ENZYMATIC EXCHANGE OF 3′-TERMINAL AMP IN tRNA

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1. Introduction

Recently, we have found that crude enzyme preparations from yeast contain a nuclease that rapidly removes the 3′-terminal AMP residue from tRNA. Since these enzyme preparations also contain strong nucleotidyl transferase activity, there is no net change in the tRNA when ATP is present in the reaction mixture (e.g., in aminoacylation assays of tRNA), and the nucleolytic effect goes unnoticed. As described in this communication, a rapid exchange of the 3′-terminal AMP residue can be demonstrated by incubating tRNA with crude enzyme preparations from various sources in the presence of radioactive ATP. This provides a very simple procedure for labeling the terminal residue of tRNA. The labeled tRNA provides a useful substrate for detecting nucleolytic activity in enzymatic preparations used to study structure–action relationships. In addition, it is potentially valuable for studying reactions such as tRNA pyrophosphorolysis, ribosome binding, and aminoacyl-tRNA ligase interaction.

2. Materials and methods

Yeast $tRNA^{Tyr}$ was prepared as described previously [1]. Its purity was checked as before [1] and found to be 98 to 100%, based on moles of tyrosine esterified/78 moles organic P in tRNA. Examination of the RNAase T_1 digest demonstrated that the 3′-terminal sequence ...pApCpCpA, was intact.

The enzyme preparation from yeast was obtained as described previously [2]. Extracts from *E. coli*, *Pseudomonas sp.*, *A. salina*, and rat liver were kindly supplied by Dr. W. Szer and Mr. M. Zasloff of this department.

Commercial samples of radioactive ATP were used. Determination of radioactivity was carried out in a scintillation spectrometer.

3. Results and discussion

When yeast $tRNA^{Tyr}$ was incubated at 25° with [^{14}C] ATP and a crude enzyme preparation from yeast radioactive label was incorporated as shown in fig. 1 and approached the level of 1 mole/mole tRNA. The exchange reaction shows a rather broad pH optimum, around 8.0. Increasing the ionic strength of the reaction mixture decreases the rate of exchange. Incubation at 37° gives a high initial rate, but a lower final yield of incorporation. This may be due to inactivation of one of the enzymes involved in the exchange, or to nuclease-catalyzed hydrolysis of residues other than the terminal A.

To see if the incorporation takes place exclusively at the 3′-end of the tRNA, the exchange was carried out either with [^{3}H] ATP or with [α-^{32}P] ATP, and the labeled tRNA produced was degraded with pancreatic RNAase to release the 3′-terminal residue as adenosine. In order to obtain sufficient material to characterize the radioactive products properly, the reaction mixture was scaled up 20-fold from that described in the legend for fig. 1. After 40 min at 25°, the reaction mixture was applied to a 1 × 3 cm column of DEAE-cellulose, equilibrated with 0.1 M NaCl, 0.1

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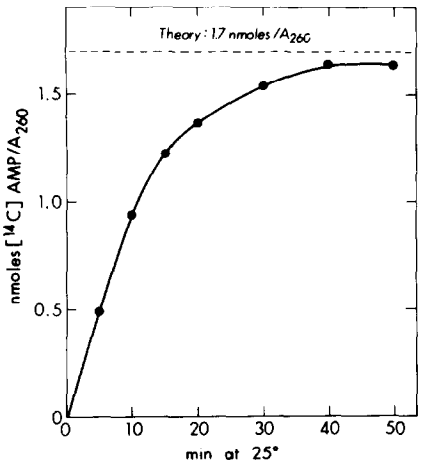

Fig. 1. Exchange of 3′-terminal AMP in $tRNA^{Tyr}$ by a crude enzyme preparation from yeast. The reaction was carried out at 25° in a mixture of 3 mM Tris-acetate, pH 8.0, 15 mM magnesium acetate, 4 mM EDTA, 2 mM [^{14}C] ATP (specific activity, 4.3 Ci/mole), 2.25 A_{260} units of $tRNA^{Tyr}$ (as measured after extensive dialysis against water), and 0.5 mg of protein in a crude enzyme preparation from yeast, in a total volume of 0.125 ml. The enzyme used was identical to the "activating enzyme" preparation of Reeves et al. [2]. At suitable time intervals, 15 μl samples were applied to Whatman 3 MM filter disks, washed, and counted as described previously [2]. A control reaction mixture, containing no tRNA, was incubated at the same time to provide the blank values. One pmole incorporation corresponded to 6.2 cpm. One A_{260} unit of $tRNA^{Tyr}$ (in H_2O) is equal to 1.7 nmole, as determined by phosphorus analysis.

sodium acetate, pH 4.5. After eluting the protein and unreacted ATP with 100 ml of the above buffer, the labeled tRNA was eluted with 2 M NaCl, 0.1 M sodium acetate, pH 4.5, containing 10% ethanol. The effluent was dialyzed against water and concentrated by evaporation at room temperature under reduced pressure. After digesting samples of the labeled tRNA with pancreatic RNAase, the radioactive products were identified by thin-layer chromatography and paper electrophoresis. When the exchange reaction was carried out with [^{3}H] ATP, adenosine was the only radioactive product in the RNAase digest. When [α-^{32}P] ATP was used, 3′-CMP was the only radioactive product found. These data show that AMP is incorporated exclusively into the 3′-end of the molecule.

To see if the exchange reaction is due to the combined action of a nuclease and nucleotidyl transferase, rather than to nucleotidyl transferase itself acting reversibly, [^{3}H] $tRNApApCpCp^*A^{Tyr}$, prepared as described above, was incubated with the crude enzyme preparation†. The enzyme was passed through Sephadex G-25 just prior to use to remove any ATP of PP_i that might have been present. After incubation at 25° for 1 hr, the ethanol-soluble products were analyzed by paper electrophoresis and thin-layer chromatography. AMP and adenosine were the only radioactive breakdown products detected. In a similar experiment employing [^{32}P] tRNApApCpC*pA AMP and P_i were the only ^{32}P-labeled alcohol-soluble products. The ratio of P_i to AMP increased during the reaction, indicating that 5′-AMP is the initial product and is subsequently broken down to adenosine and P_i. In additional experiments, it was found that PP_i is not required for, and does not stimulate, the release of acid-soluble material from the terminally-labeled tRNA; nor does inorganic pyrophosphatase inhibit the same reaction. Finally, partially purified nucleotidyl transferase, obtained by phosphocellulose chromatography of the crude enzyme preparation, is not able to release terminal AMP from tRNA under our standard conditions, but it is able to catalyze incorporation of AMP from ATP into tRNA which lacks the 3′-terminal AMP residue. These experiments demonstrate that the first step in tl exchange reaction is removal of the terminal AMP by a nuclease. This is followed by repair of tRNApCpC to give tRNApCpCpA, catalyzed by nucleotidyl transferase.

The ability to exchange the 3′-terminal AMP in tRNA is not limited to crude enzyme preparations from yeast. Similar preparations from four other sources were examined. Extracts from *E. coli* Q13, *Pseudomonas sp.* 412, embryos of *Artemia salina* (a brine shrimp), and rat liver all catalyzed the exchange reaction. The results are summarized in table 1. In these experiments, no attempt was made to find optimal conditions for each particular extract. Nevertheless, more than 50% of the 3′-terminal AMP was exchanged even with the least active preparation (rat liver).

The ability of crude enzyme preparations from various sources to catalyze the turnover of 3′-terminal AMP in tRNA suggests that this may take place

† The asterisk indicates the label is in the residue immediately to the right.

Table 1
Exchange of 3′-terminal AMP in $tRNA^{Tyr}$ by crude enzyme preparations from various sources.

Source of crude enzyme	[^{14}C] AMP incorporated (pmoles/A_{260})	
	30 min	60 min
Escherichia coli Q13	1,398	1,654
Pseudomonas sp. 412	1,467	1,558
Artemia salina	915	1,231
Rat liver	785	935

Incubation conditions and methods of sampling and counting were identical to those described in the legend for fig. 1, except that all enzymes were used at twice the concentration and the incubation temperature was 37°. All enzyme preparations were 100,000 *g* or 150,000 *g* supernatants, in some cases concentrated by $(NH_4)_2SO_4$ precipitation, dialyzed, and kept frozen before the experiment.

universally *in vivo*. In fact, it has been shown that this residue turns over in exponentially growing yeast [3] and *E. coli* [4]. Furthermore, tRNA isolated from stationary phase cells often lacks the 3′-terminal AMP. The biological significance of this turnover in growing cells is not altogether clear, though one can easily visualize a regulatory process controlled by the ATP level. In any event, the activities described here (nuclease + nucleotidyl transferase) can account for the turnover observed *in vivo*.

These reactions provide a simple method for preparing tRNA labeled at its 3′-terminus without modifying its structure in any way. The procedure is much faster and simpler than previously described methods, which require at least two steps [5–7]. All of these methods utilize partially purified enzymes, while the exchange reaction described here is carried out with a crude enzyme preparation. The yield (75%) is good under optimal conditions since the exchange is essentially complete, and the only possibility for loss of material is in the simple deproteinization step.

Finally, we have found that tRNA modified by HSO_3^- or 254 nm light is a particularly good substrate for the nuclease present in crude extracts. Furthermore, after partial degradation of the modified tRNA by the nuclease, the product is not always repairable by the nucleotidyl transferase reaction†. Since this could lead to misinterpretation of the results, it is necessary to use nuclease-free preparations of amino acid:tRNA ligases to assay aminoacylation of modified tRNA. The terminally labeled tRNA has been an indispensible substrate for detecting nuclease activity in our ligase preparations.

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† The reasons for this are somewhat complex, and an explanation must be deferred to a full paper (Kućan, Kućan and Chambers, in preparation).

References

[1] Ž. Kućan, K.A. Freude, I. Kućan and R.W. Chambers, Nature New Biol. 232 (1971) 177.
[2] R.H. Reeves, N. Imura, H. Schwam, G.B. Weiss, L.H. Schulman and R.W. Chambers, Proc. Natl. Acad. Sci. U.S. 60 (1968) 1450.
[3] R. Rosset and R. Monier, Biochem. Biophys. Res. Commun. 10 (1963) 195.
[4] M. Cannon, Biochim. Biophys. Acta 87 (1964) 154.
[5] H.C. Neu and L.A. Heppel, J. Biol. Chem. 239 (1964) 2927.
[6] R.W. Morris and E. Herbert, Biochemistry 9 (1970) 4819.
[7] J.P. Miller and G.R. Philipps, J. Biol. Chem. 245 (1971) 1274.