SEPARATION OF AMINOACYL-tRNA^{Phe} AND tRNAs^{Phe} WITH PARTIALLY HYDROLYSED 3′-END BY POLYACRYLAMIDE GRADIENT MICRO-ELECTROPHORESIS

Mathias SPRINZL, Dirk-Ingo WOLFRUM* and Volker NEUHOFF*

Abteilung Chemie und Forschungsstelle Neurochemie Max-Planck-Institut für experimentelle Medizin 3400 Göttingen, Hermann-Reinstrasse 3 West Germany*

Received 15 October 1974

1. Introduction

Using tRNA-nucleotidyl transferase it is possible to introduce modified nucleotides into the 3′-end of tRNA. Such modified tRNAs were used for spectroscopic [1,2] and biochemical investigations [3,4] as well as isomorphous substitution of tRNA^{Phe} with heavy metals for X-ray crystallographic work [5,6]. As a starting material for incorporation of CMP or AMP analogues serves a tRNA where the 3′-end is partially or completely missing. In order to achieve a specific incorporation to the required position a tRNA with a uniform 3′-terminus has to be used. Although the 3′-end nucleotide can be determined by an enzymatic digest and chromatographical analysis [5] this method requires a substantial amount of material and does not provide informations about the integrity of the whole polynucleotide chain. Using polyacrylamid gradient microelectrophoresis [7–9] it is possible to separate tRNAs in the pmol range which differ only in one nucleotide in length and allow their rapid and reproducible characterisation. Separation of aminoacyl-tRNA from the uncharged molecule is also possible by this method, which allows its identification without using radioactive labels.

Abbreviations: tRNA^{Phe}-A_{76}-native phenylalanyl specific tRNA from yeast. tRNA^{Phe}-C_{75}, tRNA^{Phe}-C_{74} and tRNA^{Phe}-A_{73} are tRNA^{Phe} species missing their A_{76}, C_{75}-A_{76} and C_{74}-C_{75}-A_{76} nucleotides, respectively. tRNA^{Phe}-C-C-3′NH_2A tRNA^{Phe}, were the 3′-terminal A_{76} residue is replaced by 3′-amino-3′-deoxy-adenosine. Phe-tRNA^{Phe}-C-C-3′NH_2A is tRNA^{Phe}-C-C-3′NH_2A enzymatically aminoacylated with phenylalanine.

2. Materials and methods

tRNA^{Phe} was purified from yeast bulk tRNA (Boehringer, Mannheim, Germany) using a described method [10]. Pure tRNA^{Phe}-C_{75} was isolated from a mixture of tRNA^{Phe}-C_{75} and 15% tRNA^{Phe}-A_{76} by chromatography on Sephadex A-25 [10]. tRNA^{Phe}-C_{74} and tRNA^{Phe}-A_{73}, respectively, were prepared by stepwise degradation of tRNA^{Phe}-C_{75} [11] and purified by chromatography on Sephadex A-25 as described [10]. The 3′-end nucleotide of the tRNAs used was determined by a chromatographical method described previously [5]. All tRNAs possessed a uniform 3′-end. The CpCpA sequence could be restored in all cases using tRNA nucleotidyl transferase and such tRNAs^{Phe} were fully aminoacylated with Phe-tRNA synthetase from yeast (1500 pmole phenylalanine/A_{260} unit tRNA^{Phe}).

Phe-tRNA^{Phe}-C-C-3′NH_2A was prepared by enzymatic aminoacylation of yeast tRNA^{Phe}-C-C-3′NH_2A which instead of the 3′-terminal adenosine contains a 3′-deoxy-3′-aminoadenosine [12]. The amino acid is bound to this tRNA by an amide linkage which is stable under conditions applied in our studies.

Micro-electrophoresis of tRNA was performed in a continuous polyacrylamide gel gradient [7] using 5 μl capillaries. Concentration of gradients was 1–40% monomer at 2% crosslinking. As gel buffer 350 mM Tris–H_2SO_4, pH 8.4 and as electrophoresis buffer 50 mM Tris–glycin, pH 8.4 was used [9]. Samples were applied on to the gel in quantities of 0.2–0.7 pmoles tRNA. Electrophoresis was performed with 100–160 V during 20 min. Toluidine blue (0.5% in water) was used for staining. Densitometer profiles

were obtained using the Joyce-Loeble doublebeam microdensitometer with 100:1 ratio of record to sample travel using a gray wedge of 0.79 d. Further details about the micro-electrophoresis on polyacrylamide gradient gels are presented elsewhere [7,8,9,13].

3. Results and discussion

Electrophoresis of single tRNA species revealed only a single band indicating a high purity of the materials used. Fig.1a shows a separation of the mixture of $tRNA^{Phe}\text{-}A_{73}$ from $tRNA^{Phe}\text{-}A_{76}$. Assignment was made by increasing the concentration of $tRNA^{Phe}\text{-}A_{73}$ whereas the concentration of $tRNA^{Phe}\text{-}A_{76}$ was left unchanged (fig.1b). The distance between the peak maxima for these two tRNAs differing by three nucleotides in chain length is 270 μm in the gel. Separation of $tRNA^{Phe}\text{-}A_{73}$ from $tRNA^{Phe}\text{-}C_{74}$ is rather poor (fig.2a). Depending on the ratio of the two tRNAs only a shoulder was observed on the peak of the major component. The distance between the interpolated maximum of $tRNA^{Phe}\text{-}A_{73}$ from the maximum of $tRNA^{Phe}\text{-}C_{74}$ is 40 μm. If the two tRNAs were applied to a gel in the same concentration no separation was detectable. On the other hand $tRNA^{Phe}\text{-}C_{74}$ can be separated well from $tRNA^{Phe}\text{-}C_{75}$ (fig.2b). The distance between the maxima is here 110 μm. After fractionation of the mixture of $tRNA^{Phe}\text{-}A_{76}$ a distance of 120 μm can be observed between the two maxima (fig.2c). As expected the mixture of $tRNA^{Phe}\text{-}C_{74}$, $tRNA^{Phe}\text{-}C_{75}$ and $tRNA^{Phe}\text{-}A_{76}$ reveals after electrophoresis on the micro-gradient gel three well separated bands (fig.2c). As shown in fig.2d the charged Phe-$tRNA^{Phe}\text{-}C\text{-}C\text{-}3'NH_2A$ can be completely separated from a mixture of $tRNA^{Phe}\text{-}A_{73}$ and $tRNA^{Phe}\text{-}A_{76}$. The

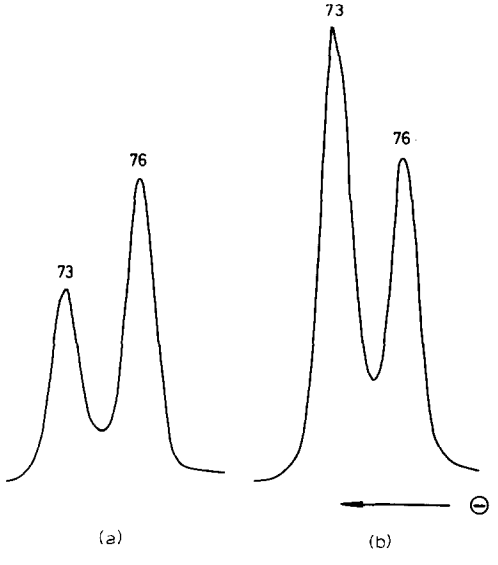

Fig.1. Microdensitogram after toluidin blue staining and fractionation of a mixture of $tRNA^{Phe}\text{-}A_{73}$ and $tRNA^{Phe}\text{-}A_{76}$ in a 5 μl polyacrylamide gradient microgel. a) Approximately equimolar amounts of both species. b) Electrophoresis of the same mixture with increased concentration of $tRNA^{Phe}\text{-}A_{73}$.

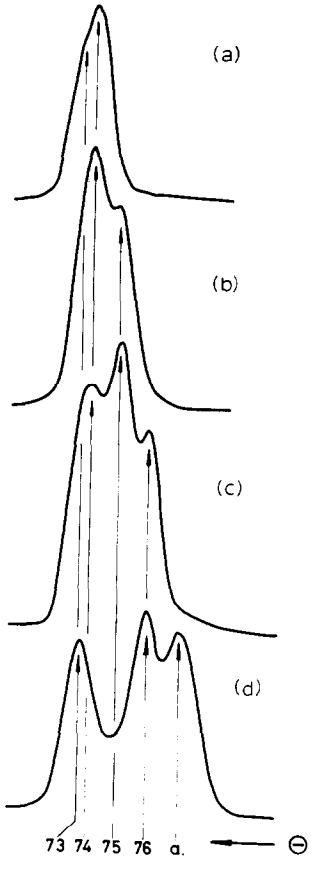

Fig.2. Separation of different mixtures of $tRNA^{Phe}$. The number of the peaks corresponds with the number of the 3'-end nucleotide of the tRNA. a. indicates the position of the aminoacylated tRNA. a) $tRNA^{Phe}\text{-}A_{73}$ + $tRNA^{Phe}\text{-}C_{74}$ b) $tRNA^{Phe}\text{-}C_{74}$ + $tRNA^{Phe}\text{-}C_{75}$ c) $tRNA^{Phe}\text{-}C_{74}$ + $tRNA^{Phe}\text{-}C_{75}$ + $tRNA^{Phe}\text{-}A_{76}$ d) $tRNA^{Phe}\text{-}A_{73}$ + $tRNA^{Phe}\text{-}A_{76}$ + Phe-$tRNA^{Phe}\text{-}C\text{-}C\text{-}3'NH_2A$.

distance between the neighbouring peaks corresponding to the aminoacylated $tRNA^{Phe}$ and the native $tRNA^{Phe}$ in the gel is 130 μm. If the positive charge of phenylalanine is eliminated by the presence of 0.05% sodium dodecyl sulfate in the system [8] the distance between the peak maxima of $tRNA^{Phe}$-A_{73} and $tRNA^{Phe}$-A_{76} remains unchanged, whereas the distance between native and aminoacyl-tRNA decreases to 100 μm.

It was shown previously that in the case of polyacrylamide gradient micro-electrophoresis there is a linear correlation between the distance of migration and the logarithm of molecular weight [7–9]. One should therefore expect that with decreased chain length of the tRNA an increased distance between the peak maxima of two molecules differing in length by one nucleotide should occur. This should be especially a case if molecules of identical primary structure are investigated. Instead of this theoretically expected result an opposite relation was found. $tRNA^{Phe}$-A_{73} is almost not separated from $tRNA^{Phe}$-C_{74}, whereas the difference in mobility between $tRNA^{Phe}$-C_{75} and $tRNA^{Phe}$-A_{76} is the largest.

It was shown that the *E. coli* ribosomal 5S RNA is able to undergo conformational changes, which can be detected by gel electrophoresis [9,14]. It is therefore possible that the observed unusual electrophoretical behavior of the tRNA molecules missing their 3'-end is also due to a change in the overall shape of the molecule. This effect appears to be most pronounced when the whole CpCpA end is missing. By a similar argumentation a conformational change can be assumed between the native and aminoacylated tRNA. Whether these behaviors have some biochemical implication e.g. in tRNA-nucleotidyl transferase reaction or ribosomal recognition needs further investigations.

Acknowledgement

The authors are indebted to Mrs E. Gaertner for preparation of some tRNA species.

References

[1] Maelicke, A., Sprinzl, M., von der Haar, F., Khwaja, A. and Cramer, F. (1974) Eur. J. Biochem. 43, 617–625.
[2] Sprinzl, M., Krämer, E. and Stehlik, D. (1974) Eur. J. Biochem., in press.
[3] Sprinzl, M. and Cramer, F. (1973) Nature 245, 3–5.
[4] Chinalli, G., Sprinzl, M., Parmeggiani, A. and Cramer, F. (1974) Biochemistry 13, 3001–3010.
[5] Sprinzl, M., von der Haar, F., Schlimme, E., Sternbach, H. and Cramer, F. (1972) Eur. J. Biochem. 25, 262–266.
[6] Sprinzl, M., Scheit, K. -H. and Cramer, F. (1973) Eur. J. Biochem. 34, 306–310.
[7] Rüchel, R., Mesecke, S., Wolfrum, D. -I. and Neuhoff, V. (1973) Hoppe-Seyler's Z. Physiol. Chem. 354, 1351–1368.
[8] Rüchel, R., Mesecke, S., Wolfrum, D. -I. and Neuhoff, V. (1974) Hoppe-Seyler's Z. Physiol. Chem. 355, 997–1020.
[9] Wolfrum, D. -I., Rüchel, R., Mesecke, S. and Neuhoff, V. (1974) Hoppe-Seyler's Z. Physiol. Chem., in press.
[10] Schneider, D., Solfert, R. and von der Haar, F. (1972) Hoppe-Seyler's Z. Physiol. Chem. 353, 1333–1336.
[11] Tal, J., Deutscher, M. P. and Littauer, U. Z. (1972) Eur. J. Biochem. 28, 478–491.
[12] Fraser, T. H. and Rich, A. (1973) Proc. Natl. Acad. Sci. U.S. 70, 2671.
[13] Neuhoff, V. (1973) Micromethods in Molecular Biology, pp. 1–83, Springer Verlag, Berlin, Heidelberg, New York.
[14] Phillips, G. R. (1974) in: Electrophoresis and Isoelectric Focusing in Polyacrylamide Gel, S. 255-264, (Allen, R. C. and Maurer, H. R., eds.) Walter de Gruyter-Verlag, Berlin, New York.