The Nucleotide Sequence of Cysteine Transfer Ribonucleic Acid from Baker's Yeast

IDENTIFICATION OF THE PRODUCTS FROM PARTIAL DEGRADATION OF THE MOLECULE AND DERIVATION OF THE COMPLETE SEQUENCE

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1. A series of large oligonucleotide fragments derived from tRNA^{Cys} were separated chromatographically and the sequence of each was deduced by examination of the products of digestion with pancreatic and T1 ribonucleases. 2. The location of the specific cleavage points in the nucleotide chain was similar to that produced by brief treatment with pancreatic ribonuclease. 3. The fragments could be arranged into two alternative sequences. The correct sequence was deduced by the sequential removal and identification of the first nine nucleotides from the 3'-end of the terminal half of the molecule.

The preparation of large batches of tRNA from baker's yeast and the subsequent chromatography by the modified RPC 1 system of Kelmers *et al.* (1965) to give tRNA^{Cys} sufficiently pure for structural studies has already been described (Holness & Atfield, 1976a). Exhaustive degradation of tRNA^{Cys} with pancreatic RNAase* and with RNAase T1 gave small oligonucleotides and the determination of the sequence of each of these was described in the preceding paper (Holness & Atfield, 1976b). However, only short regions of the sequence of the intact tRNA^{Cys} could be deduced by the overlap of these fragments.

Several procedures designed to permit specific degradation of the molecule at only a few points were investigated. During this work it was found that one sample of tRNA^{Cys} had undergone some degradation by breakage of specific internucleotide bonds similar to those broken by pancreatic RNAase.

The present paper describes the separation and the determination of the sequence of each of these fragments. This information was sufficient to enable us to elucidate the complete sequence of tRNA^{Cys}, and this has already been briefly reported (Holness & Atfield, 1974).

Experimental

Materials

Cyclohexylamine obtained from BDH, Poole, Dorset, U.K. was converted into the hydrochloride as described by Keith & Gilham (1974), crystallized from water and dried over H₂SO₄. NNN'-Tetramethylglycine amide, from Kodak, Kirkby, Liver-

* Abbreviation: RNAase, ribonuclease.

pool, U.K., was similarly converted into the hydrochloride, but could not be crystallized.

Other chemicals and reagents used were of A.R. grade or were as described in the preceding papers (Holness & Atfield, 1976a,b).

Chromatographic separation of the products resulting from the partial degradation of tRNA^{Cys}

The tRNA^{Cys} preparation (50 mg) was dissolved in 2 ml of buffer D (0.02 M-Tris/HCl, pH 7.9, in 7 M-urea) and allowed to percolate on to a column (200 cm × 1 cm) of DEAE-cellulose equilibrated with 0.05 M-NaCl in buffer D. Elution was continued with a linear gradient (500 ml) of 0.05–0.40 M-NaCl in buffer D. Fractions (1.35 ml) were collected at a rate of 2/h.

Further examination of the components liberated by the partial degradation but only partially resolved by the first chromatographic procedure

The fractions comprising each of the partially separated peaks were combined and rechromatographed on columns of DEAE-cellulose in 0.1 M-formic acid in 7 M-urea by the procedure described by Takemura *et al.* (1972). The resulting peaks of u.v.-absorbing material were desalted and the dried residues kept for further examination by methods described in detail in the preceding paper (Holness & Atfield, 1976b).

Stepwise degradation of oligonucleotides

The 3'-terminal nucleosides were removed by the cycle of reactions described by Keith & Gilham

(1974), namely periodic acid oxidation of the terminal nucleoside, base-catalysed removal of the modified nucleoside fragment and enzymic dephosphorylation of the uncovered terminal 3'-phosphate.

The modified nucleoside fragment released at each cycle was separated from the remaining oligonucleotide and from other reaction products by chromatography on a column of Bio-Gel P2 polyacrylamide beads (Keith & Gilham, 1974). It was identified by comparison of its u.v. spectrum with that of standard nucleosides.

To distinguish adenosine from uridine, which had similar spectra, their aqueous solutions were irradiated with light of wavelength 254 nm as described by Guschlbauer *et al.* (1965). Uridine was thereby converted into a compound transparent to light in the u.v.

An initial cycle of degradation and separation in the absence of the periodic acid reaction was performed to ensure complete removal of the 3'-phosphate group. During this cycle a contaminant, probably from the uncrystallized *NNN'*-tetraglycine amide, was eluted from the column of Bio-Gel P2 at the point where the eliminated nucleoside would appear. The spectrum of this substance was similar to that observed after the irradiation with short-wave u.v. light of the solution of uridine obtained at cycle 2 of procedure (b) below.

The contribution of this substance to the u.v. spectrum over the range of 250–300 nm was accordingly subtracted from the spectrum of each nucleoside released before comparison with that of the standards.

(a) *Stepwise degradation of fragment 7-2 (see below).* The oligonucleotide (7.0 E_{260} units) was subjected to five complete cycles. The nucleoside fragments isolated and identified, with yields (nmol) in parentheses were respectively C (50), U (50), C (40), G (30) and A (30).

(b) *Stepwise degradation of the terminal half.* The terminal half (107 E_{260} units) after nine successive cycles gave the following nucleosides and yields G (110), U (85), ψ (60), A (80), G (60), A (60), C (40), G (55) and A (40). Further degradation cycles were performed but the yields became small and contaminants tended to mask the spectra. Cycles 10–16 showed the presence of C, G, C, G, A, hU and G respectively.

Partial degradation of the dephosphorylated terminal half with snake venom phosphodiesterase

The dephosphorylated terminal half (200 μ g) in 0.2 ml of 0.05 M-Tris/HCl buffer, pH 8.9, containing 0.01 M-MgCl₂ was incubated for 45 min at room temperature (24°C) with a solution of snake venom phosphodiesterase (150 μ g) in 0.15 ml of the same buffer. The product was chromatographed on a

column (30 cm $\times$ 0.6 cm) of DEAE-cellulose packed in 0.02 M-Tris/HCl buffer, pH 7.5, in 7 M-urea and eluted with a linear gradient (100 ml) of 0.0–0.3 M-NaCl in the same buffer.

Fractions containing mononucleotides were combined and desalted for analysis on the analytical DEAE-cellulose acetate column as described in the preceding paper (Holness & Atfield, 1976b). The mononucleotides released were pG and pU in the ratio 1.27:1.00.

Similar digestion for 2.5 h of the same sample gave rise to a mononucleotide mixture in the molar ratio: pC, 1.0; pU + ψ , 0.8; pA, 1.2; pG, 1.5.

Results and Discussion

Fig. 1 shows the latter part of the elution profile resulting from chromatography on DEAE-cellulose in 7 M-urea at pH 3.5 of the partially degraded tRNA^{Cys}. A small peak (fraction 1) in tubes numbered 217–231, which are not shown, contained several small fragments which were not further investigated. The final part of the elution profile from fractions 336–360 consisted of a large number of components, probably degraded whole and half molecules of tRNA^{Cys}. These were difficult to resolve satisfactorily and were not further studied.

Table 1 shows the distribution of u.v.-absorbing material among the tubes whose contents were combined to form fractions 2–7 which were kept for further examination. Each of these was rechromatographed on DEAE-cellulose in 7 M-urea at pH 3.5 and

Fig. 1. Chromatography of degradation products of tRNA^{Cys} on DEAE-cellulose at pH 7.9

Degraded tRNA^{Cys} (50 mg) was chromatographed on a column of DEAE-cellulose (200 cm $\times$ 1 cm) packed in 0.02 M-Tris/HCl buffer, pH 7.9, in 7 M-urea and eluted with a linear gradient (500 ml) of 0.05–0.4 M-NaCl in the same buffer. Fractions (1.35 ml) were collected at a rate of 2/h. —, E_{260} ; ----, NaCl gradient.

Table 1. Distribution of u.v.-absorbing material among the fraction collector tubes shown in Fig. 1 whose contents were combined to form fractions 1-7

Each combined fraction was desalted before further chromatography on DEAE-cellulose at pH3.5. The detailed examination of the purified fragments isolated from these fractions is described in the text. An E_{260} unit is defined as the quantity of nucleotide or oligonucleotide which, dissolved in 1 ml of water or buffer, has an extinction of 1.0 at 260 nm when contained in a cell of 1 cm light-path-length.

Fraction	Tube numbers	Total E_{260} units
1	217-231	36
2	259-272	50
3	283-295	59
4	296-307	135
5	308-314	93
6	315-323	154
7	324-335	237

the elution profiles obtained in the cases of fractions 4-7 are shown in Figs. 2(a)-2(d).

Fractions 2 and 3 appeared homogeneous and were shown to be A-G-Cp and A-G-Tp respectively.

Fraction 4 gave rise to three fragments on re-chromatography.

Fragment 4-1: A-i⁶A-A-ψ-C-Up

Hydrolysis with pancreatic RNAase gave A-i⁶A-A-ψp and one molecule of each of Cp and Up. This fragment formed part of the longer sequence C-A-i⁶A-A-ψ-C-U-Gp previously identified among the products of complete RNAase T1 digestion of the entire molecule.

Fragment 4-2

With pancreatic RNAase this fragment gave approximately equimolar yields of the tetranucleotide G-A-G-Cp, the trinucleotide G-G-Cp, a mixture of G-m⁷G-hUp and A-Up and a large quantity of Cp and Up.

RNAase T1 digestion gave equimolar yields of the trinucleotide A-U-Gp, the dinucleotide A-Gp, the mononucleotides Cp and Gp and two other oligonucleotides spectrally similar to C-Up. One of these, eluted later than expected, could be the m⁷G-hU-m⁵C-C-U-Up.

Digestion with RNAase T2 gave a mixture of Cp, Up, Ap and Gp in the ratio 5:4:2:3 with traces of m⁷Gp and m⁵Cp.

There was insufficient of this material for further study but the fragments available suggested that fragment 4-2 was a mixture of the oligonucleotides G-A-G-C-Up, A-U-G-G-Cp and G-m⁷G-hU-m⁵C-C-U-Up.

Fig. 2. Further chromatography of selected fractions from Fig. 1 on DEAE-cellulose at pH3.5

Fractions from Fig. 1 were combined in groups as indicated in Table 1 and rechromatographed on columns of DEAE-cellulose in 0.1 M-formic acid in 7 M-urea as described in the text. (a) Fractions 296-307, (b) fractions 308-314, (c) fractions 315-323 and (d) fractions 324-335. —, E_{260} ; ----, NaCl gradient.

Fragment 4-3: A-G-hU-G-G-hUp

Pancreatic RNAase gave a mixture of the two trinucleotides A-G-hUp and G-G-hUp, whereas with RNAase T1 equal quantities of Gp, hU-Gp and A-Gp were obtained. The dihydrouridylic acid contents of the hexanucleotide and the derived trinucleotide mixture were the same, which led to the

conclusion that there was only a single hUp in the centre of the sequence. This was in agreement with the absence of the trinucleotide hU-hU-Gp from among the products of complete RNAase T1 hydrolysis of tRNA^{Cys}.

Fraction 5 afforded two components when rechromatographed.

Fragment 5-1: A-G-C-G-Cp

Digestion with pancreatic RNAase gave equal molar quantities of G-Cp and A-G-Cp. Hydrolysis with RNAase T1 gave an equimolar mixture of Cp, C-Gp and A-Gp, which can only come from the sequence shown.

Fragment 5-2: G-U-U-G-m⁷G-hU-m⁵C-C-U-Up

This component lacked adenylic acid. The base composition was: m⁷Gp, 1; m⁵Cp, 1; Cp, 1; Up, 4; Gp, 2. An oligonucleotide of this composition must form part of the longer oligonucleotide 7-5 (see below).

Fraction 6 gave three homogeneous fragments.

Fragment 6-1: G-C-G-A-G-C-U-C

This was obtained in small quantity only. It had the composition: C, 1; Cp, 2; Up, 1; Ap, 1; Gp, 3. The presence of one molecule of cytidine suggested that the fragment formed the 3'-terminus of the molecule. It contained one Gp and one Cp more than the pentanucleotide G-A-G-C-Up found among the components of fragment 4-2. To accord with the set of oligonucleotides obtained by complete enzymic digestion of the whole molecule the additional G-C must precede the sequence G-A-G-C-Up to give that shown.

Fragment 6-2: A-G-A-ψ-U-G-Cp

Further hydrolysis with RNAase T1 gave Cp, A-Gp and A-ψ-U-Gp in the ratio 1:1:0.7. Pancreatic RNAase yielded Up, G-Cp and A-G-A-ψp in the ratio 1:1:0.6. Complete hydrolysis with RNAase T2 confirmed the composition shown. This fragment formed part of the terminal half of the molecule, for both tetranucleotides had already been identified among the fragments from complete digestion of that half.

Fragment 6-3: pG-C-U-C-G-Up

This formed the 5'-terminus of the complete sequence of tRNA^{Cys}. Hydrolysis with RNAase T1 gave the tetranucleotide C-U-C-Gp and pancreatic RNAase digestion gave equal parts of pG-Cp, G-Up, Cp and Up.

Fraction 7 was the most complex, for rechromatography at pH 3.5 gave seven components.

Fragment 7-1: A-U-G-G-C-G-Cp

The composition of this heptanucleotide was: Cp, 2; Up, 1; Ap, 1; Gp, 3. Digestion with pancreatic RNAase gave equal parts of A-Up, G-Cp and G-G-Cp. The fragments A-U-Gp, C-Gp, Gp and Cp which resulted from RNAase T1 hydrolysis indicated that the sequence must be either A-U-G-G-C-G-Cp or A-U-G-C-G-G-Cp. Preliminary dephosphorylation of the fragment before digestion with pancreatic RNAase gave rise to the dinucleoside monophosphate GpC showing that the correct sequence was A-U-G-G-C-G-Cp.

The presence of the trinucleotide G-G-Cp placed this fragment in the terminal half.

Fragment 7-2: G-A-G-U-G-C-G-A-G-C-U-C

This was derived from the acceptor half of the molecule, for hydrolysis with pancreatic RNAase gave equal molar proportions of the tetranucleotides G-A-G-Cp and G-A-G-Up together with G-Cp, Up and C. RNAase T1 treatment gave C-U-C- and a mixture of mono- and di-nucleotides in the ratio Gp, 1; U-Gp, 1; C-Gp, 1; A-Gp, 2. Since we have established that fragment 6-1 has the sequence G-C-G-A-G-C-U-C then the additional tetranucleotide G-A-G-Up must be placed on the left of fragment 6-1. The sequence of five nucleotides at the 3'-terminus of this fragment was confirmed by the results of the stepwise degradation described in the Experimental section.

Fragment 7-3: C-U-G-A-G-U-G-C-G-A-G-C-U-C

The pattern of oligonucleotides resulting from pancreatic RNAase digestion was similar to that obtained from fragment 7-2, but the proportion of Cp and Up was higher. RNAase T1 digestion gave a trinucleotide C-U-Gp which replaced Gp found in the digest of fragment 7-2. The sequence of this fragment must therefore commence with this trinucleotide.

Fragment 7-4: A-i⁶A-A-ψ-C-U-G-U-U-G-m⁷G-hU-m⁵C-C-Up

Hydrolysis with RNAase T2 indicated a composition similar to that of fragment 7-5 but lacking one uridylic acid residue.

Fragment 7-5: A-i⁶A-A-ψ-C-U-G-U-U-G-m⁷G-hU-m⁵C-C-U-Up

Pancreatic RNAase digestion gave equal molar quantities of A-i⁶A-A-ψp and G-m⁷G-hUp and a

mixture of mononucleotides. Degradation with RNAase T1 gave A-i⁶A-A-ψ-C-U-Gp, U-U-Gp and an oligonucleotide with the composition m⁷G-hU-m⁵C-C-U-Up, which is part of the nonanucleotide found in the RNAase T1 digest of the acceptor half but lacking the terminal dinucleotide A-Gp.

pA, 1.2 and pG, 1.6. These mononucleotides could only arise from fragment 6-2, which must therefore complete the sequence. Two alternative arrangements are now possible for the 3'-end of the terminal half. Nucleotides are numbered from the 5'-end of the molecule.

(a)	hU - A - G - C - G - C - A - G - C - A - G - A - ψ - U - G - C														
(b)	hU - A - G - C - A - G - C - G - C - A - G - A - ψ - U - G - C														
Nucleotide	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34 35

Since the fragment G-A-i⁶A-A-ψp is not found among those produced by pancreatic RNAase hydrolysis of the intact molecule the sequence of fragment 7-5 must commence with A-i⁶A-A-ψ-C-U-Gp and be followed by U-U-Gp.

Fragment 7-6: A-G-T-ψ-C-G-m¹A-U-C-C-U-G-A-G-U-G-C-G-A-G-C-U-C

Hydrolysis with pancreatic RNAase gave the same series of fragments as that obtained from fragment 7-3 but with the addition of A-G-Tp and G-m¹A-Up. Since fragment 7-3 forms the 3'-terminus of the molecule the other components must be placed at its left hand end. G-m¹A-Up overlaps with the RNAase T1 nucleotide m¹A-U-C-C-U-Gp of which the terminal C-U-Gp sequence is already present in fragment 7-3. Likewise A-G-Tp overlaps with T-ψ-C-Gp and since this is the only remaining fragment the sequence of fragment 7-6 must be that shown.

In the preceding paper (Holness & Atfield, 1976b) the groups of fragments produced by complete hydrolysis of each of the two halves of tRNA^{Cys} with pancreatic RNAase and with RNAase T1 are summarized. Examination of the fragments derived from the acceptor half leads to the conclusion that the acceptor half consists of fragment 7-5 followed by fragment 7-6.

The sequence of the terminal half commences with fragment 6-3. The only fragment produced by RNAase T1 hydrolysis of the terminal half and starting with Up is U-A-U-Gp and so fragment 7-1 must follow fragment 6-3 to give pG-C-U-C-G-U-A-U-G-G-C-G-Cp.

Four fragments now remain to be placed in the sequence from the dihydrouridine loop to the end of the terminal half. Fragment 4-3 contains the dihydrouridylic acid nucleotides and can be placed after fragment 7-1. The three fragments 2, 5-1 and 6-2 account for all the nucleotides remaining to be sequenced. Partial hydrolysis of the dephosphorylated terminal half with snake venom phosphodiesterase gave rise to the mononucleoside 5'-phosphates pG and pU. More prolonged digestion gave a mixture of mononucleotides in the ratio: pC, 1.0; pU+pψ, 0.8;

By analogy with many other tRNA sequences the first alternative is favoured since this would lead to the placing of C at position 25 and A at position 26. The sole examples of tRNA sequences with G at position 25 are the group of tyrosine mutant tRNA species from *Escherichia coli* (Goodman *et al.*, 1968, 1970; Harada *et al.*, 1968; Doctor *et al.*, 1969). Only three examples are known of sequences with a pyrimidine at position 26, namely, yeast tRNA^{Gly} (Yoshida, 1973), bacteriophage T4-coded tRNA^{Gly} (Barrell *et al.*, 1973) and *E. coli* B tRNA^{His} (Singer & Smith, 1972).

The two sequences were distinguished by performing the repeated stepwise degradation procedure described by Keith & Gilham (1974). After nine cycles the presence of A at position 26 was confirmed thus eliminating sequence (b). Continuation of the stepwise degradation as far as cycle 16 gave results in agreement with the placing of the fragment A-G-hU-G-G-hUp after fragment 7-1.

The complete sequence of tRNA^{Cys} from baker's yeast is shown in the conventional clover-leaf form in Fig. 3.

The molecule is composed of 75 nucleotides, but, as isolated, lacks the adenosine and one cytidylic acid residue from the terminal C-C-A. The position preceding this triplet is occupied by uridine and in this respect tRNA^{Cys} is similar to tRNA^{Gly} from *E. coli* (Squires & Carbon, 1971), tRNA^{Lys} from haploid yeast (Smith *et al.*, 1973), tRNA^{Gly} from bacteriophage T4-injected *E. coli* (Barrell *et al.*, 1973), tRNA^{Asp} from yeast (Dirheimer *et al.*, 1972) and tRNA^{Gly} from *Staphylococcus* species (Roberts, 1972).

tRNA^{Cys} forms a member of that group of acceptor RNA molecules with the small extra arm. The sequence m⁷G-hU-m⁵Cp occurs at positions 31-29 from the 3'-terminus as in yeast tRNA^{Met} (Simsek & RajBhandary, 1972) and yeast tRNA^{Leu} (Madison *et al.*, 1972), but differs slightly from rabbit liver tRNA^{Phe} (Keith *et al.*, 1973; Blobstein *et al.*, 1973), yeast tRNA^{Phe} (RajBhandary *et al.*, 1967) and brewer's yeast tRNA^{Val} (Bayev *et al.*, 1967; Bonnet *et al.*, 1971) where the m⁵C is found at position 28.

There are two codon possibilities for cysteine, UGU and UGC; the sequence shown here contains

Fig. 3. Conventional clover-leaf arrangement of the nucleotide sequence of $tRNA^{Cys}$ from baker's yeast

the anticodon GCA corresponding to the latter alternative. The anticodon is followed by isopentenyladenylic acid as predicted for all tRNA species that recognize codons beginning with U (Bartz *et al.*, 1970).

The sequence in the dihydrouridine loop is the same as that reported by Yoshida (1973) for yeast $tRNA_{I}^{Gly}$. Other members of this group are yeast $tRNA^{Trp}$ (Keith *et al.*, 1972) and $tRNA_{I}^{Met}$ from a number of species.

As shown in Fig. 3 all base pairs except three are of the normal Watson-Crick type. The three others are G-U base pairs and occur one in each of three of the limbs. Such base pairs have been reported in these positions in a number of tRNA sequences.

The possibilities of tertiary interactions across the molecule accord well with a tertiary structure similar to that proposed for yeast $tRNA^{Phe}$ (Kim *et al.*, 1974; Klug *et al.*, 1974).

The sequences of most of the fragments isolated in the present work commence with adenylic acid and end in a pyrimidine 3'-phosphate. In fact all the fragments which can be liberated by breaking pyrimidine-adenylic acid internuclear bonds in $tRNA^{Cys}$ were isolated and identified. These are

shown in part (a) of Table 2. Molar yields of each fragment relative to the recovery of A-G-hU-G-G-hUp are given, but no account has been taken of the hypochromic effect when summing the molar extinctions of the oligonucleotides.

In addition to these fragments further degradation of the two large oligonucleotides from the acceptor half was noted. Some of these fragments were characterized and are shown in part (b) of Table 2. Four of these fragments resulted from the breaking of pyrimidine-guanlylic acid bonds and the remaining three from the breaking of T-ψ, U-U and U-C bonds.

The recoveries of those fragments arising from the terminal half are similar except for the low yield of A-U-G-G-C-G-Cp. The possibility of an additional pyrimidine-guanlylic acid break to give A-U-G-G-Cp is supported by the presence of A-U-G-G-Cp among the mixture of oligonucleotides unresolved in fragment 4-2. Assuming that the three components of this mixture are present in equal proportions then the recovery of fragments containing the acceptor end of the molecule is about 1.3 mol in agreement with the yield of A-G-Tp of 1.29 mol. Sequences containing the fifth loop amount to about 0.96 mol.

Table 2. Sequences and molar yields of oligonucleotides isolated from among the degradation products of tRNA^{Cys}

The first part of the Table summarizes the yields relative to that of A-G-hU-G-G-hU = 1.0 of all the fragments which resulted from pyrimidine-adenylic acid breaks. No account has been taken of any hypochromic effect when calculating total molar absorbances of the oligonucleotides. The molar absorbances for mononucleotides were those used by Holley *et al.* (1965). The second part of the Table summarizes the sequences and yields of fragments derived by further degradation of the two long oligonucleotides from the acceptor half.

(a) Fragments resulting from pyrimidine-adenylic acid breaks

Terminal half	Yield	Acceptor half	Yield
pG-C-U-C-G-Up	0.81	A-i ⁶ A-A-ψ-C-U-G-U-U-G-	
A-U-G-G-C-G-Cp	0.39	m ⁷ G-hU-m ⁵ C-C-U-Up	0.27
A-G-hU-G-G-hU	1.00	(Oligonucleotide 1)	
A-G-C-G-Cp	0.81	A-G-T-ψ-C-G-m ¹ A-U-C-C-U-	
A-G-Cp	0.86	G-A-G-U-G-C-G-A-G-C-U-C	0.23
A-G-A-ψ-U-G-Cp	1.03	(Oligonucleotide 2)	

(b) Fragments obtained by further degradation of oligonucleotides 1 and 2

Oligonucleotide 1	Yield	Oligonucleotide 2	Yield
A-i ⁶ A-A-ψ-C-Up	0.30	A-G-Tp	1.06
G-U-U-G-m ⁷ G-hU-m ⁵ C-C-U-Up	0.27	G-A-G-C-Up (see text)	
A-i ⁶ A-A-ψ-C-U-G-U-U-G-m ⁷ G-hU-m ⁵ C-C-U-Up	0.15	G-C-G-A-G-C-U-C	0.18
		G-A-G-U-G-C-G-A-G-C-U-C	0.34
		C-U-G-A-G-U-G-C-G-A-G-C-U-C	0.27

Reasons for this facile chain scission of all pyrimidine-adenylic acid linkages in tRNA^{Cys} are not clear. Secondary splits resulting in a fairly specific cleavage of oligonucleotides obtained with RNAase T1 at pyrimidine-adenylic acid bonds have been achieved during hydrolysis with RNAase T1 in Tris/chloride/EDTA buffer at pH 7.5 (Brownlee, 1972). Apparently only a small proportion of the material was unaffected and the presence of RNAase T1 was not essential for this secondary reaction.

RNAase species specific for uridylic acid linkages are known. Levy *et al.* (1973) isolated an RNAase from citrobacter species but this enzyme was specific for internucleotide bonds with uridine at either end of the linkage. A more likely possibility is that the reaction may be regarded as being similar to that resulting from brief treatment with pancreatic RNAase.

In the RNAase reaction the speed of the chain scission is influenced markedly by the nature of the neighbouring nucleotide. In a study of the rates of hydrolysis of dinucleoside monophosphates Witzel (1963) found that pyrimidine-adenylic acid bonds were more susceptible to pancreatic RNAase than other bonds. Thus in the series CpA, CpG, CpC and CpU the rates of hydrolysis were in the ratio 3000:500:240:27. Witzel (1963) considered this to be a consequence of electron interaction between the bases, increase in polarizability and nucleophilic character of the cytidine residue depending on the extent of the interaction. The series UpA, UpG, UpC and UpU behaved similarly.

Of the further internucleotide bond breaks

required to produce the additional fragments three are U-G breaks which are placed second in Witzel's (1963) order of rates of hydrolysis. The remaining three are less favoured but the linkages are in non-base-paired regions and for this reason may be more easily accessible to the hydrolytic attack.

Alternatively after chain scission at the preferential points of attack the tertiary structure may become more open making less favoured linkages more accessible to RNAase attack.

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