A SPECIFIC CHEMICAL CHAIN SCISSION OF tRNA AT 7-METHYLGUANOSINE

W.WINTERMEYER and H.G.ZACHAU
Institut für Physiologische Chemie und Physikalische Biochemie der Universität München, Germany

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1. Introduction

The study of large fragments of tRNA has led to some insight into the mode of recognition of tRNA by its cognate aminoacyl tRNA synthetase [summaries 1, 2]. Interesting results were obtained also with respect to the physical properties of the fragments and their combinations [3–5]. Most of the fragments had been obtained by partial digestion of tRNA with endonucleases, frequently at a modest yield [1, 2]. A completely specific and quantitative chemical method of chain scission had been described for preparing half molecules of $tRNA^{Phe}$ from yeast, wheat, and rat liver [6, 7].

We now describe a specific chemical method of chain scission at the m^7G position of $tRNA^{Phe}$ (fig. 1), $tRNA^{Phe}$ (HCl), $NaBH_4$ reduced $tRNA^{Phe}$ (HCl) from yeast, and the CCA halves of $tRNA^{Phe}$ from yeast and wheat. After excision of the alkali conversion product of m^7G, the chain was split with aniline under mildly acidic conditions (fig. 2–4). Pure fragments were isolated in 50–65% yield (fig. 5). The oligonucleotide analysis of a 30 nucleotide long fragment ranging from the m^7G position to the CCA end is reported (fig. 6). Combinations of this fragment with the pG half of yeast $tRNA^{Phe}$ showed considerable acceptor activity (fig. 7) although 10 nucleotides are missing. The chain scission reaction should be applicable to the many tRNAs containing m^7G.

2. Materials and Methods

$tRNA^{Phe}$ [6], $tRNA^{Phe}$ (HCl) [8], and $tRNA^{Ser}$ from yeast [9], as well as the half molecules of the $tRNA^{Phe}$ from yeast and wheat (kindly supplied by R. Thiebe) [3] were prepared as in the references. Aniline (analytical grade, E. Merck, Darmstadt) was twice distilled in vacuo from zinc dust under nitrogen. A 200-fold purified preparation of yeast Phe-tRNA synthetase was a gift of R. Hirsch of this laboratory.

If not specified otherwise, the chain scission was carried out as follows: The tRNAs were dialysed twice against 1 M NaCl, 5 mM EDTA, and three times against quartz distilled water. The tRNA (10 A_{260} units/ml in analytical experiments, 50 A_{260} units/ml $tRNA^{Phe}$ (HCl) for preparative purposes) was incubated in 0.1 M tris, pH 9.5, at 50° for 4.5 hr. After two ethanol precipitations the products were incubated in 0.3 M aniline (10–25 A_{260} units tRNA/ml), pH 3.5, at 50° for 4 hr. The mixture was extracted with ether.

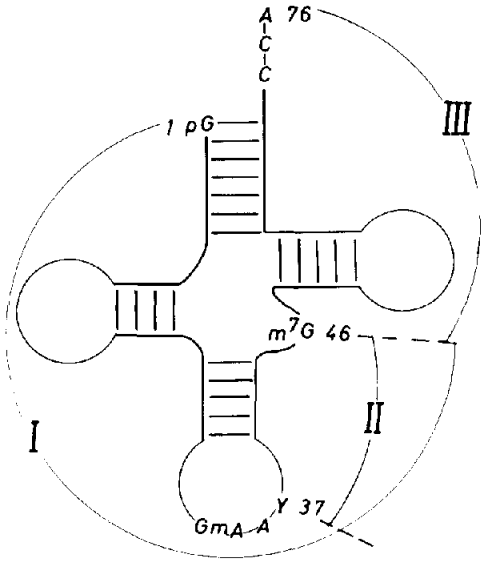

Fig. 1. Schematic cloverleaf model of yeast $tRNA^{Phe}$ according to [15]. The fragments I, II and II contain the nucleotides 1–45, 38–45, and 47–76, respectively.

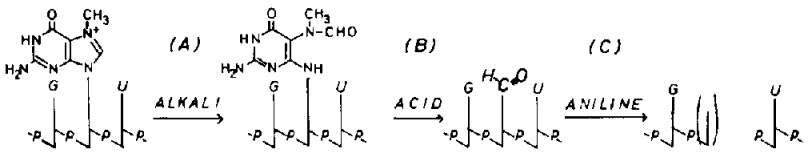

Fig. 2. Reaction scheme for the conversion of m^7G and the scission of the polynucleotide chain.

3. Results

Lawley and Brookes described the conversion by alkali of m^7G to a 4-ribosylamino pyrimidine derivative [10] (step A in fig. 2). It was now found that the glycosyl bond of this conversion product can be split easily with acid (step B). Within 4 hr at pH 9.5 and 37°, according to UV spectroscopy [10] more than 90% of the m^7G was converted to the alkali product (R_f 0.5 on cellulose thin layer in methanol–water, 7:3 [11]). Further treatment at pH 3.0 (4 hr, 37° or 15 hr, 22°) yielded a periodate negative, UV absorbing material (R_f 0.37) and ribose (R_f 0.66), indicating a split of the ribosyl bond.

In order to reach completion, the alkali conversion m^7G in tRNA required 50° (fig. 3), in agreement with the finding that this nucleoside is protected in the three-dimensional structure of tRNA [12]. m^7G reacted faster in the CCA half than in $tRNA^{Phe}$ (HCl). For obtaining chain scission, the acid and aniline treatments (steps B and C of fig. 2) were combined to an aniline treatment at pH 3–3.5. The chain splitting was followed by disc electrophoresis [6] (fig. 4). As expected no splitting could be detected with the pG half of $tRNA^{Phe}$ and with $tRNA^{Ser}$ from yeast. According to electrophoretic mobilities and to preliminary analytical data, fragments I and II comprised the nucleosides 1–45 and 38–45, respectively. In the experiments of fig. 4c and d, fragment I contained an alkali conversion product of Y or ribitol at position 37, respectively. Fragment II contained the degradation product of ribose at its 3′ end [3, 14]. Fragment III reached from position 47 to the terminal CCA; it sometimes appeared as a double band due to the partial conversion of m^1A to m^6A (see below). The low yield of fragment I in the experiment of fig. 4d was related to the formation of quarter molecules. This was probably due to a chain scission at the reduced hU, as it was observed recently [13].

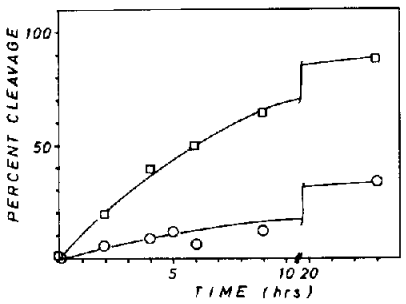

Fig. 3. Kinetics of the alkali conversion of m^7G in the $tRNA^{Phe}$ (HCl). 10 A_{260} units/ml $tRNA^{Phe}$ (HCl) were incubated at 50° in 0.1 M tris at pH 8.0 (○——○) and at pH 9.5 (□——□). At intervals aliquots were adjusted to pH 5 and precipitated with ethanol. After aniline treatment and ether extraction (Methods) the samples were subjected to disc electrophoresis. The amount of splitting was estimated by comparing the peak areas of the densitograms (examples in fig. 4). The exact shape of the curves seems to depend very much on salt concentrations.

Fragments II and III were pure after one and two column chromatographies, respectively (fig. 5). A complete T1 RNase digestion of fragment III yielded equimolar amounts (± 10%) of the expected oligonucleotides [15], which were identified by paper electrophoresis and UV spectroscopy (fig. 6). pU-C-m^5C-U-Gp (R_{Up} 1.1 at pH 2.7) gave, on digestion with pancreatic RNase [9], the expected nucleotides in equimolar amounts, expect that too little Cp and m^5Cp were found (separated as nucleosides by paper chromatography in ethyl acetate–water–propanol, 4:2:1, upper phase [16].

The pG half of the $tRNA^{Phe}$ and fragment III did not accept Phe while with combinations of the two at least 15% of the activity of $tRNA^{Phe}$ (HCl) was reached (fig. 7). This value may be increased by variations of the assay conditions.

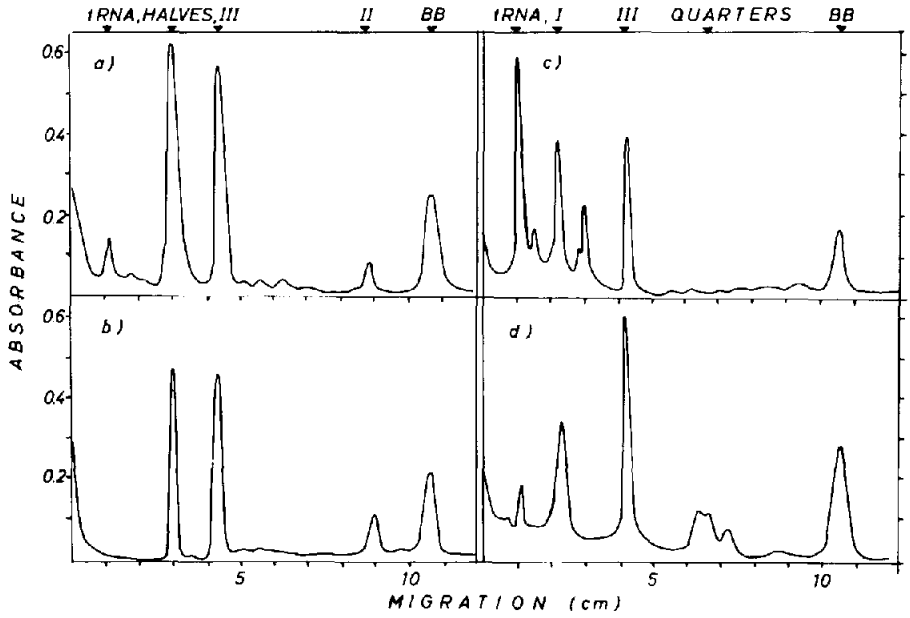

Fig. 4. Densitograms of parallel disc electrophoretic separations [6] of alkali/aniline treated a) $tRNA^{Phe}$ (HCl), b) CCA half of $tRNA^{Phe}$ (pherograms of yeast and wheat halves were nearly identical), c) $tRNA^{Phe}$, and d) $NaBH_4$ reduced $tRNA^{Phe}$ (HCl) (reduction according to [19], but in 0.3 M potassium phosphate, pH 7.5). Conditions were as in Methods, expect that in c) the aniline treatment was carried out at pH 5 because of the acid lability of Y. For the designation of the fragment peaks see fig. 1. BB is the bromphenolblue marker.

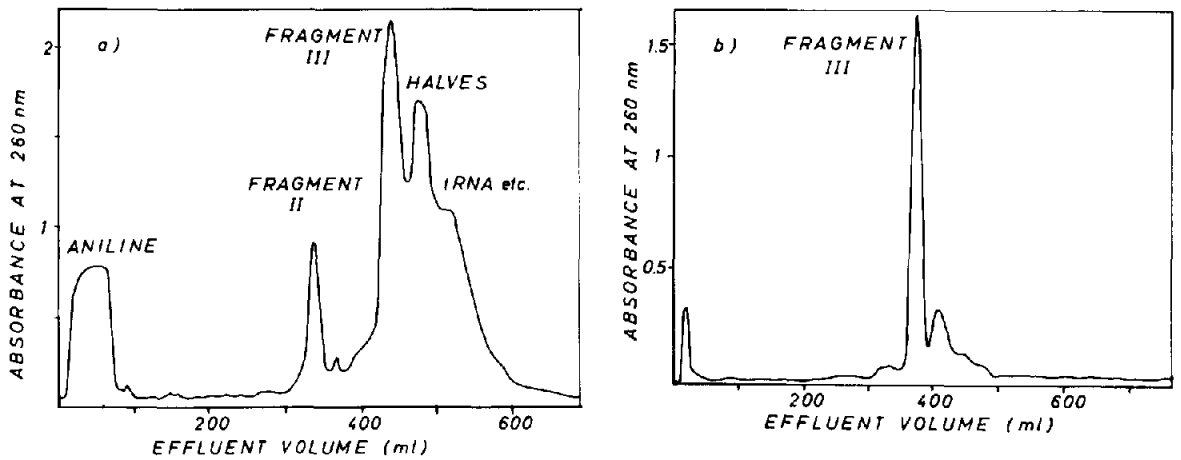

Fig. 5. a) Column chromatography of alkali/aniline treated $tRNA^{Phe}$ (HCl). Ca. 250 A_{260} units of the splitting products (Methods) in about 100 ml 7 M urea, pH 5, were applied to a column of DEAE cellulose (1 X 100 cm). The gradient was 300 ml each of 0.1 and 0.6 M NaCl, 0.01 M sodium acetate, pH 5, 7 M urea. Pure fragment II was isolated at yields of 40–65%. b) Rechromatography of fragment III on a column of DEAE Sephadex A 25 (0.5 X 70 cm). The gradient was 400 ml each of 0.1 and 0.5 M NaCl, 7 M urea, pH 3.0. Pure fragment II was isolated in a 30–40% yield. Additional 10–15% were found by rechromatography of the halves fraction (fig. 5a) on an identical column.

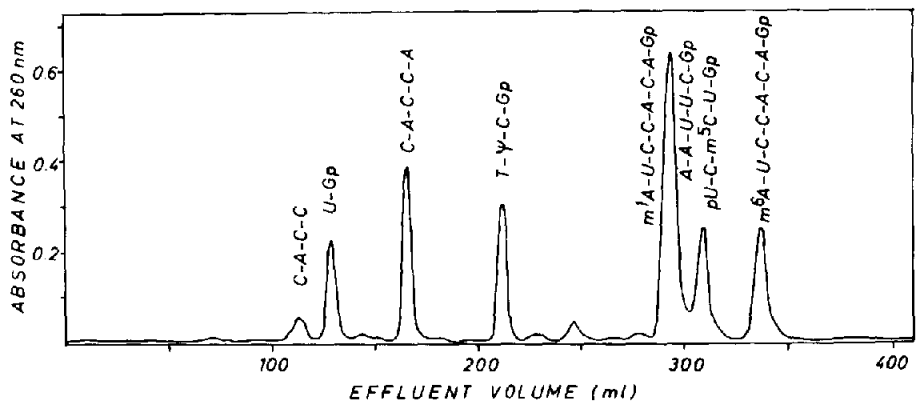

Fig. 6. Chromatography of a complete T1 RNase digest of fragment III. 12 A_{260} units were digested as described [12]. The digest was chromatographed on a column of DEAE cellulose (0.3 X 70 cm) with a linear gradient of 250 ml each of 0.01 and 0.3 M NaCl, 0.01 M tris, pH 7.4, 7 M urea.

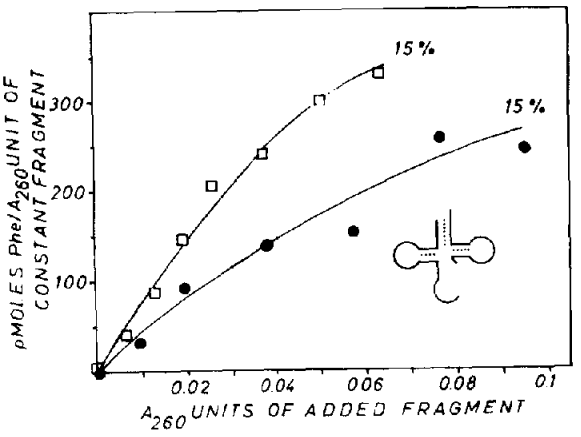

Fig. 7. Acceptor activity of the combinations of pG half and fragment III. 0.023 A_{260} units of the pG half and increasing amounts of fragment III (•——•); 0.029 A_{260} units of fragment III and increasing amounts of pG half (□——□). The assay conditions were as in [20] except that, after heating, the mixtures were held at 37° for 30 min. Incubation mixtures contained 2.5 milliunits [21] Phe-tRNA synthetase.

4. Discussion

The analytical data on fragment II and III support the conclusion that the chain scission described here proceeds analogously to the one at the position of Y [3, 6]. The mechanism of this reaction will be discussed elsewhere in detail [14].

In fragment combinations of yeast $tRNA^{Val}$ the acceptor activity was lost when nucleosides were removed from the anticodon region [17], while in yeast $tRNA_{II}^{Ala}$ large parts of the anticodon stem could be misssing without complete loss of activity [18]. In the present paper a case is reported in which the activity was not lost although several nucleotides had been excised from the anticodon loop and stem.

References

[1] R.W.Chambers, in: Progress in Nucleic Acid Research and Molecular Biology, eds. J.N.Davidson and W.E.Cohn (Academic Press, New York) in press.
[2] H.G.Zachau, Angew. Chem. 81 (1969) 645; Internat. Edit. 8 (1969) 711.
[3] W.Wintermeyer, R.Thiebe, H.G.Zachau, D.Riesner, R.Römer, and G.Maass, FEBS Letters 5 (1969) 23.
[4] K.Morikawa, M.Tsuboi, Y.Kyogoku, T.Seno, and S.Nishimura, Nature 223 (1969) 537.
[5] A.D.Mirzabekov, D.Lastity, E.S.Levina, and A.A.Bayev, FEBS Letters 7 (1970) 95.
[6] Philippsen, R.Thiebe, W.Wintermeyer and H.G.Zachau, Biochem. Biophys. Res. Commun. 33 (1968) 922.
[7] R.Thiebe and H.G.Zachau, in: Methods in Enzymology, part D, eds. L.Grossman and K.Moldave (Academic Press, New York) in press.
[8] R.Thiebe and H.G.Zachau, European J. Biochem. 5 (1968) 546.
[9] H.G.Zachau, D.Dütting, and H.Feldmann, Z. Physiol. Chem. 347 (1966) 212.
[10] P.D.Lawley and P.Brookes, Biochem. J. 89 (1963) 127.

[11] J.W.Jones and R.K.Robins, J. Am. Chem. Soc. 85 (1963) 193.
[12] T.Igo-Kemenes and H.G.Zachau, submitted to European J. Biochem.
[13] B.Beltchev and M.Grunberg-Manago, manuscript in preparation.
[14] W.Wintermeyer, R.Thiebe and H.G.Zachau, manuscript in preparation.
[15] U.L.RajBhandary, A.Stuart and S.H.Chang, J. Biol. Chem. 243 (1968) 4038; and other papers.
[16] R.H.Hall, Biochem. Biophys. Res. Commun. 12 (1963) 361.
[17] A.D.Mirzabekov, L.Ya.Kazarinova, D.Lastity, and A.A. Bayev, FEBS Letters 3 (1969) 268.
[18] N.Imura, G.B.Weiss, and R.W.Chambers, Nature 222 (1969) 1147.
[19] T.Igo-Kemenes and H.G.Zachau, European J. Biochem. 10 (1969) 549.
[20] R.Thiebe and H.G.Zachau, Biochem. Biophys. Res. Commun. 36 (1969) 1024.
[21] M.H.Makman and G.L.Cantoni, Biochemistry 4 (1965) 1434.