PREPARATION OF A pG-FRAGMENT FROM $tRNA^{Phe}_{yeast}$ BY CHEMICAL SCISSION AT THE DIHYDROURACIL, AND INHIBITION OF $tRNA^{Phe}_{yeast}$ CHARGING BY THIS FRAGMENT WHEN COMBINED WITH THE –CCA HALF OF THIS tRNA

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1. Introduction

Some insight into the specificity of the recognition reaction of tRNA-synthetase was provided by studies with large fragments of tRNA [1–4].

We are reporting here a chemical method to obtain a quarter of a $tRNA^{Phe}$ molecule (which we call pG-fragment). This fragment, 15 nucleotides long, starting from the hU residue in position 16 of yeast $tRNA^{Phe}$ and ending at the pG terminus was isolated by a chain scission at the reduced hU. Charging of yeast $tRNA^{Phe}$ is competitively inhibited by the combination of this pG-fragment with the chemically obtained -CCA portion of yeast $tRNA^{Phe}$, while each of these fragments, taken individually, has no effect.

2. Materials and methods

Purified yeast $tRNA^{Phe}$ was purchased from Boehringer (Germany). Phenylalanyl-tRNA-synthetase was a gift from Dr. J.P.Ebel (Strasbourg) and was purified according to this method [5]; its specific activity was 1200.

Splitting of tRNA into half molecules (referred to as the pG-5′-half and the 3′-half of 3′-CCA-half) was carried out as described previously [6, 7]. Polynucleotide kinase was a gift from Dr. Wright (Inst. Pasteur, Paris) and was prepared according to Richardsons's method [8].

The pG-fragment (quarter of a molecule) was prepared as follows:

a) *Reduction with sodium borohydride to convert 5,6-dihydrouracil into ureidopropanol N riboside, according to Igo-Kemenes and Zachau [9]*

The reaction mixture contained in 1.0 ml: 150 A_{260} units $tRNA^{Phe}$; 0.2 mmole KCl; 0.01 mmole $MgCl_2$; 0.2 mmole sodium borate buffer, pH 9.8; and 15 mg $NaBH_4$; The mixture was kept at room temperature in the dark for 3 hr. Excess $NaBH_4$ was destroyed by addition of about 1.0 ml 1 M CH_3COOH to the mixture (pH 4.0). The reduced $tRNA^{Phe}$ was purified by three successive ethanol purifications.

b) *Treatment with HCl in order to excise the ureidopropanol and the Y base, according to Cohn and Doherty [10]*

150 A_{260} units of reduced yeast $tRNA^{Phe}$ were incubated 2 hr at 22° in 30 ml dilute HCl (pH 2.3 instead of 1.8 as in the reference in order to minimize depurinization) the modified $tRNA^{Phe}$ was recovered by three successive precipitations with ethanol.

c) *Treatment with alkaline phosphatase in order to dephosphorylate the pG-terminus*

100 A_{260} units of HCl-treated $tRNA^{Phe}$ were incubated 2 hr at 37° in a mixture (1.0 ml) containing: 7 μmoles tris-HCl (pH 7.6) and 1800 units *E. coli* alkaline phosphatase (Worthington). The enzyme was inactivated by heating 3 min at 100°.

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d) *Treatment with polynucleotide kinase in the presence of γ^{32}P-ATP*

1 ml incubation mixture contained: 60 μmoles tris-HCl buffer (pH 7.6); 10 μmoles $MgCl_2$; 15 μmoles β-mercaptoethanol; 50 mmoles γ^{32}P-ATP (specific activity 1764 mCi/nmole); 100 A_{260} units modified $tRNA^{Phe}$, dephosphorylated as above; and 3000 units polynucleotide kinase. Incubation was for 5 min at 37°. The product was isolated by three successive ethanol precipitation and treated with aniline under acidic conditions, as described by Philippsen et al. [6], in order to split it at the excised base, thus yielding the pG-fragment (about 10 A_{260} units) and the 3′-half.

3. Results

Philippsen et al. [6] had found that treatment of $tRNA^{Phe}$ by aniline under mild acidic conditions results in a splitting at the phosphodiester bond linking the ribose moiety in which the Y base was excised. We found that the same treatment can be applied to the phosphodiester bond adjacent to the ribose after reduction of the hU and its excision; this results in a chain scission and formation of a 15 nucleotide long fragment, ranging from the 5′-P to the hU residue in position 16. The exact preparation is described in Methods.

3.1. *Chromatographic isolation of the pG-fragment and 3′-CCA-half of tRNA*

After chromatography on DEAE-Sephadex A-25, five fragments were isolated from the yeast $tRNA^{Phe}$ subjected to the treatment described above (steps a through d) (fig. 1). The complete analysis of these peaks is currently under investigation. Peak 1 was identified as a pentadecanucleotide containing the pG-terminus (pG-fragment) for the following reasons: (1) it contains all of the ^{32}P activity which is not dialysable; (2) it is excluded at the beginning of the gradient, whereas the 5′-half obtained according to the method of Philippsen et al. [6] is excluded from the same column much later (after peak 5); (3) when peak 1 is combined with the 3′-half of the molecule, the acceptor activity is not restored, as is the case when the 5′-half and 3′-half are combined.

Peak 2, 3 and 5 have not been studied, but they probably represent the fragment of the molecule from the hU to the Y base.

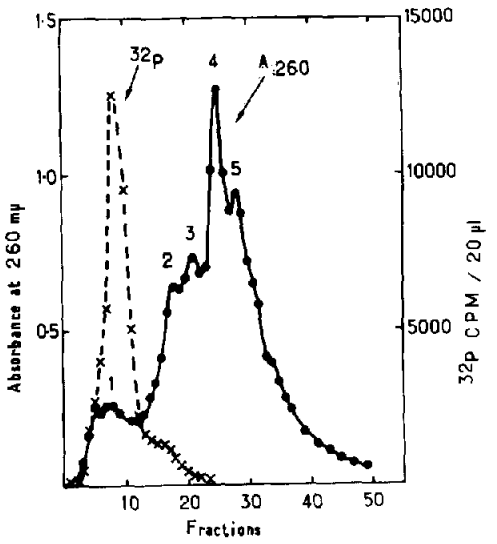

Fig. 1. Chromatographic isolation of pG-fragment of yeast $tRNA^{Phe}$. Column of DEAE-Sephadex A-25 (0.5 × 100 cm); linear gradients of 300 ml each, 0.1 and 0.5 M NaCl in 7 M urea, adjusted with HCl to pH 3.0.

Peak 4 was identified as the 3′-half of the yeast $tRNA^{Phe}$ because it is the only peak which restores the acceptor activity when added to the pG-5′-half of the yeast $tRNA^{Phe}$ as is the case with the 3′- and 5′-halves previously obtained by the method of Philippsen et al. [6]. Furthermore, this peak is excluded at the same position as the 3′-half obtained by this same method.

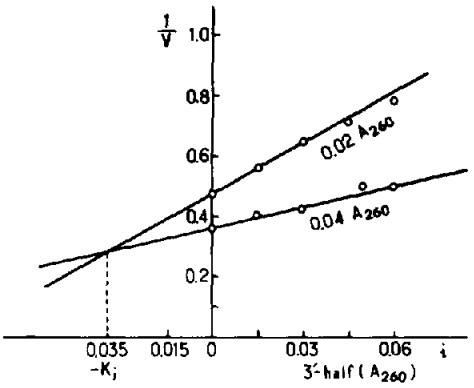

Fig. 2. Dixon plot of inhibition of the rate of charging of yeast $tRNA^{Phe}$ by combination of the 3′-half and the Gp-fragment. Charging conditions identical to those described in table 1. Concentration of $tRNA^{Phe}$ as indicated. V is expressed in pmoles tRNA charged per 2 min at 10°.

Table 1
Inhibition of the acceptor activity of yeast $tRNA^{Phe}$ by combination of the fragments from this tRNA.

	cpm / assay	Inhibition (%)
yeast $tRNA^{Phe}$		
alone (control)	860	0
+ 3′-half	800	7
+ pG 5′-half	840	3
+ pG-fragment	830	4
+ 3′-half + pG-fragment	510	41

Incubation mixture (0.1 ml): tris-HCl, 50 mM (pH 7.5); $MgCl_2$, 15 mM; ATP, 10 mM; ^{14}C-phenylalanine, S.A. = 73 × 10^3 cpm/nmole 124 000 cpm; KCl, 5 mM; β-mercaptoethanol, 5 mM; bovine serum albumin, 5 μg; yeast $tRNA^{Phe}$, 0.036 A_{260}; phenylalanyl-tRNA-synthetase, 0.05 μg protein; and, when indicated: 3′-half, 0.07 A_{260}; pG-fragment, 0.05 A_{260}; pG-5′-half, 0.07 A_{260}. Incubation 2 min at 10°.

Determination of acceptor activity was done by the method of Ingram and Pierce [11]. The samples were counted in a 5 ml toluene-based liquid scintillator containing 0.3 g 1,4-bis(*p*-phenyloxazolyl-2)-benzene, and 5 g 2,5-diphenyloxalzole per liter in a Packard liquid scintillation counter (1 μCi = 1124,000 cpm).

3.2. *Inhibition of the acceptor activity of yeast $tRNA^{Phe}$ by the combination of the 3′-half and the pG-fragment*

The results are presented in table 1. It can be seen that, none of the fragments alone have any inhibitory activity, whereas the combination of the 3′-half and the pG-fragment significantly inhibits the rate of charging of yeast $tRNA^{Phe}$. A Dixon plot of the data (fig. 2) indicates that the inhibition is competitive. The inhibition observed appears to be specific for this tRNA, since the combination of the fragments has no effect on the acceptor activity of *E. coli* $tRNA^{Tyr\ II}$ nor on that of the yeast $tRNA^{Lys}$ by their respective synthetases (not shown in table).

4. Discussion

The lack of inhibition of the charging of yeast $tRNA^{Phe}$ by the isolated halves of the molecules is in agreement with the results reported by Chang [12]. In contrast, the results of Stulberg et al. [13] with *E. coli* $tRNA^{Phe}$ indicate that fragments of this tRNA, obtained by partial degradation with snake venom phosphodiesterase (from 2–3% to 75% degradation) inhibit the charging of *E. coli* $tRNA^{Phe}$.

The present results showing the competitive inhibition of the acceptor activity of yeast $tRNA^{Phe}$ by a combination of the 3′-half and the pG-fragment of the molecule (and not by each individual fragment) may be considered in connection with the results of Chambers [14] indicating that the specific recognition site for yeast alanyl-$tRNA^{Ala}$-synthetase is located in the acceptor stem of $tRNA^{Ala}$.

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