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Note

Fractionation of transfer RNA on Sepharose 4B

Effects of Sepharose batch differences

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Fractionation of transfer RNA (tRNA) has been of central interest for many years and will probably continue to be so for many years. Among a multitude of methods developed so far, only a few come up to the demands of simplicity, high efficiency and reproducibility. Recently, Holmes *et al.*[1] introduced a promising method to fractionate tRNA on Sepharose columns with a negative linear gradient of ammonium sulphate. This procedure has since been successfully used for purification of many single tRNA species (see references in ref. 2). Extensive work has also been done by Morris[3] to compare different grades of Sepharose and Ultrogel, and by Colantuoni *et al.*[4] to establish elution profiles for *Escherichia coli* isoacceptor tRNAs from Sepharose 4B. However, comparisons between published data from different laboratories revealed great differences in resolution and elution profiles for tRNA. This prompted us to investigate different batches of Sepharose 4B for their efficiency in fractionating the isoacceptors of $tRNA^{Leu}$ from *E. coli* B. We found that the fractionation power of Sepharose 4B for tRNA varies greatly from batch to batch, but that for a given batch the reproducibility is good.

MATERIALS AND METHODS

Chemicals

L-[^{14}C(U)]Leucine (344 mCi/mmol) was from New England Nuclear. Samples of Sepharose 4B from different production batches were kind gifts from Pharmacia (Uppsala, Sweden). All other chemicals used were of reagent grade.

Bacterial strain and growth conditions

Escherichia coli B was grown in a rich medium as described[5] and harvested in late logarithmic phase. The cells were stored at −20°C.

Preparation of tRNA

Bulk tRNA was prepared from *E. coli* B as described by Zubay[6]. After deacylation at pH 9.0 in 1 *M* Tris–HCl for 1 h, high-molecular-weight RNAs were removed by chromatography on Sepharose 6B[7]. The same batch of tRNA was used throughout.

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Preparation of aminoacyl-tRNA ligases

A crude extract of aminoacyl-tRNA ligases was prepared from *E. coli* B according to Muench and Berg[8]. The preparation was stored at $-80°C$ in portions of 0.1 ml. The same batch of enzyme was used throughout.

Aminoacylation of unfractionated tRNA

The reaction mixture contained, in a final volume of 6 ml: 0.1 *M* N-2-hydroxyethylpiperazine-N′-2-ethanesulphonic acid (HEPES buffer), pH 8.0; 2 m*M* ATP; 10 m*M* $MgSO_4$; 1.2 μM [^{14}C]leucine; 20 m*M* 2-mercaptoethanol (2-ME); appropriate amounts of ligase and 16 A_{260} units of total tRNA. After incubation at 37°C for 20 min, the reaction was terminated by adding 1.5 ml of 1 *M* sodium acetate buffer, pH 4.5. The solution was diluted in 5.5 ml of 20 m*M* sodium acetate buffer, pH 4.5 (buffer A). The sample was applied on a DEAE-Sepharose column (2 cm × 0.8 cm^2), equilibrated with buffer A. The column was then washed with buffer A containing 0.3 *M* NaCl until no more UV-absorbing material was eluted. tRNA was finally eluted with buffer A containing 1 *M* NaCl, and stored at $-80°C$.

Aminoacylation of fractionated tRNA

The aminoacylation mixture contained, in a final volume of 0.1 ml: 0.1 *M* HEPES, pH 8.0; 2 m*M* ATP; 10 m*M* $MgSO_4$; 0.73 μM [^{14}C]leucine; 20 m*M* 2-ME; 12.5 μl of column fractions and an appropriate amount of aminoacyl-tRNA ligase. The incubation time was 20 min at 37°C. The reaction was terminated by adding 1 ml of cold 5% trichloroacetic acid. The samples were treated and measured as described[9].

Chromatography of tRNA on Sepharose 4B

The procedure of Holmes *et al.*[1] for fractionation of tRNA on Sepharose 4B with a negative salt gradient was adopted with minor changes as indicated below. Sepharose 4B was equilibrated at 4°C with 10 m*M* sodium acetate buffer, pH 4.5, containing 6 m*M* 2-ME, 10 m*M* $MgSO_4$ and 1 m*M* EDTA (buffer B) and poured into a column (20.5 cm × 0.8 cm^2). The column was washed with 15 ml of buffer B and then equilibrated with 40 ml of buffer B, containing 1.3 *M* $(NH_4)_2SO_4$. The flow-rate was kept at 12 ml/h.

160 A_{260} units of uncharged tRNA in 0.4 ml of water were mixed with 2.7 A_{260} units of ^{14}C-labelled leucyl-tRNA, dissolved in 0.8 ml of buffer A, containing 1 *M* NaCl. To this mixture were added 1.2 ml of a solution of 20 m*M* sodium acetate, pH 4.5, 12 m*M* 2-ME, 20 m*M* $MgSO_4$, 2 m*M* EDTA and 2.6 *M* $(NH_4)_2SO_4$. The mixture was then applied onto the column and eluted at 4°C with a gradient formed by 100 g of 1.3 *M* $(NH_4)_2SO_4$ in buffer B in the mixing chamber and 100 g of buffer B in the reservoir. Fractions of 1.6 ml were collected. The absorbance at 260 nm and the conductivity were determined at 20°C. Aliquots of 0.8 ml were withdrawn from the fractions for determination of ^{14}C-labelled leucyl-tRNA, precipitated with 0.1 ml of 50% trichloroacetic acid, dried on glass filters and counted. The fractions were also assayed directly for $tRNA^{Leu}$ as outlined above.

RESULTS AND DISCUSSION

A series of seven Sepharose 4B batches, produced in the years 1970–1979, was examined for their fractionation power for *E. coli* tRNA using the method of Holmes *et al.*[1]. Four representative chromatograms are shown in Fig. 1. The profiles of absorbance at 260 nm differ markedly both in the general appearance and the position relative to the salt gradient. Also, the profiles of leucine-accepting capacity differ between the chromatograms. Batch no. 4763 gave the best resolution of leucyl-tRNA into five separate peaks, which is comparable with the best published results. All other batches tested gave poorer resolution (at most three recognizable peaks).

The eluent concentration for the main leucine-tRNA peak varies between 1.22 and 0.65 *M* ammonium sulphate. This reflects clearly the great difference in tRNA binding properties between batches of Sepharose 4B. Also, the relative positions of any two leucine-tRNA peaks in the chromatograms vary with the Sepharose batches. We have, however, no indication from our experiments that the elution order of the isoacceptors of $tRNA^{Leu}$ varies with the Sepharose batches. On the other hand, some changes in elution order of individual tRNAs may be inferred from an analysis of the leucyl-tRNA elution volume relative to the total tRNA elution volume. Thus, the main $tRNA^{Leu}$ peak (No. IV with batch No. 4763 in Fig. 1) eluted when about 75% of the total tRNA had been eluted, while the same $tRNA^{Leu}$ was eluted from batches No. 0296 and 12380 after 59% and 65%, respectively, of the total tRNA.

To establish the relative positions in the chromatogram of the uncharged isoacceptor $tRNA^{Leu}$ species with respect to the aminoacylated tRNA, we adopted a mixed prelabelling and postlabelling technique in the chromatograms of Fig. 1. Part of the tRNA sample was charged with ^{14}C-labelled leucine before chromatography, and the fractions were analyzed for leucyl-tRNA. Aliquots of the fractions were then

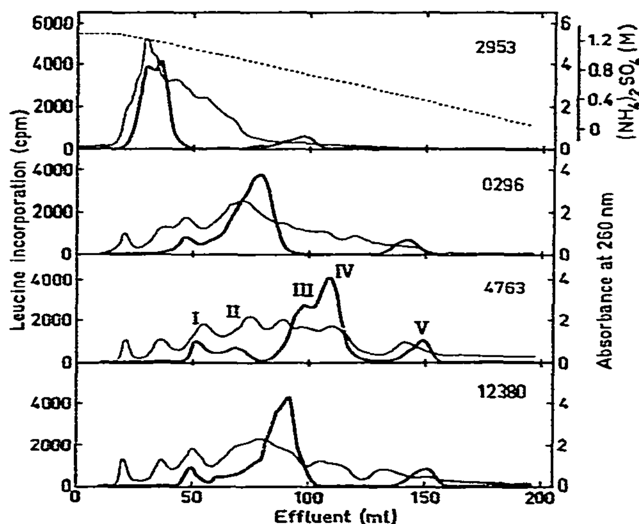

Fig. 1. Fractionation of *E. coli* B tRNA on different batches of Sepharose 4B, using a negative linear gradient of ammonium sulphate at 4°C. ——, Absorbance at 260 nm; ——, postlabelled leucyl-tRNA; - - - - -, salt gradient. Batch numbers are given at the right-hand side.

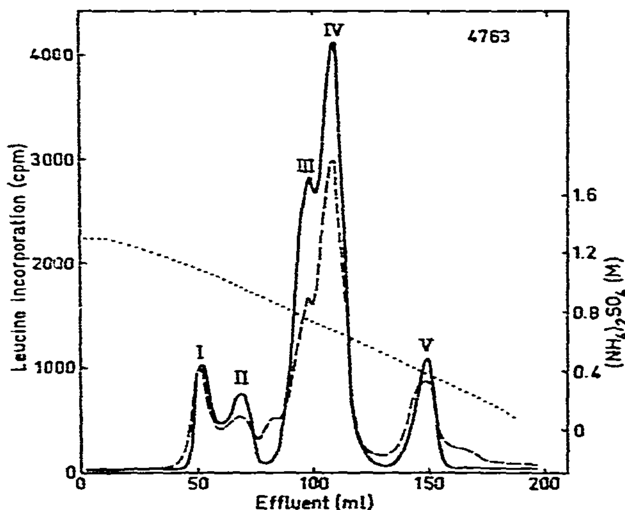

Fig. 2. Fractionation of *E. coli* B tRNA on Sepharose 4B, batch No. 4763. — — —, Prelabelled leucyl-tRNA; ——, postlabelled leucyl-tRNA; ·····, salt gradient.

assayed for $tRNA^{Leu}$ by aminoacylation. Fig. 2 shows both assays after chromatography on Sepharose 4B, batch No. 4763. There are no clear indications of positional changes due to the presence of the hydrophobic leucine moiety on the prelabelled tRNA. Only a faint tendency for leucine-tRNA to elute somewhat before the corresponding free $tRNA^{Leu}$ was noted for peaks I and V. The same tendency was found in the chromatograms of the other Sepharose batches (not shown).

The effect of cross-linking of Sepharose on tRNA fractionation has been studied by Morris[3]. We compared a cross-linked Sepharose CL-4B, batch No. 666, with its non-cross-linked parent Sepharose 4B, batch No. 0296. The chromatogram from this cross-linked Sepharose (not shown) was very similar to the chromatogram shown in Fig. 1 for batch No. 2953, both in absorbance profile, consistent with the results of Morris, and in leucyl-tRNA profile. It differs markedly from the profiles obtained with batch No. 0296 (Fig. 1).

In our study, Sepharose 4B, batch No. 4763, gave the best resolution of $tRNA^{Leu}$ isoacceptors. The reproducibility with this gel has, in our hands, been excellent with no significant changes in elution positions between several runs.

Some chromatograms obtained with batch No. 4763, employing another preparation of *E. coli* B tRNA, showed six peaks for leucyl-tRNA. The extra peak appeared just before but well separated from peak I in Fig. 2. Six isoacceptors of $tRNA^{Leu}$ have been reported by other workers, *e.g.*, Holmes *et al.*[1] using *E. coli* K tRNA.

The usefulness of Sepharose 4B for fractionation and analysis of tRNA by the method of Holmes *et al.*[1] has been demonstrated in many studies, most of which are cited in the reference list of a recent compilation of tRNA structures[2]. However, the seemingly large irreproducibility of the method must have been both confusing and a nuisance. Our study seems to explain most of the peculiarities encountered when comparing results from different laboratories using different batches of Sepharose.

We are confident that, provided appropriate batches of Sepharose are selected, this material will continue to yield excellent tRNA fractionations, both preparative and analytical.

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