AMINO ACID ACCEPTOR ACTIVITY OF BACTERIOPHAGE T4 TRANSFER RNA

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1. Introduction

In a recent study we have shown that T4 bacteriophage infection of *E. coli B* cells induces the formation of 4S RNA molecules that specifically hybridize with T4 DNA [1,2]. Moreover, the T4 4S RNA extracted from the hybrid was found to contain pseudouridylic acid [2]. Since ψ UMP is believed to be present predominantly in transfer RNA, its presence in a 4S RNA molecule coded for by the T4 genome suggested that at least some of this RNA may have amino acid acceptor function. To test this hypothesis a method was devised to recover intact, biologically active tRNA from tRNA-DNA hybrids [3]. We recently reported that the T4 4S RNA preparation extracted from a hybrid with T4 DNA has amino acid acceptor capacity [4]. In addition, Weiss et al. [5] showed that leucyl-tRNA isolated from T4-infected cells will hybridize with T4 DNA. This report describes the method used for the isolation of biologically active T4 tRNA from its hybrid with T4 DNA.

2. Experimental procedure

2.1. *Preparation of ^{32}P-labelled T4 tRNA*

E. coli B cells were infected with T4 bacteriophage in the presence of ^{32}P (0.5 mc/liter) as described previously [2]. The cells obtained from 1 liter of culture were suspended in 10 to 20 ml of a solution containing 10 mM Tris-HCl buffer, pH 7.4, 10 mM $MgCl_2$ and 10 mM NaCl and then extracted three times with an equal volume of phenol saturated with 10 mM Tris-HCl buffer, pH 7.4. To the aqueous phase two volumes of ethanol were added at 4° and the DNA removed by spooling on a glass rod. The RNA was then collected by centrifugation, washed three times with ethanol and dissolved in 2.0 ml of 1 mM NaCl. The solution was adjusted to 0.3 M Na acetate pH 7.0, and 0.54 volume of isopropanol was added at 20° and the precipitate was discarded. The 4S RNA was then precipitated by the addition of 0.34 volume of isopropanol at 0°. The precipitate obtained was washed with cold ethanol and dissolved in 1.0 ml of 10 mM Tris-HCl buffer, pH 7.4 containing 10 mM $MgCl_2$. Electrophoretically purified DNase (40 μg, Worthington Biochemicals) was added and the mixture incubated at 37° for 60 min. Pronase (200 μg, preincubated in 10 mM Tris-HCl buffer, pH 7.4 for 5 hr at 37°) was then added and the mixture incubated for 4 hr at 37°. To this solution 0.1 ml of 0.2 M EDTA was added, the mixture extracted twice with 1.0 ml of Tris-HCl saturated phenol and the RNA precipitated with 0.2 volume of 5 M NaCl and 2.2 volume of ethanol. The precipitate obtained was washed three times with ethanol and dissolved in 1.0 ml of 1 mM NaCl. Such a tRNA preparation contains usually $2–5 \times 10^3$ cpm of ^{32}P/μg RNA and has an amino acid acceptor activity of $2–3 \times 10^3$ cpm of ^{14}C/μg RNA when assayed with a mixture of fifteen ^{14}C-labelled amino acids. Radioactivity was measured in a scintillation counter using a double channel setting for counting ^{32}P and ^{14}C simultaneously.

2.2. *Isolation of tRNA from its hybrid with T4 DNA*

Between 4 to 8 mg of alkali denatured [6] T4 DNA (20 μg/ml) were hybridized in 6 × SSC solution (0.9 M NaCl and 0.09 M Na-citrate) with T4 ^{32}P-tRNA (3 μg/ml) at 70° for 60 min. The mixture was then cooled to 0° and loaded on nitrocellulose filters (type B–6, 150 mm Schleicher and Schuell). The filters were washed before use with boiling water to remove

detergents [7] and soaked in 6 X SSC. About 2 mg of T4 DNA were loaded on one filter which was then washed on each side with 200 ml of 6 X SSC. The filters were suspended in 2 X SSC and washed again on each side with 100 ml of 2 X SSC. To extract the RNA each filter was suspended in 30 ml of 10 mM of Tris-HCl buffer, pH 7.4 for 90 min at 37°. The combined extracts were concentrated by ultrafiltration through a UM-1 membrane (Diaflo ultrafiltration cell, Amicon Corp., Cambridge, Mass.). To remove excess salt, the concentrated solution was diluted with 50 ml of 2 mM NaCl solution and concentrated again. This procedure was repeated twice and the final solution (2 to 3 ml) was adjusted to 10 mM $MgCl_2$ and 10mM Tris-HCl buffer, pH 7.4. This solution was incubated with 30 μg/ml DNase for 60 min at 37°, then heated for min at 90° followed by rapid cooling to 0°. The solution was diluted to 50 ml with 2mM NaCl, and concentrated by ultrafiltration followed by repeated dilution as described above. The tRNA solution was finally concentrated to 10 to 20 μg/ml.

2.3. *Assay of amino acid acceptance*

The reaction mixture contained in a final volume of 0.1 ml: 5 μmoles of Tris-HCl buffer, pH 7.5; 1 μmole of $MgCl_2$; 0.5 μmole of KCl; 0.2 μmole of ATP; 0.5 μmole of β-mercaptoethanol; 5 μl of a mixture of 15 neutralized ^{14}C-amino acids; 0.2–1 μg of tRNA and saturating amounts of crude aminoacyl-tRNA synthetase [8]. The reaction mixture was incubated for 10 min at 37° then cooled, 0.1 ml of bovine serum albumin was added (0.1 mg) followed by 1 ml of cold 5% trichloroacetic acid. The precipitate was collected onto Millipore filters, washed with cold trichloroacetic acid, dried and counted. The ^{14}C-amino acid mixture was purchased from New England Nuclear Corp. and contained a mixture of 15 L-amino acids at approximately 40 mC/milliatom of carbon. One millicurie of this mixture contained: alanine 80 μC, arginine 70 μC, aspartic acid 80 μC, glutamic acid 125 μC, glycine 40 μC, histidine 15 μC, isoleucine 50 μC, leucine 140 μC, lysine 60 μC, phenylalanine 80 μC, proline 50 μC, serine 40 μC, threonine 50 μC, tyrosine 40 μC, and valine 80 μC.

3. Results and discussion

Table 1 demonstrates that the tRNA extracted from the hybrid has considerable amino acid acceptor activity. The amount of RNA extracted from the hybrid was determined by measuring the ^{32}P-label. Thus, 3.4 μg of T4 ^{32}P-RNA was eluted in the experiment presented.

The above isolation procedure leads to some inactivation of tRNA. In order to measure the extent of tRNA inactivation occurring during the various isolation steps, the following procedure was adopted: the excess non hybridized tRNA, which passed through the filters, was dialyzed to remove salt, digested with DNase and concentrated in exactly the same fashion as the hybridized tRNA. The amino acid acceptor capacity of this solution was then compared to that of the initial tRNA. In different experiments the extent of tRNA inactivation amounted to 20 to 40%. From these figures the corrected amino acid acceptance values were calculated (5000 cpm for the hybrid-isolated T4 tRNA, table 1). The corrected amino acid acceptance values enabled the determination of the per cent of biologically active tRNA present in the total RNA extracted from the hybrid (5000/2800 = 1.8; 100 X 1.8/3.4 = 53%). In different experiments this value varied between 50 to 85% of the total isolated ^{32}P-RNA.

The possible contamination of the T4 tRNA with host *E. coli* tRNA was also examined. Denatured T4 DNA was hybridized with *E. coli* ^{32}P-tRNA and the RNA extracted from the hybrid as described under "Experimental procedure". The results show that, based on amino acid acceptance activity, the amount of possible *E. coli* tRNA contamination in the T4 tRNA extracted from the hybrid could not have amounted to more than 10% (table 1). The true value is probably lower since the amount of *E. coli* tRNA hybridized with T4 DNA varied between 0.00 to 0.05 μg tRNA for each mg T4 DNA used. On the other hand, it was found that the amount of biologically active T4 tRNA isolated per mg T4 DNA varied between 0.4 to 1.0 μg tRNA for different experiments. The large difference found between the amount of T4 tRNA and *E. coli* tRNA attached to T4 DNA indicated that the method selects specifically for T4 tRNA. The identification of the individual amino acid specific T4 tRNA chains is now being stu-

Table 1
Amino acid acceptor activity of tRNA extracted from hybrid with T4 DNA.

Sample	RNA extracted from hybrid				
	Amount of DNA in hybrid	^{14}C-amino acid acceptance *			Amount of biologically active tRNA †
		^{32}P-RNA **	Found	Corrected ***	
	mg	μg	cpm	cpm	μg
T4 tRNA	4.46	3.4	3000	5000	1.8
E. coli tRNA	4.0	0.4	370	500	0.19

* Assay with crude aminoacyl-tRNA synthetase and a mixture of 15 ^{14}C-L-amino acids.
** 740 cpm/μg and 2300 cpm/μg for T4 tRNA and *E. coli* tRNA respectively.
*** Corrected for drop in activity of control tRNA (39.9% and 26.8% for T4 tRNA and *E. coli* tRNA respectively).
† Calculated from the amino acid acceptance values of the initial tRNA's 2810 cpm/μg and 2600 cpm/μg for T4 tRNA and *E. coli* tRNA respectively.

died. Preliminary experiments indicate that the T4 tRNA has leucine acceptor activity. Using ^{14}C-leucine (3.3×10^5 cpm/mμmole) between 200 to 1000 cpm/μg T4 tRNA were incorporated for different T4 tRNA preparations.

In the present report it was shown that a significant fraction of the 4S RNA induced by T4 phage infection has amino acid acceptor function. It still remains to be established whether all the amino acid specific tRNA's are synthesized after T4 infection or only a few selected ones.

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