SPLITTING OF PHENYLALANINE SPECIFIC tRNA INTO HALF MOLECULES BY CHEMICAL MEANS.

P. PHILIPPSEN, R. THIEBE, W. WINTERMEYER, AND H.G. ZACHAU.

Institut für Physiologische Chemie der Universität München, Germany.

Received November 11, 1968

Summary: When the base moiety of the unknown nucleoside Y of phenylalanine specific tRNA from yeast is excised, the tRNA can be split into two large fragments under the conditions of the Whitfeld degradation. The chain scission is a completely specific, almost quantitative process. The mixture of the two fragments has 50-60% of the acceptor activity for phenylalanine as compared with unmodified phenylalanine specific tRNA.

Introduction: The nucleotide sequence of $tRNA^{Phe}$ from yeast has been established by RajBhandary, Khorana, and coworkers[1]. Next to the anticodon of $tRNA^{Phe}$ the nucleoside Y was found, the structure of which is still unknown. The base moiety of Y can be excised from $tRNA^{Phe}$ by mild acid treatment without breaking the polynucleotide chain[2]. The modified tRNA which was called $tRNA^{Phe}_{HCl}$ still contains the ribose moiety of the nucleoside Y. As we pointed out before[2] the aldehyde group of the ribose offers a point of attack for chemical reagents. The reactions of the aldehyde groups in periodate oxidized oligonucleotides have been thoroughly studied. The lability in the presence of alkali or amines of the phosphodiester bond adjacent to an oxidized ribose has been exploited for the stepwise degradation of RNA and oligonucleotides[3-7]. The aldehyde group of the ribose moiety of Y in $tRNA^{Phe}_{HCl}$ may also under certain conditions labilize the neighboring

phosphodiester bond. It was now found that the phosphodiester bond linking the ribose to the next nucleotide can be split specifically in an amine catalyzed reaction producing half molecules of $tRNA^{Phe}$ in nearly quantitative yield.

Materials and Methods: $tRNA^{Phe}$ was isolated from brewer's yeast tRNA of Boehringer Co., Mannheim, by chromatography on benzoylated DEAE cellulose[8] using a second gradient for $tRNA^{Phe}$ (1.5 M NaCl, 0.05 M sodium acetate, pH 5.0, 0.01 M $MgCl_2$, 0 to 20% ethanol). Acceptor activities were above 1.0 nmole phenylalanine/A_{260}-unit. The conversion of $tRNA^{Phe}$ to $tRNA^{Phe}_{HCl}$, which did not change the acceptor activity in a homologous charging system, was carried out as before[2,9].

Splitting of $tRNA^{Phe}_{HCl}$ was performed at concentrations of 10-15 A_{260}-units/ml with three different amines. 1. Incubation in 0.3 M aniline hydrochloride, pH 5.0, for 5 hours at 25° (similar to reference 7). The reaction mixtures were adjusted to pH 9.0 with aqueous ammonia, twice extracted with ether, brought to pH 6.0 with acetic acid, and dialyzed overnight against 0.01 M sodium acetate, pH 6.0. In some experiments ^3H-aniline (100 C/M) was used as the acetate salt in 0.05 M sodium acetate, pH 5.0; spacer gel buffer in 50% saccharose was added to the incubation mixtures prior to electrophoresis. 2. Incubation in 0.4 M cyclohexylamine acetate, pH 10.2, for 2 hours at 45°[4,6]. The reaction mixtures were extracted and dialyzed as above. 3. Incubation in 0.4 M lysine hydrochloride, pH 8.5, for 2 hours at 45°[6]. The reaction mixtures were dialyzed as above. (Shorter reaction times than those given left some $tRNA^{Phe}_{HCl}$ undegraded.)

Phosphomonoesterase treatment of tRNA fractions was performed as described by Harkness and Hilmoe[10]. Inorganic phosphate was determined according to Ames and Dubin[11]. Disc electrophoresis was performed in a vertical gel electrophoresis cell EC 490 (E-C Apparatus Corp., Philadelphia). The gel system was modified from Richards, Coll, and Gratzer[12]: Reservoir solution, 30 mM cacodylic acid, 2.5 mM imidazole (pH 4.9); spacer gel, 50 mM HCl, 50 mM imidazole (pH 4.1), 5% acrylamide, 0.25% bisacrylamide; running gel, 50 mM HCl, 62.5mM imidazole (pH 6.4), 18% acrylamide, 1.5% bisacrylamide; both gels had 6 M urea, 3 mM ammonium persulphate, 4 mM tetramethyl ethylenediamine. Electrophoresis was run for 3 hours at 0-4° with ca. 300 V and 100 mA. Staining was performed with 1-ethyl-2-(3-(1-ethylnaphtho(1,2d)thiazolin-2-ylidene)-2-methylpropenyl)-naphtho(1,2d)thiazolium bromide according to a personal communication of Dr. A.C. Peacock. Some details of the staining procedure will be published shortly by A.E. Dahlberg, C.W. Dingman, and A.C. Peacock. For $tRNA^{Phe}$ and large fragments satisfactory staining was also obtained with toluidine blue and other stains.

Results: When $tRNA^{Phe}$, $tRNA^{Phe}_{HCl}$, and aniline treated $tRNA^{Phe}$ were incubated with phosphomonoesterase, approximately one mole phosphate was released per mole tRNA (Fig. 1). Aniline treatment of $tRNA^{Phe}_{HCl}$, however, made one more mole of phosphate sensitive to monoesterase indicating one break per chain. This was confirmed by disc electrophoresis of $tRNA^{Phe}_{HCl}$ with and without aniline treatment (Fig. 2). $tRNA^{Phe}$ with and without aniline treatment moved on disc electrophoresis as $tRNA^{Phe}_{HCl}$. When the chain scission of $tRNA^{Phe}_{HCl}$ was performed with lysine or cyclohexylamine, the electrophoresis pattern was identical with

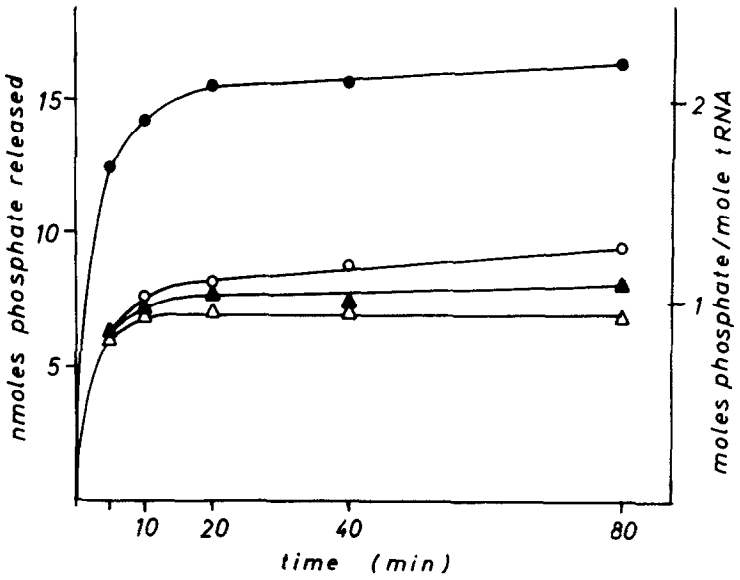

Fig. 1: Release of inorganic phosphate with phosphomonoesterase from 4.2 A_{260}-units each of $tRNA^{Phe}$ (Δ—Δ), $tRNA^{Phe}_{HCl}$ (O-O), aniline treated $tRNA^{Phe}$ (▲—▲), and aniline treated $tRNA^{Phe}_{HCl}$ (●-●). The molecular weight of $tRNA^{Phe}$ was taken as 24,957 and 1 mg $tRNA^{Phe}$ as 23 A_{260}-units.

---

the one of aniline treated $tRNA^{Phe}_{HCl}$. The hexanucleotide 2'OMeGpApApYpApΨ from the anticodon loop of $tRNA^{Phe}$ was stable in these amine solutions. 2'OMeGpApAp-pApΨ, on the other hand, from which the base moiety of Y had been excised[2], was completely converted to degradation products by all of the above amines.

The mixture of the two fragments from aniline treated $tRNA^{Phe}_{HCl}$ which according to disc electrophoresis contained no trace of tRNA still accepted 50-60% phenylalanine compared to untreated $tRNA^{Phe}_{HCl}$. In preliminary experiments the acceptor activity was not changed by heating and fast or slow cooling of the

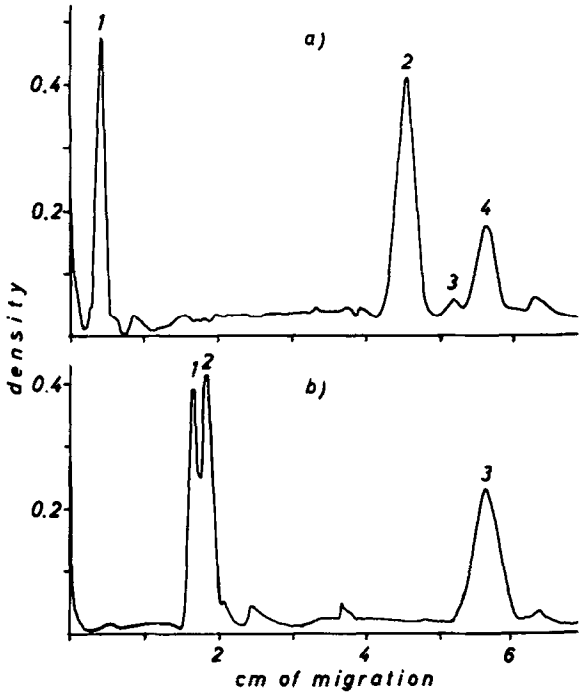

Fig. 2: Densitometer traces at 578 mμ of parallel gels with a. $tRNA^{Phe}_{HCl}$ (1), the oligonucleotides 2'OMeGpApApΨpApΨ (2) and 2'OMeGpApAp-pApΨ (3) (cf. reference 2), bromphenol blue (4); b. $tRNA^{Phe}_{HCl}$ after treatment with aniline (1,2), bromphenol blue (3).

---

fragments. The acceptor activity of fragments produced by lysine or cyclohexylamine treatment was below 50%. When aniline treated $tRNA^{Phe}_{HCl}$ was charged with ^{14}C-phenylalanine and subsequently run on disc electrophoresis all radioactivity was found in the fragment peaks. A certain amount of aniline was incorporated into the fragments during the splitting reaction as was shown by the use of ^{3}H-aniline and subsequent disc electrophoresis.

Discussion: Nishimura and Novelli[13] found that tRNA samples with enzymatic splittings still accepted amino acids; the

chain scissions were probably in the anticodon regions, since the transfer activities were decreased. Bayev et al.[14] produced half molecules of $tRNA^{Val}_{yeast}$ with T1-RNase and reported full acceptor activity on recombination of the fragments.

In the present paper the first completely specific chemical splitting of a RNA molecule into large fragments is reported. The chain scission occurs next to the anticodon of $tRNA^{Phe}$ since it takes place only after a specific modification in this region of the molecule. This conclusion is in agreement with results from model experiments on hexanucleotides from the anticodon region of $tRNA^{Phe}$. Fragments of about equal length are formed from $tRNA^{Phe}_{HCl}$ according to disc electrophoresis. The detailed mechanism of the reaction is not yet clear. The aldehyde group of the ribose may be present as hydrate or semiacetal in part of the $tRNA^{Phe}_{HCl}$ molecules. It is likely that, in analogy to the Whitfeld degradation, the phosphodiester bond in the ß-position to the aldehyde group is cleaved in the amine solutions. This would leave the ribose attached to the 36 nucleotide pGp-containing fragment and the internucleotide phosphate attached to the 5'-terminal hydroxyl group of the 39 nucleotide CCA-containing fragment. The finding of acceptor activity in the mixture of the two fragments offers new possibilities for the study of synthetase recognition sites in $tRNA^{Phe}$.

References:

1. RajBhandary, U.L., Chang, S.H., Stuart, A., Faulkner, R.D., Hoskinson, R.M., and Khorana, H.G., Proc. Natl. Acad. Sci. U.S. 57 (1967) 751.

2. Thiebe, R. and Zachau, H.G., Europ. J. Biochem. 5 (1968) 546.

3. Whitfeld, P.R. and Markham, R.,
Nature, 171 (1953) 1151.

4. Yu, C.-T. and Zamecnik, P.C.,
Biochim. Biophys. Acta, 45 (1960) 148.

5. Khym, J.X. and Cohn, W.E.,
J. Biol. Chem. 236 (1961) PC 9.

6. Neu, H.C. and Heppel, L.A.,
J. Biol. Chem. 239, (1964) 2927.

7. Steinschneider, A. and Fraenkel-Conrat, H.,
Biochemistry, 5 (1966) 2735.

8. Gillam, I., Millward, S., Blew, D., von Tigerstrom, M.,
Wimmer, E., and Tener, G.M.,
Biochemistry, 6 (1967) 3043.

9. Thiebe, R. and Zachau, H.G.,
Biochem. Biophys. Res. Commun. 33 (1968) 260.

10. Harkness, D.R. and Hilmoe, R.J.,
Biochem. Biophys. Res. Commun. 9 (1962) 393.

11. Ames, B.N. and Dubin, D.T.,
J. Biol. Chem. 235 (1960) 769.

12. Richards, E.G., Coll, J.A., and Gratzer, W.B.,
Analyt. Biochem. 12 (1965) 452.

13. Nishimura, S. and Novelli, G.D.,
Proc. Natl. Acad. Sci. U.S. 53 (1965) 178.

14. Bayev, A.A., Venkstern, T.V., Mirzabekov, A.D., Krutilina,
A.I., Axelrod, V.D., Li, L., Fodor, I., Kasarinova, L.Y.,
and Engelhardt, V.A., "Structure and Function of Transfer
RNA and 5S-RNA" Universitetsforlaget, Oslo, and Academic
Press, London and New York (1968) p. 17.