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Purification of mammalian tyrosyl-tRNA synthetase by high-performance liquid chromatography

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Aminoacyl-tRNA synthetases catalyse the specific aminoacylation of cognate tRNA by amino acids. Aminoacyl-tRNA synthetases of higher eukaryotes, including mammals, have been less studied than the bacterial and yeast enzymes, mainly owing to difficulties in their purification. Usually the concentration of mammalian aminoacyl-tRNA synthetases in tissue is about 0.1 – 0.01% of total protein[1,2], and about 1000 – 10 000-fold purification is required in order to obtain homogeneous enzymes. In addition, aminoacyl-tRNA synthetases are very susceptible to endogeneous proteolysis and special precautions should be taken to obtain pure native enzyme. Normally, procedures for the purification of mammalian enzymes require stage(s) of affinity chromatography or numerous conventional chromatography stages[1–5] and are time consuming. Recently we described the successful application fast protein liquid chromatography (FPLC) to the purification of mammalian valyl-tRNA synthetase[6], which made the purification procedure rapid and reproducible.

Several attempts to purify tyrosyl-tRNA synthetase have been made[7–9]. However, the reported molecular weights and properties of the enzyme vary significantly. Here we present a rapid method for the purification of mammalian tyrosyl-tRNA synthetase by FPLC.

EXPERIMENTAL

Materials

We used dithiotreitol (DTT), phenylmethylsulphonyl fluoride (PMSF) from Serva (Heidelberg, F.R.G.) diisopropyl fluorophosphate (DFP) from Merck (Darmstadt, F.R.G.) and ^{14}C-labelled tyrosine from Chemapol (Prague, Czechoslovakia). All other reagents were of analytical-reagent grade.

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Enzyme assay

Enzyme acitivity was measured in 50 μl of a mixture containing 100 m*M* Tris–HCl (pH 7.5) (37°C), 5 m*M* $MgCl_2$, 25 m*M* KCl, 0.1 m*M* DTT, 3 m*M* ATP, 5 μM [^{14}C]tyrosine (400 Ci/mol) and 50 – 100 μg of rabbit liver tRNA. Reaction was initiated by addition of the limiting amount of enzyme. Samples were incubated for 3 min at 37°C, reaction was stopped by the addition of 5% trichloroacetic acid, samples were filtered through GF/C glass-fibre filters (Whatman) and the filters were dried and counted in a toluene scintillator.

Purification procedure

Separation was effected with an FPLC system (Pharmacia, Uppsala, Sweden) on a DEAE-Toyopearl 650S (20 cm x 2 cm I.D.) prepacked column (Toyo Soda, Tokyo, Japan) and Mono S HR 5/5 and Mono Q HR 5/5 columns (5 cm × 5 mm I.D.) (Pharmacia). Chromatography was performed at room temperature.

Liver extract was obtained by homogenization of liver in a Waring blender for 60 s at 8000 rpm in two volumes of cold buffer [50 m*M* Tris–HCl (pH 7.5), 5 m*M* $MgCl_2$, 1 m*M* DTT, 10% glycerol] containing 1 m*M* of both PMSF and DFP. Protease inhibitors were were added as 0.2 *M* acetone solutions just before homogenization. The homogenate was centrifuged for 20 min at 10 000 *g*, filtered through four layers of gauze and used for PEG-6000 fractionation.

Polyethylene glycol (PEG) 6000 (Merck) solution (50%, w/v) was slowly added to the extract up to the final concentration of 5%. Precipitated protein was removed by centrifugation at 10 000 *g* for 10 min and PEG solution was added to the supernatant to obtain a concentration of 9%. A pellet obtained as above was dissolved in buffer A [25 m*M* Tris–HCl (pH 7.5), 1 m*M* $MgCl_2$, 1 m*M* DTT, 10% glycerol], centrifuged for 10 min at 10 000 *g* and applied to the DEAE-Toyopearl 650S column at a flow-rate of 3 ml/min. The column was washed with two volumes of starting buffer and proteins were eluted by a linear 0–0.3 *M* KCl gradient in 200 ml of buffer A. Active fractions that eluted at 60–90 m*M* KCl concentration were pooled, diluted twice with buffer A and applied to the Mono S column. Chromatography was performed at a flow-rate of 1 ml/min. Proteins were eluted with 20 ml of a 0–0.4 *M* KCl gradient in buffer A. Active fractions that eluted at 80 m*M* KCl concentration were pooled, diluted twice with buffer A and applied to the Mono Q column. Chromatographic conditions for the Mono Q step were the same as for Mono S. Active fractions containing purified tyrosyl-tRNA synthetase were stored frozen in liquid nitrogen.

Sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE) was performed on a PhastSystem (Pharmacia) using Phast Gradient Gels 10–15. After separation the gels were stained according Pharmacia recommendations[10].

Protein was determined according to Bradford[11].

RESULTS AND DISCUSSION

A rapid four-step purification method has been developed for the isolation of mammalian tyrosyl-tRNA synthetase. The procedure consist of the PEG-6000 precipitation and three consecutive steps of high-performance ion-exchange chromatography on DEAE-Toyopearl, Mono S and Mono Q columns. Purification data are summarized in Table I.

TABLE I

PURIFICATION OF TYROSYL-tRNA SYNTHETASE

Purification starts from 95 g of rabbit liver.

Purification step	*Protein (mg)*	*Total activity (U)*[a]	*Specific activity (U/mg)*[a]	*Yield (%)*	*Purification (fold)*
Extract	7100	70.3	0.0099	100	1
PEG-6000 (5–9%) precipitation	2200	40.8	0.018	58.5	1.8
DEAE-Toyopearl	82	22.1	0.27	31.7	27.3
Mono S	0.51	15.9	31.7	22.6	3200
Mono Q	0.11	15.4	140	22.0	14140

[a] 1 Unit (U) corresponds to the formation of 1 nmol of aminoacyl-tRNA per minute at 37°C.

The first step of the purification procedure is PEG-6000 precipitation. This method is better than the commonly used ammonium sulphate precipitation as it does not require dialysis or desalting prior to the ion-exchange chromatographic step. Tyrosyl-tRNA synthetase precipitates at high PEG-6000 concentrations (5–9%), which is characteristic of the 'soluble' cytoplasmic aminoacyl-tRNA synthetases.

The next step is chromatography on a DEAE-Toyopearl column. Tyrosyl-tRNA synthetase binds to this matrix and elutes at KCl concentrations of 60–90 m*M* as a single sharp peak [Fig. 1]. Active fractions after two-fold dilution are applied to the Mono S cation-exchange column [Fig. 2]. This step is extremely effective in the purification of tyrosyl-tRNA synthetase (see Table I) as more than 95% of the applied protein remains in the flow-through fraction, whereas tyrosyl-tRNA synthetase

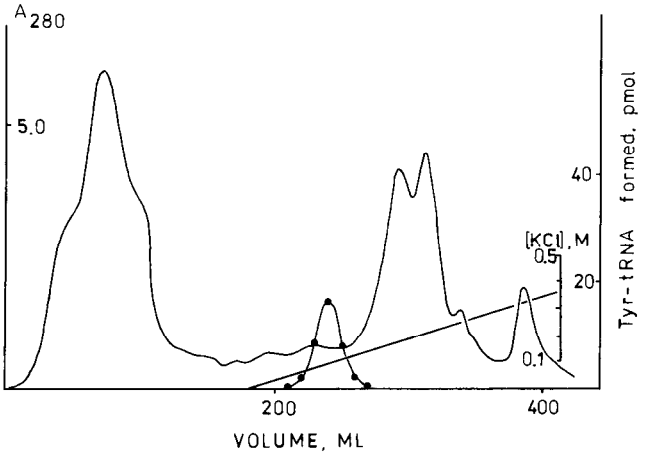

Fig. 1. Chromatography of tyrosyl-tRNA synthetase on the DEAE-Toyopearl 650S prepacked column (20 × 2 cm I.D.). Proteins from PEG-6000 (5–9%) precipitate were dissolved in buffer A and applied to the column at a flow-rate of 3 ml/min. Elution was performed with a linear KCl gradient from 0 to 0.3 *M* in 200 ml of buffer A. ● = Activity of tyrosyl-tRNA synthetase.

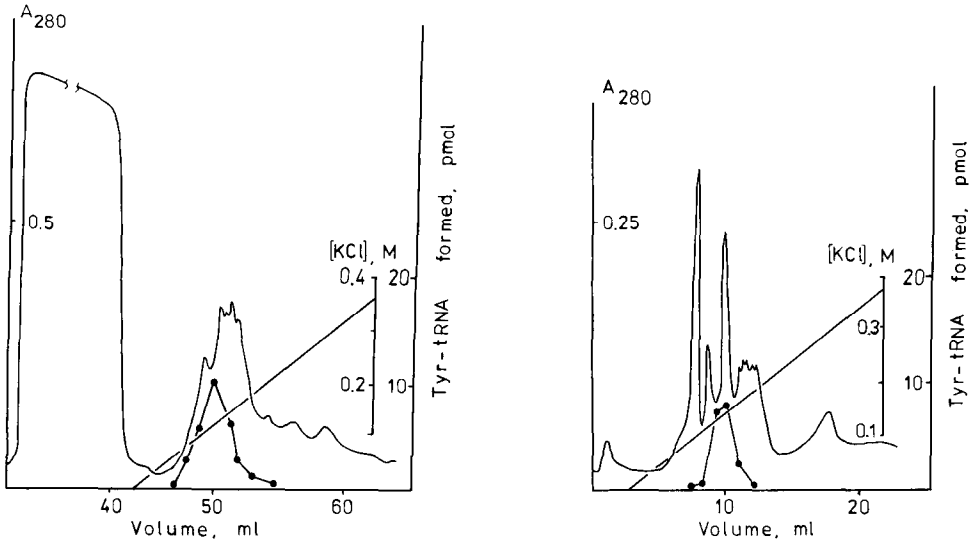

Fig. 2. Chromatography of tyrosyl-tRNA synthetase on the Mono S HR 5/5 column. Active fractions after DEAE-Toyopearl chromatography were applied to the Mono S column. Elution was performed with a linear KCl gradient from 0 to 0.4 *M* in 20 ml of the buffer A. Flow-rate, 1 ml/min. ● = Activity of tyrosyl-tRNA synthetase.

Fig. 3. Chromatography of tyrosyl-tRNA synthetase preparation on the Mono Q HR 5/5 anion-exchange column. Tyrosyl-tRNA synthetase preparation from the Mono S column was applied to the Mono Q column. Chromatographic conditions as in Fig. 2.

activity binds completely to the matrix. Enzyme elutes from the Mono S column at a KCl concentration of 80 m*M*. Tyrosyl-tRNA synthetase is finally purified by anion-exchange chromatography on a Mono Q column, where it elutes as a sharp peak well separated from the other protein peaks (Fig. 3).

SDS-PAGE of this fraction reveals a single polypeptide band with molecular weight *Mr* 70 000 (Fig. 4). Gel filtration of the purified enzyme also reveals a single peak corresponding to *Mr* 140 000 (data nog shown), indicating that mammalian

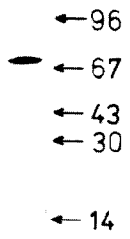

Fig. 4. SDS-page of the Mono Q tyrosyl-tRNA synthetase preparation. Arrows indicate the position of molecular weight markers: 96 = phosphorylase *b* (96 000 daltons); 67 = bovine serium albumin (67 000 daltons); 43 = ovalbumin (43 000 daltons); 30 = carboanhydrase (30 000 daltons); 14 = lactalbumin (14 000 daltons).

tyrosyl-tRNA synthetase has the same α_2 structure as all known tyrosyl-tRNA synthetases[12]. The molecular weight of the enzyme is higher than that previously reported, which suggests that previously reported preparations may be degraded by proteolysis.

The method described allows 14 000-fold purification of the enzyme to be achieved with a yield of about 22% (Table I). Enzyme with the same properties was also obtained from beef liver by the same method.

Several points should be noted. First, the time required to complete the purification is a single working day and the same buffer system is used throughout the purification. Second, this method can be easily scaled up by increasing the dimensions of the DEAE-Toyopearl column only, as the protein loading of the Mono S HR 5/5 column may be increased up to 10-fold.

In our laboratory we have successfully purified tyrosyl-tRNA synthetase from 0.5 kg of rabbit liver using the described method within a single working day.

Easy purification makes mammalian tyrosyl-tRNA synthetase a suitable material for kinetic and structural studies.

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REFERENCES

1 J. P. Pailliez and J. P. Waller, *J. Biol. Chem.*, 259 (1984) 15491.
2 T. Mizutani, T. Narihard and A. Hashimoto, *Eur. J. Biochem.*, 143 (1984) 9.
3 R. Rauchut, H.-J. Gabius and F. Cramer, *Biosystems*, 19 (1986) 173.
4 O. Kellerman, H. Tonetti, A. Brevet, M. Mirande, J.-P. Pailliez and J.-P. Waller, *J. Biol. Chem.*, 257 (1982) 11041.
5 D. E. Godar and D. C. H. Yang, *Biochemistry*, 27 (1988) 2181.
6 Y. A. Motorin, A. D. Wolfson, A. F. Orlovsky and K. L. Gladilin, *FEBS Lett.*, 220 (1987) 363.
7 Y. S. Prasada Rao and P. R. Srinivasan, *Nucl. Acid Res.*, 4 (1977) 3887.
8 F. Deak and G. Denes, *Biochem. Biophys. Acta*, 526 (1978) 626.
9 J. M. Clark and J. P. Eyzaguirre, *J. Biol. Chem.*, 237 (1962) 3698.
10 *PhastSystem Owners Manual*, Pharmacia, Uppsala, 1987.
11 M. M. Bradford, *Anal. Biochem.*, 72 (1976) 248.
12 L. L. Kisselev, O. O. Favorova and O. I. Lavrik, *Protein Biosynthesis from Amino Acids to Aminoacyl-tRNA*, Nauka, Moscow, 1984, p. 22.