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Purification of valyl-tRNA synthetase high-molecular-mass complex from rabbit liver

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A high-molecular-mass complex containing valyl-tRNA synthetase has been purified to homogeneity from rabbit liver. The molecular mass of the complex is about 800 kDa. The complex consists of four polypeptides of 130, 50, 40 and 30 kDa.

Valyl-tRNA synthetase; High-molecular-mass complex; HPLC

1. INTRODUCTION

One of the intriguing features of the higher eukaryotic aminoacyl-tRNA synthetases is their ability to form high-molecular-mass complexes. A complex containing 8-9 aminoacyl-tRNA synthetases, specific towards Arg, Asp, Gln, Glu, Ile, Leu, Met and Pro, has been purified from several sources and well characterised [1]. The only aminoacyl-tRNA synthetase which was also observed in a high-molecular-mass form, but was not present in the purified complex is valyl-tRNA synthetase [2,3]. This enzyme was supposed to form homocomplexes consisting of several subunits of the enzyme [4]. Here, we have succeeded in purification of the high-molecular-mass complex of valyl-tRNA synthetase and show that it is composed of several different polypeptides.

2. EXPERIMENTAL

We used columns of Sepharose CL-6B, prepacked Mono Q HR 5/5, Mono S HR 5/5 and Superose 6 HR 10/30 (Pharmacia), phenylmethylsulfonyl fluoride (PMSF) (Serva), diisopropyl fluorophosphate (DFP) (Merck), β-mercaptoethanol (Loba Chemie) and [^{14}C]valine (200 Ci/mol). The other reagents were of analytical grade.

The activity of valyl-tRNA synthetase was determined as described in [5]. One unit of enzyme activity is defined as the amount required to form 1 nmol aminoacyl-tRNA per min at 37°C.

Rabbit liver extract was obtained as in [5] except that the concentrations of PMSF and DFP were 1 mM.

The extract was further centrifuged at 20000 × g for 20 min. The supernatant (50 ml) was immediately applied onto the Sepharose CL-6B column (2.6 × 95 cm), equilibrated with 25 mM potassium phosphate buffer (pH 7.5), with 1 mM $MgCl_2$, 10% glycerol and 2 mM β-mercaptoethanol (buffer A). Elution was performed at a flow rate of 20 ml/h. The size of fractions was 10 ml. Active fractions (70 ml) were combined and applied on the Mono S column. This and other procedures were performed at room temperature. Proteins were eluted with a linear gradient of KCl from 0 to 0.5 M in buffer A at a flow rate of 1 ml/min. Active fractions were combined, diluted twice with buffer A and immediately applied on the Mono Q column. Elution was performed with

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KCl from 0 to 1.0 M in buffer A. Active fractions were stored frozen at −12°C. The specific activity of valyl-tRNA synthetase was about 40 U/mg.

Analytical gel filtration was performed using the Superose 6 HR 10/30 column. Protein samples (500 μl) were loaded onto the column and eluted at a flow rate of 0.5 ml/min in buffer A with 0.3 M KCl.

Electrophoresis was performed according to Laemmli [6].

Renaturation experiments were carried out as described by Waller et al. [7].

3. RESULTS AND DISCUSSION

The purification of valyl-tRNA synthetase includes 3 steps - gel filtration of the extract and two stages of high-performance ion-exchange chromatography. Gel filtration of the extract revealed that practically all the activity is eluted as a single peak corresponding to approx. 800 kDa (fig.1).

The fractions containing the highest specific activity were used for further purification. During chromatography on Mono S (fig.2) some of the valyl-tRNA synthetase activity flows through the column. This is due to the presence in the fraction applied of a considerable amount of RNAs, including tRNA, which are able to elute aminoacyl-tRNA synthetase from cation exchangers [8]. Final purification was achieved by the use of Mono Q chromatography (fig.3). The enzyme is eluted as a single peak at extremely high concentration of KCl

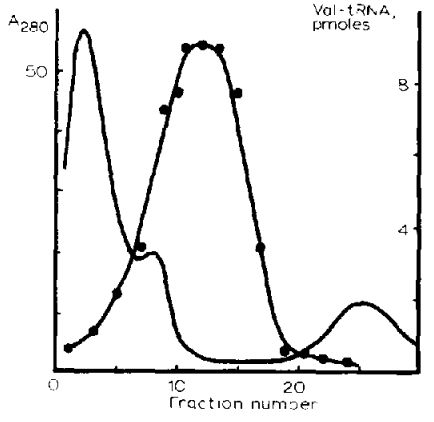

Fig.1. Chromatography of the extract on the Sepharose CL-6B column. A_{280} (——), valyl-tRNA synthetase activity (●——●).

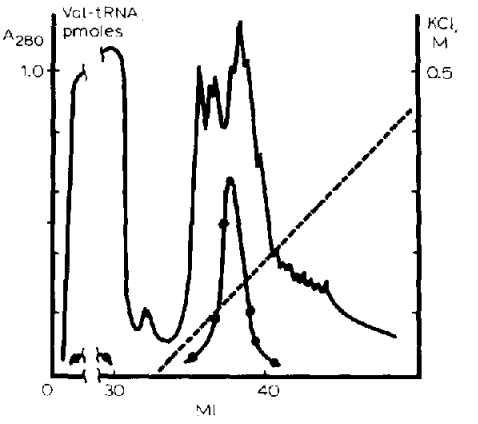

Fig.2. Chromatography of valyl-tRNA synthetase on the Mono S HR 5/5 column. A_{280} (——), valyl-tRNA synthetase activity (●——●).

- about 0.6 M. The specific activity of the enzyme after this step was about 40 U/mg and the purification about 1900-fold.

The fraction thus obtained contains four polypeptides of 130, 50, 40 and 30 kDa, approximately in stoichiometric quantities (fig.4). During gel filtration all polypeptides and valyl-tRNA synthetase activity were revealed as a single peak corresponding to 800 kDa with a shoulder at about 400 kDa (fig.5). Valyl-tRNA synthetase from the extract elutes exactly at the same position as the purified complex. The minor peak has the same

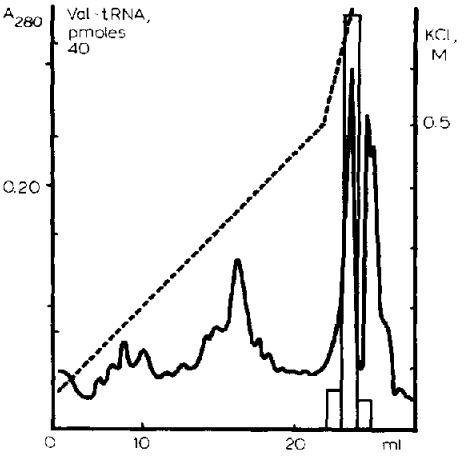

Fig.3. Chromatography of valyl-tRNA synthetase on the Mono Q HR 5/5 column. A_{280} (——); bars indicate valyl-tRNA synthetase activity.

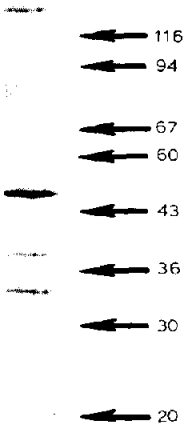

Fig.4. SDS electrophoresis of the valyl-tRNA synthetase complex after Mono Q chromatography using 10% gel. Arrows indicate the positions of molecular mass markers: *E. coli* β-galactosidase (116 kDa), phosphorylase *b* (94 kDa), albumin (67 kDa), catalase (60 kDa), ovalbumin (43 kDa), lactate dehydrogenase (36 kDa), carboanhydrase (30 kDa) and soybean trypsin inhibitor (20 kDa).

polypeptide composition and may result from partial dissociation of the complex.

The co-elution of these four polypeptides during gel filtration and two different steps of high-performance chromatography, and the identity of the enzyme's molecular mass in the extract and purified preparation lead us to consider that these polypeptides exist in the form of a single complex.

This is supported by the fact that introduction of an additional hydroxyapatite stage after, or heparin-Sepharose before, gel filtration did not change the polypeptide composition of the purified complex. Heparin-Sepharose chromatography was found to destabilise the complex.

Since the sum of the molecular masses of the subunits is 250 kDa, the stoichiometry of the complex is not clear. It may contain from 2 to 4 sets of subunits, if they are present stoichiometrically.

The single candidate for valyl-tRNA synthetase is the 130 kDa polypeptide, since the molecular mass of the yeast enzyme is 130 kDa [9] and those of yeast and mammalian enzymes are similar [7].

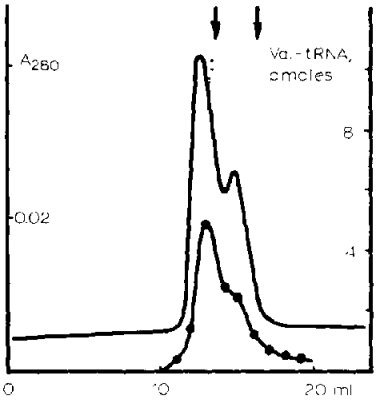

Fig.5. Gel filtration of the purified valyl-tRNA synthetase complex on the Superose 6 HR 10/30 column. Arrows indicate the elution positions of markers: thyroglobulin (699 kDa) and pyruvate kinase (235 kDa).

This fact is supported by the experiments on renaturation of the enzyme after SDS electrophoresis.

The function of the other polypeptides remains unknown. For example, they may be factors of protein biosynthesis, the existence of which in the high-molecular-mass form has been reported [2]. Work on the stability of the complex and identification of the polypeptides is in progress in our laboratory.

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