PURIFICATION OF TRANSFER-RNA-NUCLEOTIDYLTRANSFERASE FROM E. COLI B*

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Summary: Transfer-RNA-nucleotidyltransferase has been purified 1400-fold from E. coli B by liquid polymer phase fractionation and chromatography on DEAE-cellulose, hydroxylapatite, and QAE-Sephadex. The final preparation contains no detectable RNase I, RNase II, polynucleotide phosphorylase, ATPase, CTPase, or any of the aminoacyl-tRNA synthetases. The enzyme is extremely labile. It can be stabilized by tRNA, but not rRNA.

The 3'-terminal AMP of E. coli tRNA exhibits a measurable turnover in vivo (Cannon, 1964, 1966; Rosset and Monier, 1965). An enzyme capable of catalyzing the exchange of the terminal AMP, and the following two CMP moieties has been purified from E. coli (Preiss, et al., 1961; Furth, et al., 1961), rat liver (Canellakis and Herbert, 1960; Daniel and Littauer, 1963), rabbit muscle (Starr and Goldthwait, 1963), and baker's yeast (Lebowitz, et al., 1966). The purification so far reported for the E. coli tRNA-nucleotidyltransferase (E. C. 2. 7. 7. 20) was only 100-fold (Preiss, et al., 1961; Furth, et al., 1961). This report describes a new

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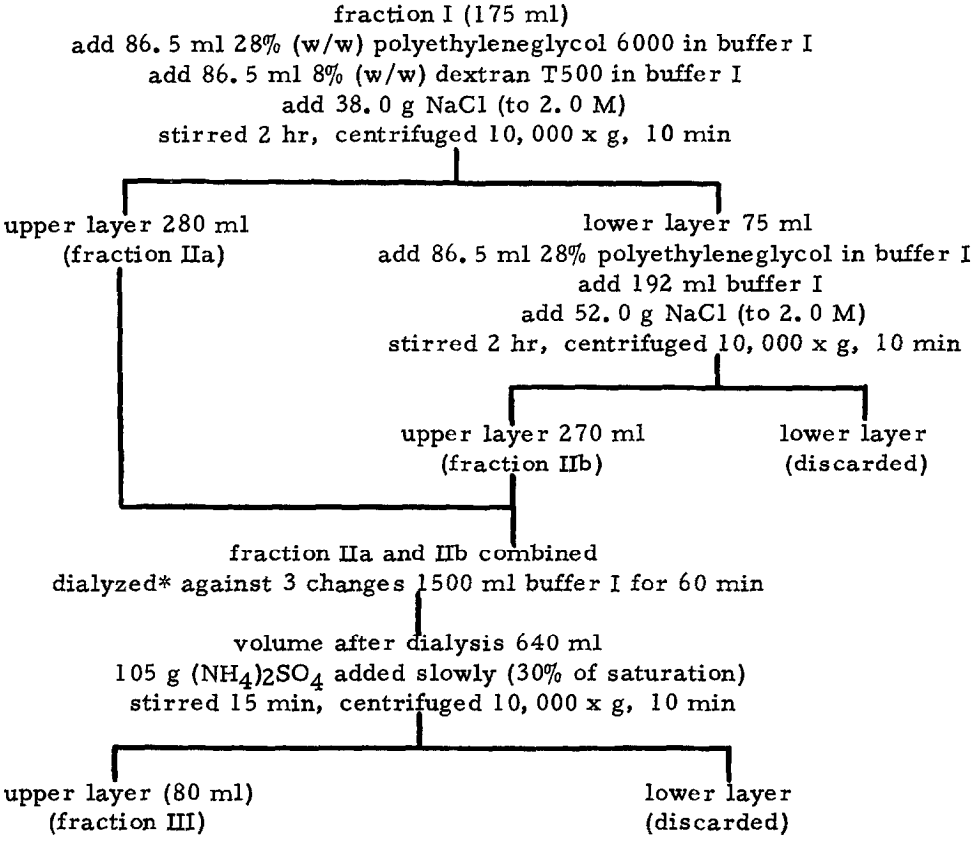
procedure which yields a highly purified enzyme essentially free from any interfering contaminants.

Materials and Methods. E. coli B cells, 1/2 log phase, were from Grain Processing Co., Muscatine, Iowa. Commercially obtained tRNA was further purified and treated with venom phosphodiesterase (Philipps, 1970) to remove either the 3' terminal-pA or -pCpA. For the estimation of tRNA concentration, an average molecular weight of 26, 000 and an absorbance in 0.2 M NaCl of $A_{260}^{1\%}$ = 220 was used (von Ehrenstein and Lipmann, 1961).

The standard assay for nucleotidyltransferase activity contained the following in 0.1 ml: 5.0 μmoles glycine-NaOH, pH 9.2; 1.0 μmole Mg^{2+} acetate ; 1.0 μmole reduced glutathione; 1.6 nmoles venom phosphodiesterase-treated tRNA (tRNApCpC to measure AMP addition and tRNApC for CMP addition) and 0.1-5.0 μg protein. The assay for AMP incorporation contained 10.4 nmoles (12.4 nCi) ATP-8-^{3}H, while the assay for CMP incorporation contained 15.4 nmoles (23.2 nCi) CTP-5-^{3}H. Incubation was for 2, 4, and 6 min at 37°. The reaction was stopped by addition of 2.0 ml 20 mM EDTA containing 0.2 mM ATP followed by 2.0 ml of 10% trichloroacetic acid. For a control, the reaction mixture was precipitated at zero time. The precipitate was collected on glass fiber filters and washed with trichloroacetic acid and ethanol. The dried filters were counted in 3 ml toluene based scintillator in a Packard 3380. One unit of enzyme is defined as that amount which incorporates 1.0 nmole of nucleotide into tRNA in 1.0 min at 37°.

Results and Discussion. Purification of tRNA-nucleotidyltransferase. All procedures were performed at 4°. E. coli B cells (100 g) were thawed and suspended in 100 ml buffer I (10 mM tris-HCl, pH 7.8 (4°); 12 mM Mg^{2+} acetate; 2 mM EDTA; 5 mM 2-mercaptoethanol; 10% (v/v) glycerol). They

were broken in a French pressure cell at 15, 000 p. s. i. Bentonite (2. 5 mg) was added and the mixture stirred for 10 min. The extract was centrifuged at 44, 000 x g for 30 min in a Sorvall SS 34 rotor and the supernatant (fraction I) subjected to the following fractionation:



*Dialysis was performed with sufficient agitation to keep material within the dialysis bag homogeneous.

Fraction III was immediately applied to a 90 x 5 cm Sephadex G-25 column equilibrated with buffer I. The void volume was 560 ml. The next 350 ml containing all nucleotidyltransferase activity were pooled and immediately applied to a 10 x 2 cm column of DEAE-cellulose (Whatman DE-52) previously equilibrated with buffer I. The column was washed with

buffer II (10 mM tris-HCl, pH 7.8; 10 mM Mg^{2+} acetate; 5 mM 2-mercaptoethanol; 10% glycerol) until the A_{280} mμ of the eluate was less than 0.01. The tRNA-nucleotidyltransferase activity was eluted with 85 ml of buffer II containing 0.15 M KCl. This material (fraction IV) was immediately applied to a 3 x 2 cm hydroxylapatite column (Tiselius et al., 1956) previously equilibrated with buffer III (5 mM sodium phosphate, pH 7.4; 10 mM Mg^{2+} acetate; 5 mM 2-mercaptoethanol; 10% glycerol. The column was successively eluted with 20 ml each of buffer III containing 5 mM, 15 mM, 30 mM, and 45 mM sodium phosphate pH 7.4. Normally most of the activity was eluted with 15 mM and 30 mM phosphate. Fractions containing enzymatic activity were pooled and stored (fraction V). The purification is summarized in Table 1.

Table 1. Purification of tRNA-nucleotidyltransferase.

Fraction	Total protein (mg)	Specific activity (units/mg)		Purification (-fold)	
		AMP	CMP	AMP	CMP
I	4575	0.0774	0.156	--	--
IIa	2080	0.216	0.406	2.8	2.8
IIb	665	0.209	0.218	2.7	1.4
III	356	1.12	2.03	14.5	13.0
IV	83	13.2	25.7	170	165
V	31	54.5	112	704	718

Liquid polymer phase fractionation (Albertsson, 1960) was crucial since it yielded tRNA-nucleotidyltransferase completely free of tRNA. In the presence of tRNA multiple enzyme fractions were obtained on DEAE-

cellulose. If the purification was performed without Mg^{2+}, fraction V lost all activity in one week at 4°. If glycerol was omitted, both recovery of enzyme and the specific activity were lower.

Properties of the purified enzyme. Fraction V contained no detectable activity for RNase I, polynucleotide phosphorylase, ATPase, CTPase, or any of the aminoacyl-tRNA-synthetases. A small amount of nuclease activity was probably due to RNase II; after exposure of polyadenylic acid to the enzyme, only 5'-AMP could be identified by thin layer chromatography. The ability of this residual nuclease activity to remove the 3'-terminal-pCpA nucleotides from tRNA was determined with tRNA·pCpA labeled with tritium either in the terminal AMP or the following CMP. This nuclease activity was negligible since under the same conditions where one AMP or CMP were added to tRNA in 10 min less than 2 percent of either nucleotide were removed. In preliminary experiments fraction V was further purified on QAE-Sephadex.* This enzyme fraction was approximately 1400-fold enriched and contained no RNase II activity.

The purified enzyme is very unstable. Storage for 2 months at -20° in buffer III containing 35 mM Na phosphate, pH 7.4, results in a 98 percent loss of AMP - and 48 percent loss of CMP-incorporating activity. Addition of tRNA during storage (2 mg tRNA/mg protein) completely prevented this loss, while r-RNA was without effect. This specificity is consistent with recent observations by Honda (1969) that E. coli tRNA-nucleotidyltransferase will form a complex with tRNA but not with r-RNA or MS2-RNA.

Fraction V showed five distinct bands, two of which were prominent on analytical electrophoresis in polyacrylamide gel. We are currently investigating the possibility that AMP and CMP are added by different enzymes.

* diethyl-2-hydroxypropylaminoethyl-Sephadex (Pharmacia)

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