IN VITRO INCORPORATION OF 2'-DEOXYADENOSINE AND 3'-DEOXYADENOSINE INTO YEAST $tRNA^{Phe}$ USING tRNA NUCLEOTIDYL TRANSFERASE, AND PROPERTIES OF $tRNA^{Phe}$-C-C-2'dA AND $tRNA^{Phe}$-C-C-3'dA

Mathias Sprinzl, Karl-Heinz Scheit[+], Hans Sternbach, Friedrich von der Haar, and Friedrich Cramer

Max-Planck-Institut für experimentelle Medizin, Abteilung Chemie

34 Göttingen, Hermann-Rein-Str. 3, BRD

[+]Max-Planck-Institut für biophysikalische Chemie

Karl-Friedrich-Bonhoeffer-Institut

34 Göttingen, Am Faßberg, BRD

Received February 27,1973

SUMMARY

2'-Deoxyadenosine and 3'-deoxyadenosine (cordycepin) can be incorporated into the 3'-terminal position of $tRNA^{Phe}$ by tRNA nucleotidyl transferase. $tRNA^{Phe}$-C-C-2'dA and $tRNA^{Phe}$-C-C-3'dA, missing the cis-diol group at the 3'-terminal end are resistant to periodate oxidation and are not able to form borate complexes. In aminoacylation experiments only the $tRNA^{Phe}$-C-C-3'dA proved to be chargeable.

Abbreviations: $tRNA^{Phe}$ = $tRNA^{Phe}$-C-C-A = phenylalanine transfer RNA; $tRNA^{Phe}$-C-C = $tRNA^{Phe}$ lacking the 3'-terminal AMP; $tRNA^{Phe}$-C-C-2'dA and $tRNA^{Phe}$-C-C-3'dA = $tRNA^{Phe}$ with 3'-terminal 2'-deoxyadenosine and 3'-deoxyadenosine, respectively.

INTRODUCTION

The C-C-A end of tRNA is of particular interest in the investigation of the mechanisms of aminoacylation and protein biosynthesis. Modification of the 3'-end of tRNA, which can be performed by introduction of modified nucleotides by tRNA nucleotidyl transferase is a valuable tool for the study of the structure and function of this exposed part of the tRNA molecule.

We have used this method for the introduction of heavy atoms (1), acceptors of heavy metal ions (2,3) or spectroscopic labels (4) into the 3'-end of $tRNA^{Phe}$. We wish to report that introduction of 2'-deoxyadenosine or 3'-deoxyadenosine (the antibiotic cordycepin (5)) into the 3'-terminal position of $tRNA^{Phe}$ is also possible using this enzyme yielding $tRNA^{Phe}$-C-C-2'dA and $tRNA^{Phe}$-C-C-3'dA, respectively, which may serve as tools in mechanistic studies of protein biosynthesis.

MATERIALS AND METHODS

3'-Deoxyadenosine-5'-phosphate: The phosphorylation of 3'-deoxyadenosine (Sigma Chem.Co., St. Louis, USA) was carried out according to (6). The crude reaction product was separated on a DEAE-cellulose column using a linear gradient of triethylammonium bicarbonate. The nucleotide was isolated as the disodium salt in 33 % yield. $C_{10}H_{12}N_5Na_2O_7P \times 5\ H_2O$ (465.2), calculated: P 6.7 %, found: P 6.8 %. Ultraviolet absorption in water at pH 7: λ_{max} 259 nm, λ_{min} 227 nm; ε_p (259 nm): 16 000; absorbance ratios: $A_{280}/A_{260} = 0.125$, $A_{260}/A_{250} = 1.25$.

3'-Deoxyadenosine-5'-triphosphate: The preparation starting from 3'-deoxyadenosine-5'-phosphate followed a standard procedure (7). The triphosphate was obtained as the amorphous disodium salt in 50 % yield. $C_{10}H_{14}N_5Na_2O_{12}P_3 \times 5\ H_2O$ (645.2), calculated: P 14.4 %, found: P 14.8 %. Ultraviolet absorption in water at pH 7: λ_{max} 259 nm, λ_{min} 228 nm; ε_p (259 nm): 15 800; absorbance ratios: $A_{280}/A_{260} = 0.126$, $A_{260}/A_{250} = 1.25$.

2'-Deoxyadenosine-5'-triphosphate was obtained from Boehringer GmbH (Mannheim, Germany).

Enzymes: Phenylalanyl-tRNA synthetase (E.C. 6.1.1.-) was purified from baker's yeast by affinity elution up to a specific activity of 800 units/mg (1 unit = incorporation of 1 nmole phenylalanine into $tRNA^{Phe}$/min) (8).

tRNA nucleotidyl transferase (E.C. 2.7.7.-) purified from baker's yeast had a specific activity of 12 000 units/mg (1 unit = incorporation of 1 nmole ATP into $tRNA^{Phe}$-C-C/min) (9). Pancreatic ribonuclease (E.C. 2.7.7.16.) 1 mg/ml and snake venom phosphodiesterase (E.C. 3.1.4.1.) 1 mg/ml were obtained from Boehringer GmbH (Mannheim, Germany).

$tRNA^{Phe}$ species: $tRNA^{Phe}$-C-C-A from yeast was prepared according to (10). Partial degradation of the -C-C-A end with snake venom phosphodiesterase (1) followed by incorporation of CMP into positions 74, 75 with tRNA nucleotidyl transferase (10) gave $tRNA^{Phe}$-C-C. Cellulose-bound phenylboronic acid (11) was a generous gift of Dr. W. Brümmer (Merck, Darmstadt, Germany).

Incorporation of 2'dATP and 3'dATP into $tRNA^{Phe}$-C-C: 66 A_{260} units $tRNA^{Phe}$-C-C were incubated with 2 µmole nucleoside triphosphate and 50 µg tRNA nucleotidyl transferase in 1 ml solution containing 0.01 M $MgSO_4$, 0.1 M Tris-HCl pH 9.0, and 0.1 M KCl at 37°C for 1 h. The reaction mixture was worked up by Sephadex A 25 chromatography at pH 5.2 and Biogel P-2 filtration. Yield 52.0 A_{260} units $tRNA^{Phe}$-C-C-2'dA and 55.8 A_{260} units $tRNA^{Phe}$-C-C-3'dA, respectively. Further characterization of these tRNAs is given under Results.

RESULTS AND DISCUSSION

3'-Deoxyadenosine-5'-triphosphate, 3'dATP, was obtained from 3'-deoxyadenosine by chemical synthesis and was found to be a substrate for tRNA nucleotidyl transferase. Compared to the natural substrate, ATP, the incorporation of 3'dAMP proceeds with much lower velocity and requires a large excess of highly purified enzyme. Even then the incorporation was not complete. The structural isomer of 3'dATP, 2'-deoxyadenosine-5'-triphosphate, 2'dATP, is also a substrate for tRNA nucleotidyl transferase. Under the same conditions used for the incorporation of 3'dAMP, 2'dAMP is also incorporated into the 3'-terminus of $tRNA^{Phe}$. Both modified tRNA's were purified by chromatography on Sephadex A 25 where a complete separation of excess

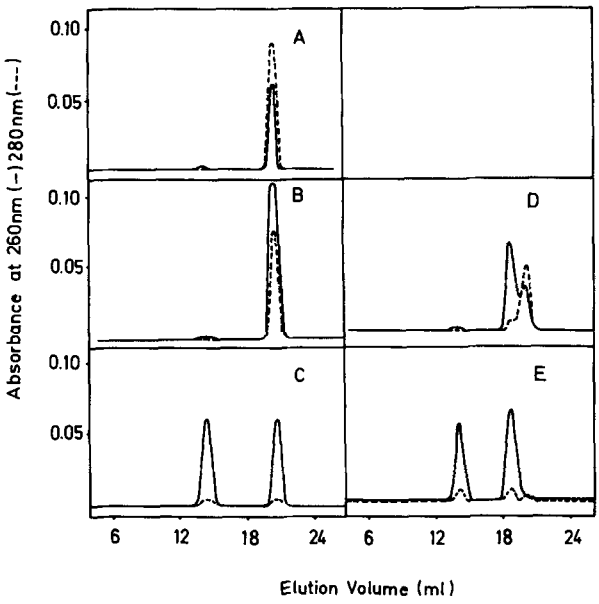


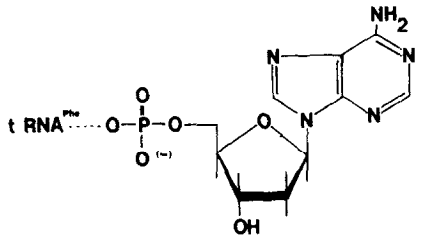
Fig. 1: Analysis of the 3'-end nucleoside of $tRNA^{Phe}$ species. 5 A_{260} units of $tRNA^{Phe}$ were incubated in a 30 µl mixture containing 5 µl pancreatic RNAase in 0.2 M ammonium acetate pH 7.5 at 37°C for 3 hrs. The hydrolysate was applied to a column of Beckman M-71 ion exchanger and eluted with 0.4 M ammonium formate buffer pH 4.75. Chromatograms were evaluated as in (1). Position of the peaks: adenosine 14.5 ml, 3'-deoxyadenosine 19.4 ml, 2'-deoxyadenosine and cytidine 20.5 ml. A: $tRNA^{Phe}$-C-C contaminated with less than 2 % $tRNA^{Phe}$-C-C-A; B: Mixture of 51 % $tRNA^{Phe}$-C-C and 49 % $tRNA^{Phe}$-C-C-2'dA; C: Mixture of 51 % $tRNA^{Phe}$-C-C-A and 49 % $tRNA^{Phe}$-C-C-2'dA obtained by regeneration of a $tRNA^{Phe}$ mixture (analyzed in Fig. 1B) with tRNA nucleotidyl transferase and ATP; D: Mixture of 42 % $tRNA^{Phe}$-C-C and 58 % $tRNA^{Phe}$-C-C-3'dA; E: Mixture of 42 % $tRNA^{Phe}$-C-C-A and 58 % $tRNA^{Phe}$-C-C-3'dA obtained by regeneration of a $tRNA^{Phe}$ mixture (analyzed in Fig. 1D) with tRNA nucleotidyl transferase and ATP.

---

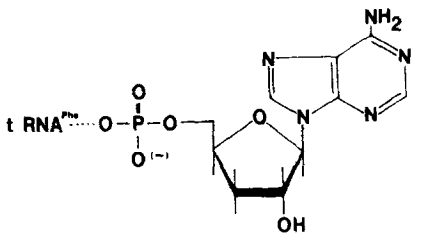
monomers could be achieved. The presence of modified nucleosides at the 3'-terminus of $tRNA^{Phe}$ was checked by pancreatic RNAase digestion of tRNA and chromatographic analysis of the 3'-terminal nucleosides (1). Fig. 1A shows the analysis of the starting $tRNA^{Phe}$-C-C where a single peak of cytidine was obtained. Partial incorporation of 3'dAMP leads to a mixture of 58 % $tRNA^{Phe}$-C-C-3'dA and 42 % $tRNA^{Phe}$-C-C (Fig. 1D). The remaining $tRNA^{Phe}$-C-C could be completely regenerated by reincubation with tRNA

nucleotidyl transferase and ATP. Analysis of the mixture obtained gave well-resolved peaks of adenosine and 3'-deoxyadenosine in the expected ratio (Fig. 1E). The same procedure was used for the analysis of the mixture of 51 % $tRNA^{Phe}$-C-C and 49 % $tRNA^{Phe}$-C-C-2'dA (Fig. 1B) which was converted to a mixture of $tRNA^{Phe}$-C-C-A and $tRNA^{Phe}$-C-C-2'dA (Fig. 1C).

$tRNA^{Phe}$-C-C-3'dA, which does not possess a terminal cis-diol group, is not able to form a complex with boronic acid. In consequence of this, $tRNA^{Phe}$-C-C-3'dA is not retarded on a column of cellulose-bound phenylboronic acid (11) and can be separated from $tRNA^{Phe}$-C-C (Fig. 2). Similarly



t RNA^{Phe} – C – C – 2'dA



t RNA^{Phe} – C – C – 3'dA

$tRNA^{Phe}$-C-C-2'dA and $tRNA^{Phe}$-C-C-3'dA are resistant to periodate oxidation of the C2'-C3' linkage (12,13). Under conditions where $tRNA^{Phe}$-C-C-A is completely oxidized, the 3'-terminal end of both modified tRNA's remains intact. This can be used for investigation of the aminoacylation properties of the $tRNA^{Phe}$-C-C-2'dA and $tRNA^{Phe}$-C-C-3'dA in the presence of native tRNA, since $tRNA^{Phe}$-C-C-A can be deactivated by periodate oxidation (Table 1). In this connection one must stress that periodate oxidized $tRNA^{Phe}$-C-C-A is neither substrate nor inhibitor of phenylalanyl-tRNA synthetase (12).

Results given in Table 1 show that the $tRNA^{Phe}$ missing the 2'-hydroxyl group on the terminal adenosine cannot be aminoacylated. On the other hand, the lack of the hydroxyl group on the 3'-position of terminal adenosine does not influence the extent of aminoacylation. This would suggest that native $tRNA^{Phe}$-C-C-A is also aminoacylated specifically on the 2'-hydroxyl group. In order to confirm this, a careful comparison of both modified tRNA's in

TABLE 1: Maximum aminoacylation with [^{14}C]Phenylalanine^{+} (pmol/A_{260} unit $tRNA^{Phe}$)

$tRNA^{Phe}$ species	before oxidation with $NaIO_4$		after oxidation with $NaIO_4$$^{++}$	
	without NTase	with NTase^{+++}	without NTase	with NTase
$tRNA^{Phe}$-C-C-A	1460 (100%)	1450 (99.5%)	0	0
$tRNA^{Phe}$-C-C	0	1450 (99.5%)	0	0
$tRNA^{Phe}$-C-C-2'dA$^{\Phi}$	0	780 (53 %)	0	0
$tRNA^{Phe}$-C-C-3'dA$^{\Phi}$	820 (56 %)	1440	800 (55 %)	820 (56 %)

$^{+}$Aminoacylation assay as in (8).

$^{++}$Periodate oxidation as in (13).

$^{+++}$tRNA nucleotidyl transferase and ATP present in aminoacylation assay as in (1).

$^{\Phi}$$tRNA^{Phe}$ samples tested were the mixtures analyzed in Fig. 1B and 1D, respectively.

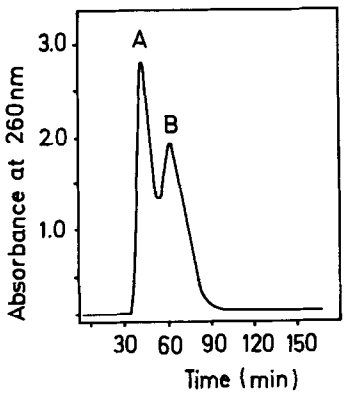


Fig. 2: Separation of $tRNA^{Phe}$-C-C from $tRNA^{Phe}$-C-C-3'dA on a column of cellulose-bound phenylboronic acid (0.5 x 120 cm) (11), equilibrated with 0.05 M morpholine·HCl pH 8.5 in 0.1 M $MgCl_2$. 30 A_{260} units of a mixture of 58 % $tRNA^{Phe}$-C-C-3'dA and 42 % $tRNA^{Phe}$-C-C were eluted with the same buffer at a constant flow rate of 0.3 ml/min. 8 A_{260} units consisting of 85 % $tRNA^{Phe}$-C-C-3'dA and 15 % $tRNA^{Phe}$-C-C were isolated from peak A, and 11.3 A_{260} units of pure $tRNA^{Phe}$-C-C from peak B; the area where the peaks overlap was not analyzed. Both samples were desalted by Biogel P-2 filtration and analyzed for the 3'-end nucleoside as in Fig. 1.

aminoacylation is necessary. Work on this and on its biological implications is in progress.

ACKNOWLEDGEMENT

We are deeply indebted to Dr. D. Gauss for discussions and Miss E. Gaertner as well as Mrs. R. Sprinzl for an excellent assistance during this work.

REFERENCES

1. Sprinzl, M., von der Haar, F., Schlimme, E., Sternbach, H., and Cramer, F. 1970. Eur.J.Biochem. 25:262-266.
2. Sprinzl, M., Scheit, K.H., and Cramer, F. 1973. Eur.J.Biochem., in press.
3. Schlimme, E., von der Haar, F., Eckstein, F., and Cramer, F. 1970. Eur.J. Biochem. 14:351-356.
4. Maelicke, A., Sprinzl, M., von der Haar, F., Khwaja, T., and Cramer, F. 1973. J.Biol. Chem., submitted.
5. Suhadolnik, R.J.: Nucleoside Antibiotics, Wiley Interscience, New York, 1970.
6. Yoshikawa, M., Kato, T., and Takanishi, T. 1967. Tetrahedron Letters, 5065-5068.
7. Faerber, P., and Scheit, K.H. 1971. Chem.Ber. 104:456-468.
8. von der Haar, F. 1973. Eur.J.Biochem., in press.
9. Sternbach, H., von der Haar, F., Schlimme, E., Gaertner, E., and Cramer, F. 1971. Eur.J.Biochem. 22:166-172.
10. Schneider, D., Solfert, R., and von der Haar, F. 1972. Hoppe-Seyler's Z. physiol.Chem. 353:1333-1336.
11. Rosenberg, M., and Gilham, P.T. 1971. Biochim.Biophys.Acta 246:337-340.
12. von der Haar, F., Schlimme, E., Gomez-Guillen, M., and Cramer, F. 1971. Eur.J.Biochem. 24:296-302.
13. Cramer, F., von der Haar, F., and Schlimme, E. 1968. FEBS-Letters 2:136-139, 354.