Proteins of the *Thermus thermophilus* ribosome

Purification of several individual proteins and crystallization of protein TL7

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The procedure of selective removal of eight proteins from the 50 S ribosomal subunit of the extreme thermophilic bacterium *Thermus thermophilus* has been developed based on extraction at 60°C in the presence of 0.5 M or 1 M NH₄Cl and 50% ethanol. CM-Sepharose CL column chromatography of the protein mixture under non-denaturing conditions yielded five proteins with a purity of 95% or higher. Crystals of one of these proteins, namely TL7 (probably an analog of L6 protein from the *Escherichia coli* ribosome) have been obtained using the 'hanging drop' method with ammonium sulphate as a precipitant.

1. INTRODUCTION

*Thermus thermophilus* ribosomes were first isolated by Ohno-Iwashita et al. [1] in 1975. In 1986 Gogia et al. [2] developed a method of purifying *T. thermophilus* ribosomes free of membrane fragments and active in the cell-free system. Here ribosomal proteins from *T. thermophilus* were analyzed by two-dimensional electrophoresis and their nomenclature defined. Recently *T. thermophilus* ribosomes were obtained in a crystalline form [3]. In this paper we report a procedure of selective removal of eight proteins from the 50 S ribosomal subunit of *T. thermophilus* ribosomes and of CM-Sepharose CL column chromatography for fractionation of these proteins. As a result five proteins were yielded with a purity of 95% or higher. Protein TL7 (probably an analog of L6 protein from *E. coli* ribosomes) was crystallized.

2. MATERIALS AND METHODS

The growing of *T. thermophilus* HB 8 and purification of ribosomes were performed as described in [2]. Ribosomal subunits were isolated as in [2] with the exception that the salt composition of the sucrose gradient was 0.01 M MgCl₂, 0.4 M NaCl, 0.001 M Na₂EDTA, 0.2 M Tris-HCl, pH 7.5 at 20°C. Ribosomal proteins were extracted according to [4] with some modifications. An equal volume of ethanol at 60°C was added to a solution of 50 S subunits (10 mg/ml, at the same temperature) containing 0.2 M MgCl₂, 1 M NH₄Cl, 0.0005 M Na₂EDTA, 0.04 M Tris-HCl, pH 7.5 at 20°C. The mixture was incubated for 50 min at 60–61°C with moderate mixing and centrifuged at 35,000 × g. The supernatant was collected and the derivative ribosomal particles were dissolved in a buffer with 2 M NH₄Cl and repeated ethanol treatment.

Fractionation of prepared protein mixture was done by CM-Sepharose CL column chromatography under non-denaturing conditions in a sodium-acetate buffer at pH 5.6, the NaCl gradient concentration being from 0.04 to 0.7 M.
The protein composition of extracts and identification of proteins were determined by two-dimensional electrophoresis [5]. The purity of the proteins was tested by SDS-electrophoresis [6].

3. RESULTS AND DISCUSSION

Fig. 1 which was borrowed from [2] shows the two-dimensional electrophoretic separation of ribosomal proteins from *T. thermophilus* and *E. coli*. They are shown here because we have used the nomenclature for *T. thermophilus* ribosomal proteins proposed by the authors of that paper [2].

The standard and most effective ribosomal protein isolation procedures of selective and cooperative removal of protein groups by high

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Fig. 1. Two-dimensional electropherograms of ribosomal proteins from *T. thermophilus* and *E. coli*: (a, b, c) proteins from 70 S ribosomes, 50 S and 30 S ribosomal subunits of *T. thermophilus*; (d) proteins from 70 S ribosomes of *E. coli* (illustration from [2]).
concentrations of lithium chloride [7] or sodium chloride [8] proved to be unsuitable for our purpose.

Our technique of selective removal of proteins from *T. thermophilus* ribosomes is based on removal of the \( (L7/L12)_2L10 \) protein complex from *E. coli* ribosomes [4] and consists of treating ribosomes with 50% ethanol in the presence of 0.5 M NH₄Cl and 0.01 M MgCl₂. Some modifications in the procedure allowed quantitative removal of 8 proteins from the 50 S subunit. Protein extraction was done in two steps. The first was the treatment of 50 S subunits with 50% ethanol in the presence of 0.5 M NH₄Cl and 0.01 M MgCl₂ at 60°C. The second step was the treatment with 50% ethanol and 1 M NH₄Cl and 0.01 M MgCl₂ at 60°C of the ribosomal particles obtained from the first step. Thus, two fractions of ribosomal proteins were obtained. Two-dimensional electrophoresis indicates that the first fraction (proteins extracted with 0.5 M NH₄Cl and 50% ethanol) consisted of proteins TL2, TL4, TL5, TL6, TL7, TL8, TL11* (analog of the complex \( (L7/L12)_2L10 \) from *E. coli*) and another protein, probably TL14 (identification of this protein by two-dimensional electrophoresis could not be determined reliably).

The second fraction (proteins extracted with 1 M NH₄Cl and 50% ethanol) consisted of the same proteins, an amount of proteins TL21,
Fig. 4. Micrograph of TL7 protein crystals.

TL27/TL28 and also of proteins TS14, TS19 and TS20; the presence of the latter can be explained by contamination of the 50 S subunit sample with 30 S subunits. Analysis of the protein composition of ribosomal particles after extraction shows that the proteins TL11* and TL7 were removed completely, the TL5 and TL4 ones up to 90–95% and the TL6, TL8 and TL2 proteins up to 80–85%. The rest (TL21, TL14, TL27/TL28) were extracted partly (fig. 2). It is noteworthy that a decrease of extraction temperature to 55°C led to a sharp fall in the yield of all extracted proteins with the exception of TL11*.

The chromatographic procedure gave proteins TL11*, TL7, TL2, TL4 and TL5 with a purity of 95% or higher, the value for proteins TL11, TL6 and TL8 being 75–85%. Chromatographic data and results of electrophoretic analysis of protein peaks are shown in fig. 3.

One of the prepared proteins, TL7 (probably an analog of protein L6 from E. coli ribosomes) was crystallized. Crystals in the form of hexagonal rods with a ratio of length to thickness of about 10:1 were obtained using the ‘hanging drop’ vapour diffusion technique. Ammonium sulphate was used as a precipitant. The crystals were grown in a solution consisting of 0.05 M Mes-NaOH, pH 6.0–6.1, and 40–43% ammonium sulphate saturated at a protein concentration of 2–4 mg/ml. The addition of 0.001 M MgSO4 to the protein solution decreased the crystal length to thickness ratio to about 4:1 (fig. 4). At present, work is being done to prepare crystals of protein TL7 for X-ray analysis.

REFERENCES