SEPARATION OF AMINOACYL-TRANSFER RNAs by POLYACRYLAMIDE GEL ELECTROPHORESIS

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SUMMARY : A polyacrylamide gel electrophoresis system for separating E. coli tRNAs and aminoacyl-tRNAs is described. The tRNA was separated into 6 discrete bands which contained varying amounts of tRNA and therefore varying numbers of tRNA species. In order to locate specific tRNAs, tRNA was charged with a ^{14}C amino acid and the aminoacyl-tRNA was located by autoradiography. With several amino acids, 2 isoaccepting species were found. In total, 30 aminoacyl-tRNAs were located.

Polyacrylamide gel electrophoresis provides a very sensitive separation of RNA. It has been noted that even transfer RNA (tRNAs) species which differ very little in composition and molecular weight can be partially separated from each other by polyacrylamide gel electrophoresis (1, 2). In one system (1) the Escherichia coli tRNAs consisting of about 56 species (3) separate into 7 bands. These bands are of varying intensitives which indicates that the bands contain varying amounts of tRNA and varying numbers of tRNA species. It seemed that the tRNA separation on polyacrylamide gels would be useful in the purification of tRNA and possibly for a partial characterization of the tRNA profile. This requires some method to locate specific tRNAs among the bands on a gel. We have used aminoacylation of bulk tRNA with a ^{14}C labelled amino acid to locate the various E. coli tRNAs. It was necessary to modify the electrophoresis system which was previously used (1) to be able to carry out the electrophoresis under conditions where the ^{14}C aminoacylated tRNAs are stable.

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The system used and the localization of E. coli tRNAs specific for each of 20 amino acids are described in this paper.

METHODS : Amino Acids. All [^{14}C] labelled amino acids were purchased from New England Nuclear except Tryptophan was purchased from Amersham/Searle. Their specific activities (μc/ mole) were as follows: Ala, 156; Arg, 311; Asp, 170; AspN, 161; Cys, 271; Glu, 209; GlN, 218; Gly, 83; His, 252; Ile, 250; Leu, 311; Lys, 256; Met, 222; Phe, 383; Pro, 233; Ser, 128; Thr, 164; Try, 58; Tyr, 397; Val, 248.

Transfer RNA and aminoacyl-tRNA synthetase. Unfractionated tRNA was prepared from Escherichia coli K12 (strain CA244) by Zubay's method (4) except that chromatography on a DEAE-cellulose column was included as the final step.

Unfractionated aminoacyl-tRNA synthetase was obtained by a DEAE-cellulose column fractionation as the final step in the procedure of Muench and Berg (5). Pure $tRNA_2^{Glu}$ and $tRNA^{fMet}$ were a gift of Dr. A. D. Kelmers.

Radioactive aminoacyl-tRNA. Radioactively labeled aminoacyl-tRNAs were prepared from unfractionated E. coli tRNA with the homologous aminoacyl-tRNA synthetase. The incubation mixture contained, per ml.: 6 A_{260} units of tRNA, 100 μmoles of sodium cacodylate (pH 7.2), 10 μmoles of magnesium acetate, 10 μmoles of KCl, 2 μmoles of ATP, about 300 μg of aminoacyl-tRNA synthetase and radioactive amino acids (1 to 4 nmoles with specific activites described above).

When ^{14}C glutamine and ^{14}C asparagine were charged, the reaction medium contained excess ^{12}C glutamate and ^{12}C aspartate, respectively. Incubation was carried out at 37° for 15 min., followed by mixing with an equal volume of water-saturated phenol. The ethanol precipitable materials were recovered from the water-phase and finally dissolved in 75 μl of distilled water. They were stored at -20° until used. These samples contained radioactivity at 700-170 cpm/μl when counted in a liquid scintillation counter.

Separation of aminoacyl-tRNA. The tRNAs were separated at pH 5.8 on 8.5% polyacrylamide gels containing 7 M urea. The following stock solutions were used:

A. 19 g acrylamide, 1 g bis acrylamide, 48 g urea per 100 ml of solution

B. 8 M urea

C. 6.4% v/v solution of DMAPN (dimethylaminopropionitrile)

D. 1.6% w/v ammonium persulfate

E. 5.5 g boric acid, 0.93 g Na_2EDTA, 17.0 g MES [(2-N-morpholino)-ethanesulfonic acid] per 100 ml. of solution. The pH is adjusted to 3.5 with 1 M NaOH.

To prepare the 8.5% gels, 4.25 ml. of solution A, 0.30 ml. of solutions C and D, 4.15 ml. of solution B, and 1.0 ml. of solution E are mixed. The pH of this mixture is adjusted to pH 5.8 with 1 M NaOH.

10 cm. gels are prepared in the usual way.

2-5 µg (about 1 A_{260} unit) of carrier RNA and 1,000 cpm or more of aminoacyl-tRNA in 10-50 µl to which a few crystals of urea and some bromophenol blue have been added are layered on the surface of a gel.

The chamber buffer is 5.5. g boric acid, 0.93 g Na_2EDTA and 2.09 g MES per liter. The pH is adjusted to 5.8 with 1 M NaOH.

Electrophoresis is carried out in the cold room at 2-3 mA per gel until the bromophenol blue marker reaches the end of the gel. This usually requires about 3 hours.

Gels are then stained with methylene blue (1). Stained gels are sliced lengthwise, dried and autoradiograms prepared (6).

The running pH of this system was determined by running a blank gel. After electrophoresis, the gel was ground up in distilled water. The pH of the aqueous phase was 5.8.

RESULTS : Escherichia coli tRNA was separated on 8.5% acrylamide (5%

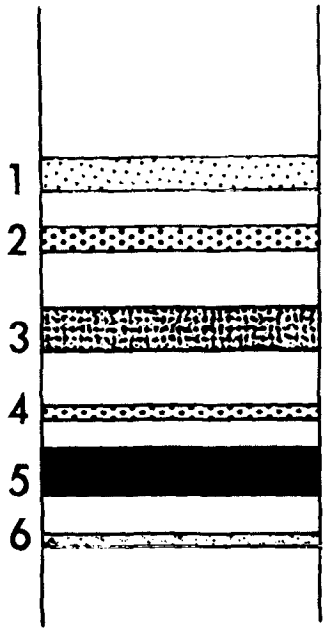

Fig. 1: Separation of E. coli tRNA by polyacrylamide gel electrophoresis. Electrophoresis was done as described in Methods.

crosslinking) gels containing 7 M urea. The buffer system used provided a running pH of 5.8. A representation of the profile of methylene blue stained material in the tRNA region of gels prepared and run as described above is shown in Fig. 1. There are 6 bands of varying width and intensity. The bands are numbered from 1 to 6. The slowest moving band is number 1 and the fastest moving is number 6. The determine the location of tRNAs in relation to the stained bands, the bulk tRNA was aminoacylated with one ^{14}C amino acid. This was done in turn with all the usual amino acids - 20 in all. Autoradiograms were prepared from each gel in order to locate the aminoacyl-tRNA. In all cases the autoradiogram coincided with a stained band.

The assignment of the various aminoacyl-tRNA bands located by autoradiography to the stained bands is given in Table 1. With 10 of the aminoacyl-tRNAs, two bands were found on the autoradiogram. Thus a total of 30 aminoacyl-tRNAs were found. This is about one-half of the total E. coli tRNAs (3). In Table 1 an estimate of the proportion of the total radioactivity in each of the two bands

TABLE 1

LOCATION OF E. COLI AMINOACYL-tRNAs

Band	1	2	3	4	5	6
Aminoacyl-tRNA	Ser 2/3	Ser 1/3	Val 1/3	Arg	Gly	Glu 2/3
		Thr 1/2	Thr 1/2	Cys	GlN 1/3	GlN 2/3
		Leu	Phe	Try	Ile 1/5	Met 2/3
			Ile 4/5	Ala 1/3	Ala 2/3	
			Asp	Lys 1/3	Pro	
			AsN	His 4/5	Val 2/3	
			Tyr		His 1/5	
			Glu 1/3			
			Lys 2/3			
			Met 1/3			

is given. Some bands probably contain only one of the isoacceptor tRNAs for a given amino acid while others most likely contain 2, or more isoacceptor tRNAs. For example, Ile 1/5 probably represents a single Ile tRNA.

On the other hand, only one band was found for leucine tRNA. It is known that there are 5 leucyl-tRNAs in E. coli (7). However, since 3 of the leucyl-tRNAs comprise less than 10% of the total leucyl-tRNA, they may not have been detectible in these experiments.

The tryptophanyl tRNA is a unique problem to locate. This (aminoacyl-)tRNA appears to aggregate (8) and only a small percentage of the total enters the gel.

To substantiate the above results, gels were run with charged, pure $tRNA^{fMet}$ and $tRNA_2^{Glu}$. In both cases only one stained band was found and the autoradiographs also showed one band which coincided with the stained band. Gels were also run with pure, charged tRNA mixed with marker RNA, cold E. coli tRNA. Both the $tRNA_2^{Glu}$ and the $tRNA^{fMet}$ were found in band 6. The location of one Glu-tRNA and one Met-tRNA was thus confirmed. Moreover, the relative proportion of $tRNA^{fMet}$

(in band 6) to the other Met-tRNA (in band 3) agrees well with the known proportions of these two tRNAs (9).

DISCUSSION : A method for partially separating tRNAs on polyacrylamide is described. This method should be useful for obtaining a semi-qualitative and semi-quantitative profile of tRNAs present in a bulk tRNA sample from various sources. Gel separation of tRNA should also be useful in purifying tRNA. For certain E. coli tRNAs (for example, serine, band 1, Fig. 1) 33-50% pure tRNA could be obtained in one step. For other tRNAs polyacrylamide gel separation coupled with a column chromatography step may provide extensive purification since the basis for tRNA separation in the two steps may differ.

The advantages of the gel separation of tRNA are that it is quick and requires only a small amount of material.

The basis for the separation of E. coli into 6 discrete bands on polyacrylamide gels is not clear.

While it is true that smaller tRNAs, about 75 base residues, migrate faster than larger tRNAs, about 85 base residues, this is not adequate to explain the separation obtained. There is a good possibility that conformational differences may play an important role in the separation.

Polyacrylamide gel separation of tRNAs from several other sources is currently being investigated.

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