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Mammalian valyl-tRNA synthetase forms a complex with the first elongation factor

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The high-molecular-mass form of valyl-tRNA synthetase is associated with the first elongation factor activity. It includes two polypeptides of about 50 kDa and two others of 40 and 30 kDa, identified as α, β, γ and δ subunits of eEF-1H. The complex of valyl-tRNA synthetase with eEF-1H is suggested to be a novel form of the first elongation factor.

valyl-tRNA synthetase; First elongation factor; High-molecular-mass complex

1. INTRODUCTION

One of the characteristic features of eukaryotic aminoacyl-tRNA synthetases is their ability to form high-molecular-mass complexes. The complex of nine aminoacyl-tRNA synthetases is now well characterised [1]. Valyl-tRNA synthetase is the only aminoacyl-tRNA synthetase, which exists in a high-molecular-mass form different from this complex [1–3]. Recently we succeeded in purifying the valyl-tRNA synthetase complex from rabbit liver [4]. This complex consists of polypeptides with molecular masses of 130, 50, 40 and 30 kDa and has a molecular mass of about 800 kDa. A complex with the same polypeptide composition was also isolated from rabbit liver by Waller et al. [5] and several other mammalian sources in our laboratory. The 130 kDa polypeptide was identified as valyl-tRNA synthetase. In this work we have identified the low-molecular-mass polypeptides of the complex as subunits of the heavy form of the first elongation factor (eEF-1H).

2. MATERIALS AND METHODS

Preparations of eEF-1 and eEF-2 were the kind gift of A.G. Ryasanov (Institute of Protein Research, Puschino). 80 S ribosomes from rabbit reticulocytes were isolated by a conventional procedure [6].

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The valyl-tRNA synthetase complex was purified by gel filtration on Sepharose CL-6B and chromatography on Mono S and Mono Q columns (Pharmacia) as described previously [4]. The activity of valyl-tRNA synthetase was determined as described in [7].

EF-1 activity was assayed with the poly(U)-dependent translational system in 50 μl of a mixture which contained 20 mM Tris-HCl (pH 7.6), 10 mM $MgCl_2$, 100 mM KCl, 2 mM DTT, 1.2 mM GTP, 0.1 mg/ml poly(U), 10 pmol [^{14}C]Phe-tRNA, 0.2 A_{260} units of ribosomes and 1 μg of EF-2.

Analytical gel filtration was performed on a Superose 6 HR 10/30 column (Pharmacia) in 25 mM potassium phosphate (pH 7.6), 1 mM $MgCl_2$, 300 mM KCl, 2 mM β-mercaptoethanol, 10% glycerol. The flow rate was 0.5 ml/min and the sample volume 200 μl.

SDS-gel electrophoresis and two-dimensional gel electrophoresis were performed on minigels with PhastSystem (Pharmacia) according to Pharmacia recommendations [8,9]. Gels were stained with silver according to [10].

3. RESULTS AND DISCUSSION

Apart from the 130 kDa polypeptide identified earlier as valyl-tRNA synthetase, the complex contains polypeptides of 50, 40 and 30 kDa according to SDS electrophoresis. Since the SDS-electrophoresis pattern of the complex closely resembled that of eEF-1H (fig.1), we have suggested that the valyl-tRNA synthetase forms a

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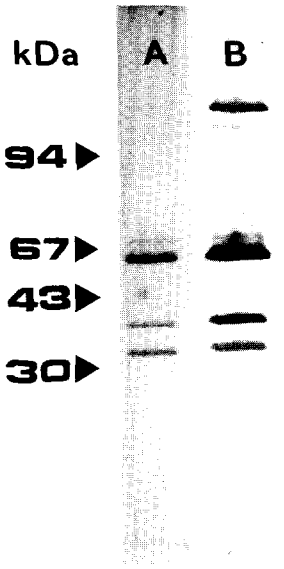

Fig.1. SDS gel electrophoresis on PhastGradient Gel 10–15. (A) eEF-1H from rabbit reticulocytes, (B) preparation of rabbit liver valyl-tRNA synthetase complex. Arrowheads indicate the position of molecular mass markers.

complex with eEF-1H. In order to prove this hypothesis several experiments were carried out.

The eEF-1 activity of the purified complex was assayed in the poly(U)-dependent translational system without eEF-1. The activity of the valyl-tRNA synthetase complex was approximately the

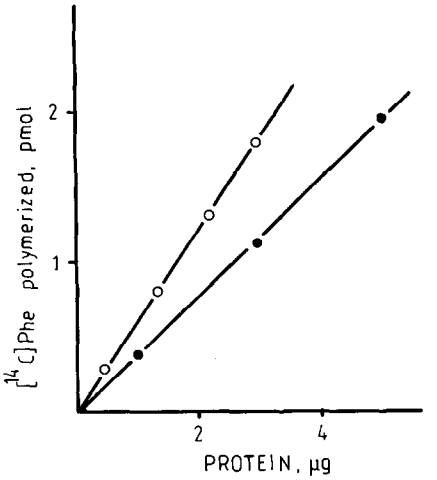

Fig.2. Stimulation of polyphenylalanine synthesis in poly(U)-dependent translational system. eEF-1H (○); valyl-tRNA synthetase complex (●).

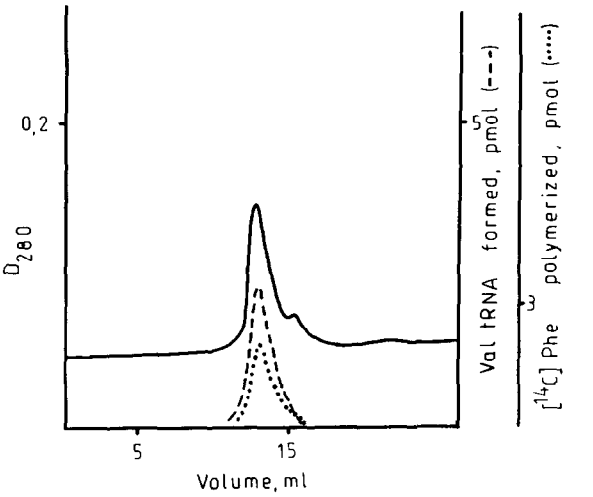

Fig.3. Gel filtration of the purified valyl-tRNA synthetase complex.

same as the conventional eEF-1H preparation (fig.2). The activities of eEF-1 and valyl-tRNA synthetase coelute upon gel filtration of the

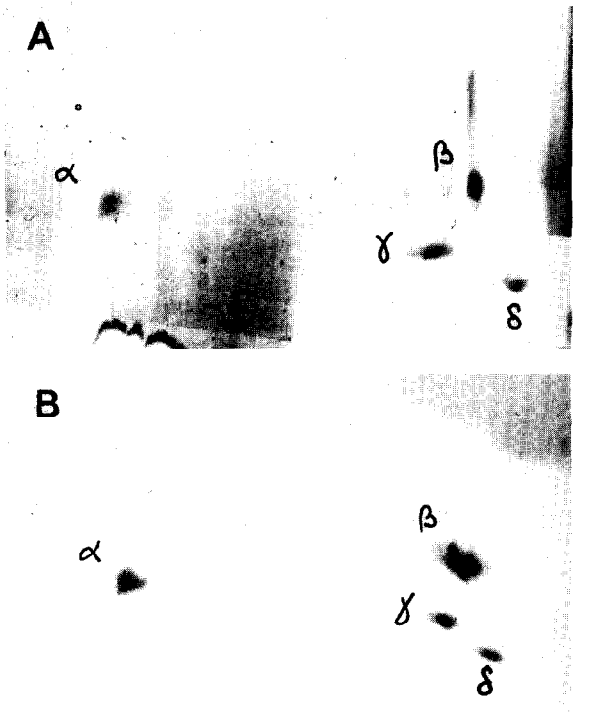

Fig.4. Two-dimensional gel electrophoresis of valyl-tRNA synthetase complex (a) and of eEF-1H (b) from rabbit reticulocytes.

purified complex on Superose 6 (fig.3). The coelution of both activities was also observed during the isolation procedure (not shown). The valyl-tRNA synthetase complex was analysed by two-dimensional gel electrophoresis for the identification of its polypeptides as subunits of eEF-1H (fig.4). Two polypeptides of about 50 kDa and p*I* values of about 9.0 and 5.0 are present in the complex (fig.4a). The comparison of the two-dimensional electrophoresis data for the complex and eEF-1H shows that they are practically identical (fig.4). All the subunits of eEF-1H (α, β, γ and δ [11]) are present in the complex. The spot corresponding to valyl-tRNA synthetase was not observed on two-dimensional gels probably due to the high proteolytic lability of this enzyme.

Since all valyl-tRNA synthetase in the extracts of various eukaryotic cells exists exclusively in high-molecular-mass form ([2–4] and unpublished) and is tightly associated with the subunits of eEF-1H, the high-molecular-mass complex is probably a special form of EF-1, which exists in the extracts as well as 400 kDa eEF-1H and 50 kDa eEF-1L. Further studies will provide understanding of the function of this particular form of elongation factor.

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