Isolation of gram quantities of isoleucyl-tRNA synthetase from an overproducing strain of *Escherichia coli* and its use for purification of cognate tRNA

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The *ileS* gene coding for isoleucyl-tRNA synthetase was cloned on a runaway-replication plasmid. From the cells harboring the plasmid, gram quantities of the synthetase were isolated using two column procedures. The synthetase was used for the purification of cognate tRNA. Isoleucine $tRNA_{GAU}$ of greater than 90% purity was easily isolated by taking advantage of a specific complex formation of the synthetase with cognate tRNA.

Isoleucyl-tRNA synthetase *Overproducing strain* *tRNA isolation* *Complex formation*

1. INTRODUCTION

Recently it was discovered that the *ileS* gene coding for isoleucyl-tRNA synthetase and the *lspA* gene for lipoprotein signal peptidase constitute a cotranslational unit in the order of promoter-*ileS*-*lspA* [1,2]. For the study of tRNA recognition using isoleucyl-tRNA synthetase, we tried to obtain a large amount of isoleucyl-tRNA synthetase. One of the simplest ways to do this is to clone this gene on a plasmid which has a very high copy number. Here, we describe one such strain from which gram quantities of isoleucyl-tRNA synthetase can be easily isolated. Purification of isoleucine tRNA by means of the complex formation with the synthetase is also described.

2. MATERIALS AND METHODS

2.1. *Materials*

Crude tRNA (*Escherichia coli* MRE 600) was obtained from Boehringer Mannheim. Purified $tRNA^{Ile}_{GAU}$ (*E. coli* B) was obtained from Subriden RNA. *Eco*RI was from Takara Shuzo.

Abbreviations: PAGE, polyacrylamide gel electrophoresis; DTT, dithiothreitol; kb, kilobase pairs

2.2. *Bacterium and plasmid*

E. coli JE5506 used as a host cell was described in [2]. Antibiotic medium 3 (Difco) was used in the presence of kanamycin (50 μg/ml). Plasmid pKD15 was constructed by insertion of the *ileS*- and *lspA*-carrying 4.3-kb fragment of *E. coli* into the *Eco*RI site of pSY343, a runaway-replication plasmid vector [2–4]. Cells harboring plasmid pKD15 were grown at 30°C to 0.05 A_{660nm}. The temperature was then changed to 37°C for amplification of the plasmid and cells were harvested 5 h after the temperature change. About 4 g (wet wt) cells per l were typically obtained.

2.3. *Enzyme assay*

The assay for isoleucyl-tRNA formation was carried out in 0.05 M Tris-HCl (pH 7.5) containing 4 mM ATP, 40 mM KCl, 10 mM $MgCl_2$, 25 μM [U-^{14}C]isoleucine (50 mCi/mmol) and 1 mg crude tRNA in a total volume of 150 μl as described in [5].

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2.4. *Purification of the enzyme*

Cells (200 g) were suspended in 300 ml of 0.01 M potassium phosphate buffer (pH 7.0) containing 0.5 mM DTT. The cells were then disrupted using a Cell-Mill and glass beads as in [6]. The lysate was clarified by centrifugation. The supernatant was charged to a DEAE-Sephacel column (5.8 × 16 cm) preequilibrated with 0.05 M potassium phosphate buffer (pH 7.0) containing 0.5 mM DTT and 0.05 M KCl. The column was eluted with a 2800 ml linear gradient of KCl (0.05–0.25 M) containing the potassium phosphate buffer and 0.5 mM DTT. The enzyme was eluted at about 0.18 M KCl. Partially purified enzyme was subjected to a hydroxyapatite column (5.8 × 16 cm) preequilibrated with 0.1 M potassium phosphate buffer (pH 7.0) containing 1 mM DTT and 25% glycerol. The column was eluted with a 2800 ml linear gradient of potassium phosphate (pH 7.0, 0.1–0.35 M) containing 1 mM DTT and 25% glycerol. Two protein peaks were obtained. The latter peak had isoleucyl-tRNA synthetase activity.

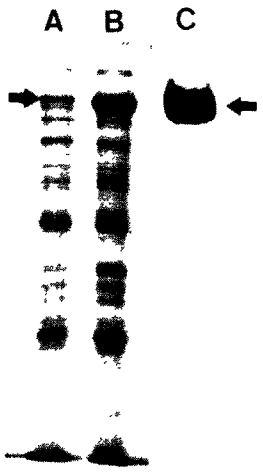

Fig.1. SDS-polyacrylamide (10%) gel electrophoresis of unfractionated cell extracts and purified isoleucyl-tRNA synthetase. Proteins were stained with Coomassie brilliant blue. JE5506/pKD15 was harvested at 1 h (lane A) and 5 h (lane B) after the temperature shift. Lane C, synthetase purified here. The arrow indicates the position of isoleucyl-tRNA synthetase of 115 kDa.

3. RESULTS AND DISCUSSION

3.1. *Purification of isoleucyl-tRNA synthetase*

JE5506 harboring pKD15 grown at 30°C was further grown at 37°C for induction of plasmid replication. Samples were harvested and crude extracts were subjected to SDS-PAGE [7].

A striking demonstration of overproduction of the synthetase is illustrated in fig.1. Thermal induction resulted in the appearance of isoleucyl-tRNA synthetase with maximal levels at 5 h after the shift to higher temperature. Enzymatic assay indicated an overproduction of 60-fold relative to strain JE5506. The synthetase was purified by a two-column procedure (fig.1 and table 1). This simple procedure is a modification of the method of Eldred and Schimmel [8], whose procedure consisted of (1) ammonium sulfate fractionation, (2) Sephadex G-150 gel filtration, (3) DEAE-cellulose column chromatography and (4) hydroxyapatite column chromatography. The yield of the syn-

Table 1

Summary of the purification of isoleucyl-tRNA synthetase from *E. coli* (200 g)

Step	Protein (g)	S.A.[a] (U/mg)	Activity (units)	Fold	Yield (%)
S-100	14	122	17×10^5	1	100
DEAE-Sephacel column chromatography	3.8	404	15.4×10^5	3.3	90
Hydroxyapatite column chromatography	1.37	1070	14.7×10^5	8.8	86

[a] Specific activity: one unit of the enzyme has the capacity of aminoacylating 1 μmol of isoleucine tRNA for 1 min at 37°C

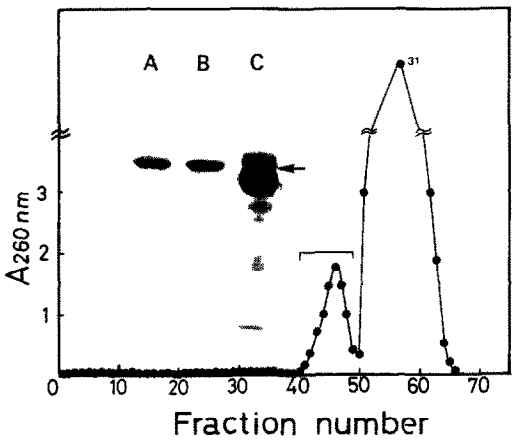

Fig.2. Isolation of tRNA complexed with isoleucyl-tRNA synthetase by gel filtration. The synthetase (5.7 mg) was incubated with 200 A_{260nm} units of unfractionated tRNA in 0.65 ml of 0.02 M Tris-HCl buffer (pH 7.0) containing 8 mM $MgCl_2$, 0.1 M KCl, 1 mM DTT and 10% glycerol for 1 h at 0°C. The whole solution was subjected to an Ultragel AcA 34 column (0.9 × 56 cm), which was preequilibrated with the buffer used above. The first peak was treated with phenol to remove proteins and subjected to PAGE. Inset figure; polyacrylamide (10%) gel profiles of RNA in the first peak (A), authentic $tRNA^{Ile}_{GAU}$ (B) and unfractionated *E. coli* tRNA (C). The position of $tRNA^{Ile}_{GAU}$ is indicated by the arrow. RNAs were stained with methylene blue.

thetase in the present work was 1.37 g from 36.6 g (dry wt) *E. coli* cells. The 3.7% yield was far better than the 0.1% or the 0.06% as in [8] and [9], respectively.

3.2. *Isolation of isoleucine specific tRNA*

Aminoacyl-tRNA synthetases are well known to form specific complexes with cognate tRNA [10]. We tried to develop a simple method for tRNA isolation. The synthetase (5.7 mg) was incubated with 200 A_{260nm} units of crude *E. coli* tRNA at 0°C for 1 h. The complex of the synthetase with the tRNA was isolated as in fig.2. The RNA (3 A_{260nm} units) was recovered from the first peak and analyzed by 10% PAGE as in [11]. The RNA component was found to be identical to authentic $tRNA^{Ile}_{GAU}$ (fig.2 inset). In addition, the isoleucine acceptance activity was 1500 pmol/A_{260nm} unit, showing that the isolated tRNA has a purity of 90% or more. In *E. coli*, there are two isoleucine tRNA species, $tRNA^{Ile}_{GAU}$ and $tRNA^{Ile}_{NAU}$. Since the amount of $tRNA^{Ile}_{NAU}$ in *E. coli* cells is known to be less than 5% of that of $tRNA^{Ile}_{GAU}$ [12,13], it may be difficult to detect the $tRNA^{Ile}_{NAU}$ band in PAGE of fig.2.

Since it is simple, our present procedure will be generally applicable to the purification of tRNA from various organisms. In addition, this is the first success of a single-step complete purification.

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