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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 NH_4Cl 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.

Ribosomal protein; Crystallization; Purification; (*Thermus thermophilus*)

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.

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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 $MgCl_2$, 0.4 M NaCl, 0.001 M Na_2EDTA, 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 $MgCl_2$, 1 M NH_4Cl, 0.0005 M Na_2EDTA, 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 $35\,000 \times g$. The supernatant was collected and the derivative ribosomal particles were dissolved in a buffer with 2 M NH_4Cl 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.

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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

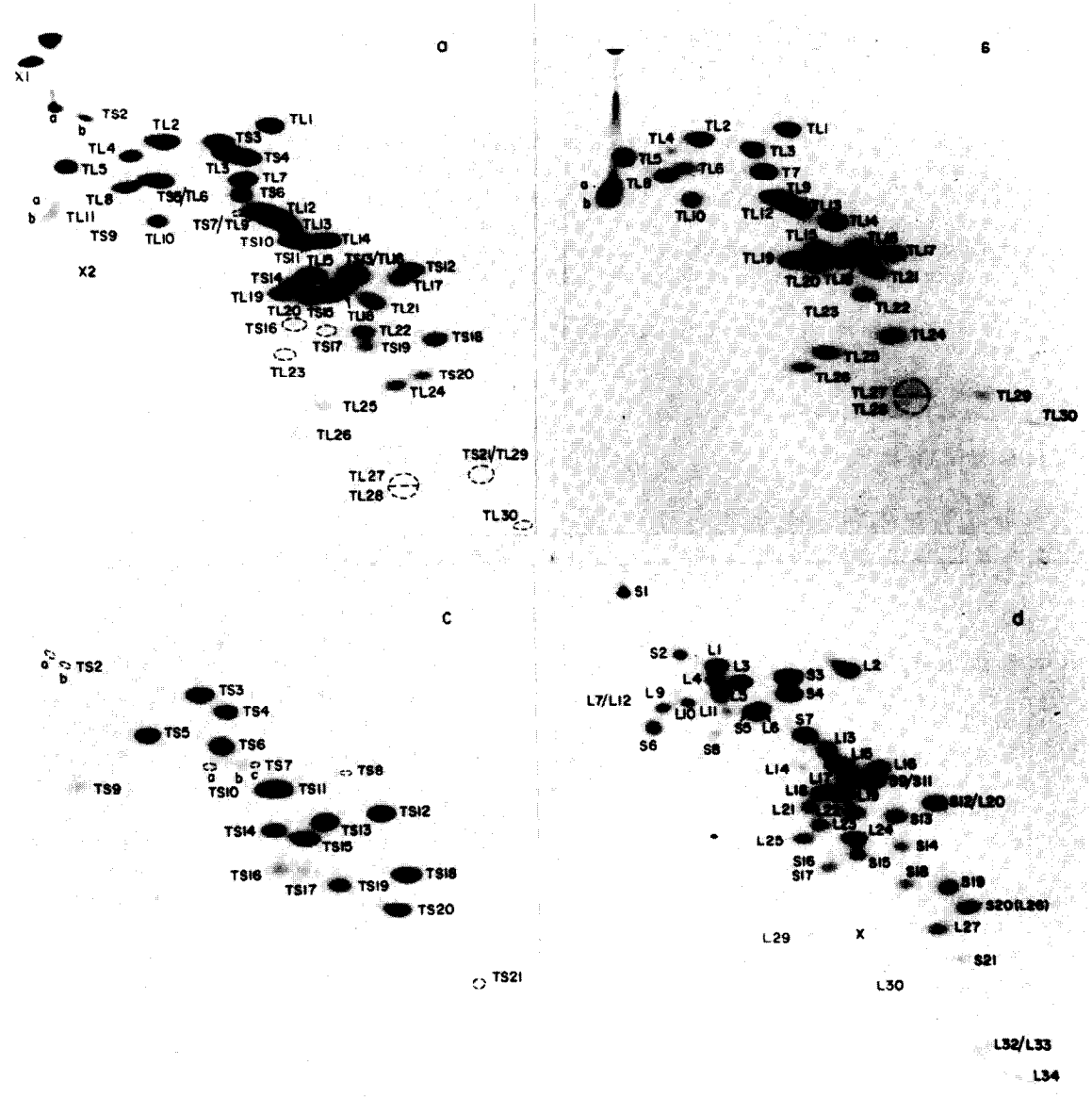

Fig.1. Two-dimensional electropherograms of ribosomal proteins from *T. thermophilus* and *E. coli*: (a,b,c) proteins from 70 S ribosomes, 50 S and 30 S ribosomal subunits of *T. thermophilus*; (d) proteins from 70 S ribosomes of *E. coli* (illustration from [2]).

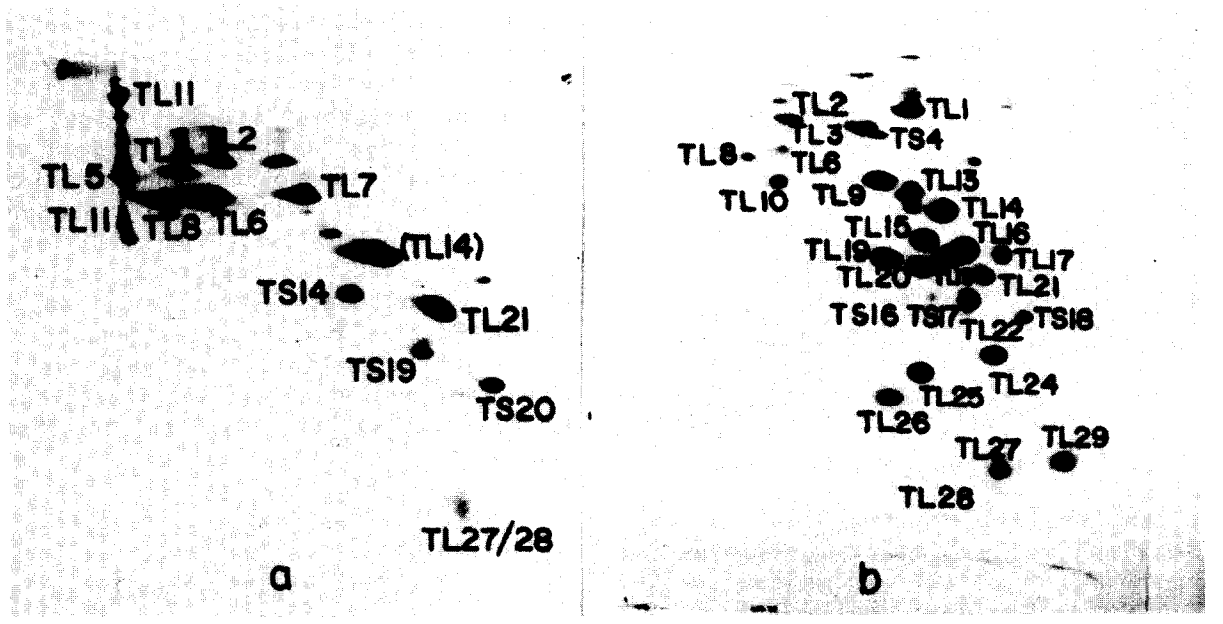

Fig.2. Two-dimensional electropherograms of ribosomal proteins from *T. thermophilus*: (a) protein composition of the second protein fraction removed from the 50 S subunits in the presence of 1 M NH_4Cl and 50% ethanol; (b) protein composition of ribonucleoprotein particles prepared as a result of this treatment.

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 NH_4Cl and 0.01 M $MgCl_2$. 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 NH_4Cl and 0.01 M $MgCl_2$ at 60°C. The second step was the treatment with 50% ethanol and 1 M NH_4Cl and 0.01 M $MgCl_2$ 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 NH_4Cl 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 NH_4Cl and 50% ethanol) consisted of the same proteins, an amount of proteins TL21,

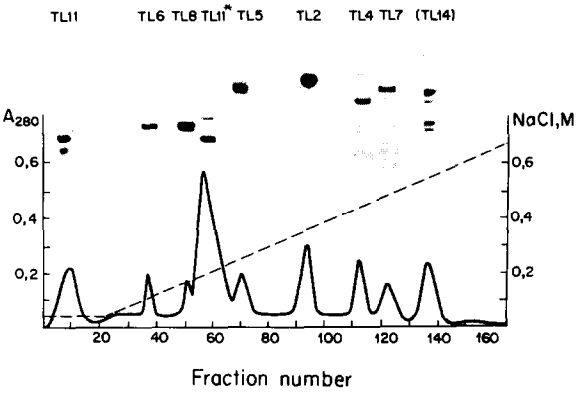

Fig.3. Fractionation of the protein mixture prepared by treatment of 50 S subunits with 0.5 M NH_4Cl and 50% ethanol (the first fraction). The CM_2Sepharose CL column had a volume of 30 ml. The amount of protein used was 150 mg. A 900 ml NaCl gradient from 0.04 to 0.7 M in a sodium-acetate buffer at pH 5.6 was used for protein elution. The elution rate was 10 ml/h and the fraction volume 5.8 ml. The protein composition of fractions was examined by SDS-electrophoresis.

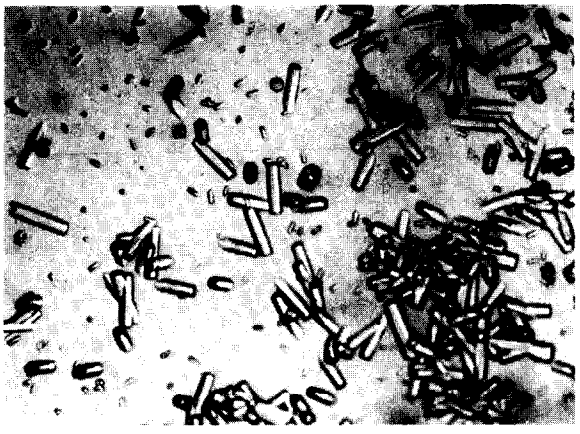

Fig.4. Micrograph of TL7 protein crystals.

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 $MgSO_4$ 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.

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