Heterologous mischarging as a means of tRNA fractionation. II. Isolation of E. coli $tRNA_1^{Val}$ and $tRNA_1^{Ala}$

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Summary. Highly purified E. coli $tRNA_1^{Val}$ and $tRNA_1^{Ala}$ have been isolated, based on the properties of heteroaminoacylated tRNAs and their behaviour on BD-cellulose chromatography.

It is known that Neurospora crassa and yeast phenylalanyl-tRNA synthetase (PRS) can charge phenylalanine to several E. coli tRNA species[2–5]. We have reported previously that E. coli $tRNA_1^{Val}$ heterologously mischarged with phenylalanine showed chromatographic properties on BD-cellulose which were attributed to the hydrophobic nature of the amino acid and that the observed behaviour could be used as a means for tRNA purification[6]. To prove that the mischarging reaction can be used to purify other tRNA species, which also undergo phenylalanine esterification with an heterologous enzyme, we have extended our work to isolate E. coli $tRNA_1^{Ala}$. We present here a combined procedure which results in the isolation of E. coli $tRNA_1^{Ala}$ and $tRNA_1^{Val}$.

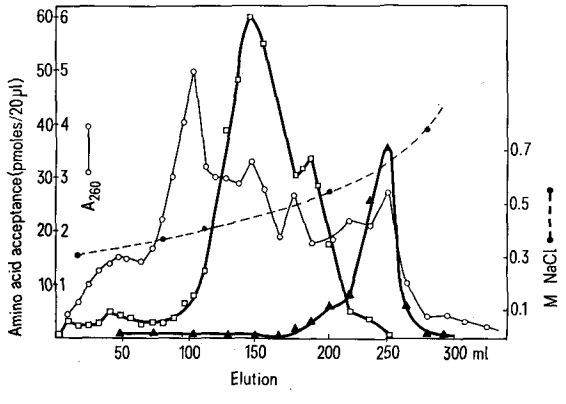

Fig. 1. Chromatography of E. coli tRNA enriched in $tRNA_1^{Val}$ and $tRNA_1^{Ala}$ on Plaskon. A sample of 1096 A_{260} units of the enriched tRNA was eluted from a 0.9 × 90 cm column with a gradient as indicated. Fractions were analyzed for homologous amino acceptance activity. □—□ valine; ▲—▲ alanine.

Materials and methods. ^{14}C Valine (50 mCi/mM), ^{14}C Phenylalanine (45.6 mCi/mM), ^{3}H Phenylalanine (99.4 mCi/mM) and ^{14}C Alanine (38.7 mCi/mM) were products from Amersham/Searle. Benzoylated-DEAE cellulose Lot Y-2495 and unfractionated E. coli B tRNA were obtained from Schwarz/Mann. The material for Plaskon chromatography (RPC-5) was a generous gift from Dr M. A. Q. Siddiqui.

Assays for E. coli homologous amino acid activity were carried out in 0.1 ml reaction mixture containing 100 mM Tris-HCl buffer pH 7.5, 10 mM $MgCl_2$, 10 mM KCl, 10 mM β-Mercaptoethanol, 2 mM ATP, 0.02 mM of labeled amino acid, tRNA and activating enzymes[7], as described before. Heterologous aminoacylation was performed according to Strickland and Jacobson[8]. The assays were carried out in 0.1 ml reaction mixtures containing 50 mM potassium cacodylate buffer, pH 6.3, 7.5 mM $Mg(OAc)_2$, 0.5 mM ATP, 0.025 mM ^{3}H or ^{14}C Phe and E. coli tRNA. The mixtures were incubated at 25 °C for 20 min using partially purified N. crassa PRS[2] in

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Purification of E. coli $tRNA_1^{Val}$ and $tRNA_1^{Ala}$

	$tRNA_1^{Val}$ A_{260} units	Specific activity*	Purification factor	Yield**	$tRNA_1^{Ala}$ A_{260} units	Specific activity*	Purification factor	Yield**
Crude tRNA	3600	54.4	1	100	3600	29.2	1	100
Pool after 1st BD-cellulose (pool I)	1102	164	3	92.3	1102	88	3	92.3
Pools after RPC-5								
Fractions: 118–206 (pool II)	473.4	276	5.1	66.7	–	–	–	–
Fractions: 220–265 (pool III)	–	–	–	–	131.7	400	13.7	50.1
Pool II after 2nd BD-cellulose	41	1368	25.1	28.6	–	–	–	–
Pool III after 2nd BD-cellulose	–	–	–	–	25	1301	44.6	30.9

* Specific activity is expressed as pmoles of amino acid accepted per A_{260} units of tRNA; ** Expressed in terms of the pmoles recovered.

saturating amount[6]. Large scale heterologous mischarging of enriched $tRNA_1^{Val}$ and $tRNA_1^{Ala}$ was done by scaling up the standard assay systems. The products were isolated by ethanol precipitation after a phenol extraction and chromatography of a combined silicic acid Sephadex G-25 column[9]. Radioactivity measurements of the column fractions were performed using a Triton X-Toluene-scintillation mixture.

Results and discussion. In order to isolate an enriched fraction of E. coli tRNA containing the $tRNA_1^{Val}$ and $tRNA_1^{Ala}$ species from other valine and alanine isoacceptor tRNAs, as well as from $tRNA^{Phe}$ species which are able to be mischarged with PRS from N. crassa[3], a BD-cellulose column chromatography was performed with 3600 A_{260} units of crude E. coli tRNA as described previously[5]. The $tRNA_1^{Val}$ and $tRNA_1^{Ala}$ containing peak (pool I, 1096 A_{260} units) was loaded on a RPC-5 column previously equilibrated with 10 mM Tris-HCl buffer, pH 7.0, 10 mM Mg^{++}, 1 mM $Na_2S_2O_3$ and 0.3 M NaCl. Elution was performed at 4 °C according to Roe et al.[10].

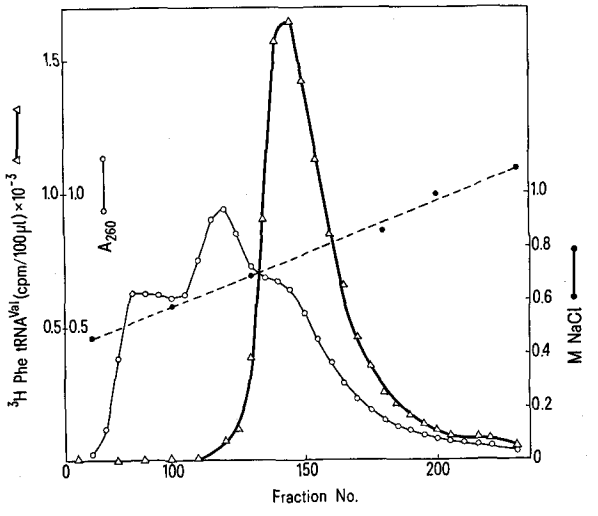

Fig. 2. Purification of $tRNA_1^{val}$. A sample of 327 A_{260} units of ^{3}H-Phe-$tRNA_1^{val}$ was loaded on a 0.9×90 cm BD-cellulose column at 4 °C. Elution was done as indicated in the text. Fractions (4 ml) were collected at a flow rate of 30 ml/h and analyzed for soluble radioactivity.

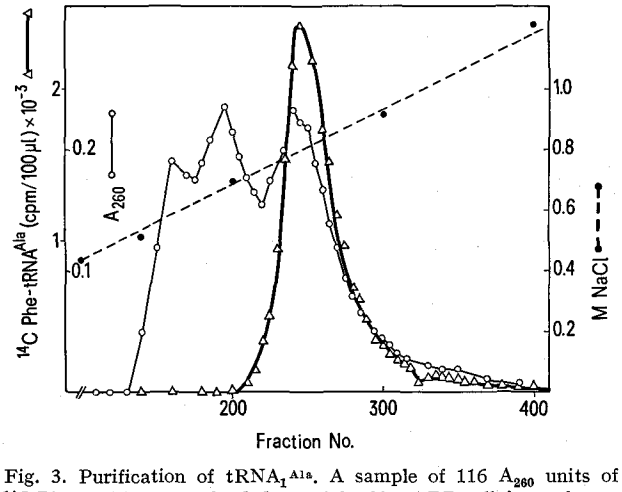

Fig. 3. Purification of $tRNA_1^{Ala}$. A sample of 116 A_{260} units of ^{14}C-Phe-$tRNA_1^{Ala}$ was loaded on a 0.9×90 cm BD-cellulose column. The chromatography and analysis was performed as in figure 2.

As seen in figure 1, 2 enriched fractions were resolved. The first one is a composed peak of valine acceptance activity. The minor peak is probably due to the modified form of $tRNA_1^{Val}$ which is characterized by a cross-link between 4-thiouridine in the 8th position of the tRNA sequence and the cytosine residue on the 13th position[11]. Although the results of our RPC-5 column could be attributed to other factors which would require a specific investigation, it is interesting to note that chromatographic heterogeneity as a result of cross-linked tRNA molecules has been reported for E. coli $tRNA^{fMet}$ using BD-cellulose chromatography[12].

Pools were made with $tRNA_1^{Val}$ (pool II) and $tRNA_1^{Ala}$ fractions (pool III), as indicated in the table. Pool II accepted 276 pmoles of valine and pool III 400 pmoles of alanine per A_{260} unit of tRNA.

In order to introduce a hydrophobic amino acid residue in the enriched fractions obtained from the RPC-5 chromatography, each pool was heterologously mischarged using PRS from N. crassa as described under 'material and methods'. The products containing either ^{3}H Phe-$tRNA_1^{Val}$ or ^{14}C Phe-$tRNA_1^{Ala}$ were separately loaded on BD-cellulose columns equilibrated with 5 mM sodium formate buffer, pH 4.0, 10 mM $MgSO_4$ and 0.3 M NaCl. Elution was performed with the same buffer using a NaCl linear gradient (figures 2, 3). As expected, the heteroaminoacylated tRNAs were clearly separated from the unacylated ones by virtue of the incorporation of an aromatic moiety.

The $tRNA_1^{Val}$ and $tRNA_1^{Ala}$ peaks from each BD-cellulose column were pooled, giving a mean specific acceptance activity of 1368 and 1301 pmoles per A_{260} unit for $tRNA_1^{Val}$ and $tRNA_1^{Ala}$ respectively. Quantitative data, purification factors and yields are summarized in the table.

A widely used general procedure for the isolation of tRNA species, developed by Gillam and Tener[13], involves enzymatic aminoacylation, chemical phenoxyacetylation and isolation of the derivatized material by chromatography on BD-cellulose column. It is known that phenoxyacetylation can modify some minor nucleosides[14,15] and also that the heterologous mischarging reaction is blocked when cross-linked tRNAs are used as substrates[16]. Because of this, phenylalanine heteroaminoacylation appears to be an attractive and profitable alternative procedure for the isolation of several tRNA species.

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