at least 400 μ g of tRNA/ml and the total incorporation of cysteine was doubled. However, with purified $tRNA^{Cys}$, as obtained from reversed-phase chromatography, incorporation of cysteine by $tRNA^{Cys}$ even in the presence of CTP showed a marked departure from linearity above 3 μ g/ml of assay medium. This led to difficulties in the assay procedure, in particular of $tRNA^{Cys}$ in column fractions, where for convenience, standard samples from each fraction were used. At the highest concentrations of $tRNA^{Cys}$ the incorporation of [^{14}C]cysteine was depressed leading to an apparent fall in the specific activity. It is possible that this could result from an inefficiency in the charging enzyme, for it had been demonstrated that the incorporation of [^{14}C]cysteine could be improved slightly by increasing the volume of enzyme preparation added. There was a far greater increase in the relative uptake of [^{14}C]cysteine when the concentration of $tRNA^{Cys}$ used in the assay was less than 1 μ g/ml. The resulting uptake was then very close to that expected for a preparation that was substantially pure $tRNA^{Cys}$. This apparent depression in the uptake of [^{14}C]cysteine could in extreme cases lead to a second peak of radioactivity suggesting a second component (Fig. 6).

An alternative explanation invokes a competing second-order or pseudo-second-order reaction, such as the formation of a dimer of $tRNA^{Cys}$, giving rise to a product which can no longer take part in the aminoacylation reaction.

Fig. 7. Chromatography of tRNA^{Cys} on a column of Bio-Gel P100 polyacrylamide beads

tRNA^{Cys} (6mg) obtained from tubes 340–358 of the countercurrent-distribution experiment (Fig. 4) was applied to the column (90cm × 1cm) and eluted with 0.05M-Tris/HCl (pH 7.0)/0.01M-MgCl₂/0.45M-NaCl. Fractions (1.4ml) were collected at a rate of 3/h. Cysteine acceptor ability (○) was assayed as described in the Experimental section. ●, E₂₆₀.

Table 4. Nucleotide composition of tRNA^{Cys} preparations

tRNA preparations 1 and 2 were samples of 'enriched' tRNA^{Cys} and were hydrolysed with 0.3N-KOH as described by Holness & Atfield (1971). Preparations 3 and 4 were obtained by using 'graded' Celite in the reversed-phase chromatography and preparations 5 and 6 were respectively the 'active' and 'inactive' forms of tRNA^{Cys} resulting from chromatography on Bio-Gel P100 polyacrylamide beads. Preparations 3–6 were hydrolysed with ribonuclease T2.

tRNA ^{Cys} preparation	...	Composition (E ₂₆₀ %)					
		1	2	3	4	5	6
Nucleotide							
m ⁷ Gp*						2.0	3.3
Cp + m ⁵ Cp	12.0	13.5	17.1	15.9	16.6	16.5	
m ¹ Ap*						2.2	1.9
Up + Tp + Ψp	28.3	25.8	23.2	24.0	22.7	22.6	
Ap + i ⁶ Ap	26.2	26.8	26.1	27.9	24.3	24.0	
Gp	33.5	34.0	33.6	32.8	32.3	32.3	

* The percentage of m¹Ap and m⁷Gp present in the hydrolysates was determined only for preparations 5 and 6.

Such a conversion may occur during efforts to purify tRNA^{Cys} further. A much less active component was demonstrated by chromatographing tRNA^{Cys} on Bio-Gel P100 polyacrylamide beads (Fig. 7) and this suggests either that the inactive molecule is the larger of the two or that there is a difference in conformation allowing greater non-specific adsorp-

tion between the active tRNA^{Cys} and the polyacrylamide molecules than is possible with the inactive tRNA^{Cys}. There was no interconversion between the two under the conditions used on the Bio-Gel P100 columns.

The base compositions of these two species of tRNA^{Cys} were almost identical and are shown in columns 5 and 6 of Table 4. The analyses shown in columns 1 and 2 are the result of alkaline hydrolysis of some early preparations of tRNA^{Cys}. The deamination of cytidylic acid caused by the strongly alkaline conditions becomes clear when these results are compared with those in columns 3 and 4 which were determined after ribonuclease T2 hydrolysis of purified samples of tRNA^{Cys}.

The following paper (Holness & Atfield, 1976) reports the results of complete pancreatic ribonuclease hydrolysis and of complete ribonuclease T1 hydrolysis of tRNA^{Cys}.

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