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5S rRNA binding ribosomal proteins from *Thermus thermophilus*: identification and some structural properties

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Abstract An unusual acidic ribosomal protein from *Thermus thermophilus*, TL5, that binds to 5S rRNA specifically and strongly, has been investigated. The N-terminal sequence of TL5 does not reveal any homology with known ribosomal proteins. Two large tryptic fragments of TL5 have been isolated and characterized. 5S rRNA protected TL5 and its unstable N-terminal fragment against trypsin action. The 5S rRNA binding ability of TL5 is probably inherent in its N-terminal part. The other 5S rRNA binding ribosomal protein from *T. thermophilus*, TL4, has been identified as a homolog of the ribosomal protein L5 from *Escherichia coli*.

Key words: 5S RNA; Ribosomal protein; *Thermus thermophilus*

1. Introduction

The extreme thermophile *Thermus thermophilus* is being used successfully as a source of proteins and nucleoprotein complexes for crystallization and structural studies. An impressive success has been reached in crystallization of ribosomes and ribosomal subunits from this microorganism (see, e.g. review [1]). Two 5S rRNA binding proteins TL4 and TL5 from *Thermus thermophilus* have been found using a reconstruction technique [2]. Highly stable hybrid complexes of the 5S rRNA from *E. coli* and *Bacillus stearothermophilus* with proteins TL4 and TL5 from *T. thermophilus* have been obtained. In the reconstruction experiments protein TL4 replaced protein L5 from the *Escherichia coli* 5S rRNA-protein complex, therefore it was suggested that TL4 is homologous to *E. coli* L5. The rather large acidic ribosomal protein TL5 specifically replaced two small ribosomal proteins L18 and L25 from the *E. coli* 5S rRNA-protein complex. This protein has not been characterized and in this work we aimed to identify this protein by the N-terminal amino acid sequence and to investigate the nature of its high affinity to 5S rRNA.

N-terminal amino acid sequencing of both TL4 and TL5 was done in this work. It was confirmed that TL4 is a homolog of *E. coli* L5. The first 53 amino acid residues of TL5 did not reveal any homology with known ribosomal proteins. However, extremely high homology has been found for the N-terminal part of TL5 and the earlier described 5S rRNA binding protein from *T. flavus* [3-5]. 2D electrophoresis also shows that the spot of protein TL5 is not similar to any spot in the electrophoretic picture of *E. coli* ribosomal proteins: TL5 is more acidic than all proteins from *E. coli* 50S ribosomal subunits except L7/L12. Probably this protein is a special 5S rRNA binding protein in extreme thermophiles. A limited trypsinolysis approach allowed us to split TL5 into two big fragments. It was shown that the 5S rRNA binding protects TL5 against trypsin action. Moreover, the unstable 8 kDa N-terminal fragment of TL5 was protected by the 5S rRNA against trypsinolysis.

2. Materials and methods

2.1. Materials

Sephadex G-100, CM-Sepharose CL-6B and epoxy-activated Sepharose 6B were purchased from Pharmacia Fine Chemicals (Sweden). All other reagents were purchased from Fluka (Switzerland), Serva (Germany) and Sigma (USA).

2.2. Isolation of ribosomal 50S subunits and ribosomal proteins TL4 and TL5 from T. thermophilus and the 5S rRNA from B. stearothermophilus

Ribosomal 50S subunits were obtained from *T. thermophilus* as described earlier [6]. Proteins TL4 and TL5 from *T. thermophilus* 50S ribosomal subunits were purified by CM-Sepharose CL-6B column chromatography under non-denaturing conditions as in [6]. The 5S ribosomal RNA from *B. stearothermophilus* was isolated by Sephadex G-100 gel filtration from the total RNA preparation obtained by standard phenol extraction procedure described earlier [7].

2.3. Trypsin digestion of protein TL5 and its complex with the 5S rRNA

Samples of ribosomal protein TL5 and the 5S rRNA *B. stearothermophilus* were prepared in reconstitution buffer (20 mM Tris-HCl, pH 7.5, 10 mM MgCl, 100 mM NaCl and 0.1 mM EDTA) at a concentration of 1–2 mg/ml. The 5S rRNA was renatured at 60°C for 5 min. The 5S RNA-protein complex was prepared by incubation of 5S rRNA with protein TL5 (mol/mol) at 40°C for 5 min according to [2]. Protein TL5 or its complex with 5S rRNA was treated with trypsin at a 1/100 enzyme/protein ratio (w/w) for 5–30 min at 37°C. The reaction was stopped by an addition of 2% SDS or trypsin inhibitor (3× over trypsin quantity).

2.4. Preparation of the 5S rRNA-Sepharose gel and affinity chromatography

Immobilization of RNA was done according to the previously published method [8]. We used 0.5–1.0 ml of the RNA gel containing 10–20 A260 units 5S rRNA. The native ribosomal protein TL5 or its mixture with its fragments after limited trypsinolysis was loaded onto the column in the reconstitution buffer. Elution was performed with the same buffer using a linear NaCl gradient from 0.1 M to 3.0 M.

2.5. Isolation of fragments of protein TL5 by reversed-phase HPLC

The products of tryptic hydrolysis of protein TL5 were separated on a Hi-Pore RP-318 column (240×4.6 mm) (Bio-Rad, USA). A linear gradient of acetonitrile with 0.1% trifluoroacetic acid was used. Elution profiles were scanned with a 226 nm detector. Fractions

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collected were desiccated in a Speedvac (Savant Inc., USA) vacuum centrifuge and stored dry at 4°C. All samples were tested by SDS-PAGE according to [9].

2.6. N-terminal sequencing

Sequence analysis of proteins was performed by automatic Edman degradation on an Applied Biosystems (Foster City, CA, USA) gas phase sequence. Data were analyzed using the software 610A Data Program, Version 1.2.

3. Results and discussion

The N-terminal amino acid sequence of ribosomal protein TL4 from *T. thermophilus* is shown in Fig. 1A. Alignment with L5 from *E. coli* [10] shows that these are two homologous proteins.

The 53 aa N-terminal sequence of ribosomal protein TL5 from *T. thermophilus* is shown in Fig. 1B. This sequence has not revealed any homology with known ribosomal proteins. However, the 5S rRNA-binding ribosomal protein from *T. flavus* with a practically identical N-terminal sequence was determined previously [3-5] (Fig. 1B). We suggest that this protein could be an evolutionary acquisition of extremely thermophilic bacteria.

The RNA-binding properties of ribosomal protein TL5 have been investigated using the limited trypsinolysis approach. The scheme of experiments is presented in Fig. 2A. The free protein TL5 was split by trypsin into two fragments: HFr, a high molecular mass (about 15 kDa) fragment, and LFr, a low molecular mass (about 8 kDa) fragment (Fig. 2B, a, lane 5 min). HFr was very stable to trypsin action, but LFr was unstable and quickly disappeared (Fig. 2B, a, lanes 15 min and 30 min). An addition of the 5S rRNA to the incubation mixture after 5 min trypsinolysis (see Fig. 2A) protected LFr against further hydrolysis (Fig. 2B, b). The native protein TL5 in complex with the 5S rRNA was very stable to trypsin action (Fig. 2B, c).

Experiments on binding the products of TL5 limited trypsinolysis to the 5S rRNA gel have been carried out. The mixture contained the native TL5 and a nicked TL5 (HFr and LFr with excess of HFr). It has been found that HFr alone did not bind to the gel, but the nicked TL5 as well as the intact TL5 bound to this gel and were eluted from the column using a linear NaCl gradient (Fig. 3). The nicked TL5

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A

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TL4.   PLDLALKRKYYEEVRPELIRRFGYQNVXEVPRLEKVVIN
EcoL5.    AKLHDYYKDEVVKKLMTEFNYNSVMQVPRVEKITLN

B

     ************************************  *  *** * ***
TL5. MEYRLKAYYREGEKPSALRRAGKLPGVMYNRHLNRKVYV-DLVEFDKVF
TfN. MEYRLKAYYREGEKPSALRRAGKLPGVMYNRHLNRXXYXXDLVQFRKVF

     *** *
     RQAXI
     RQASIHHVIVLQLRRXTRLSPE
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Fig. 1. (A) The N-terminal amino acid sequence of protein TL4 from *T. thermophilus* as compared with the N-terminal sequence of protein L5 from *Escherichia coli*. (B) The N-terminal amino acid sequence of protein TL5 from *T. thermophilus* as compared with the N-terminal sequence of the 5S rRNA binding protein from *T. flavus* (TfN).

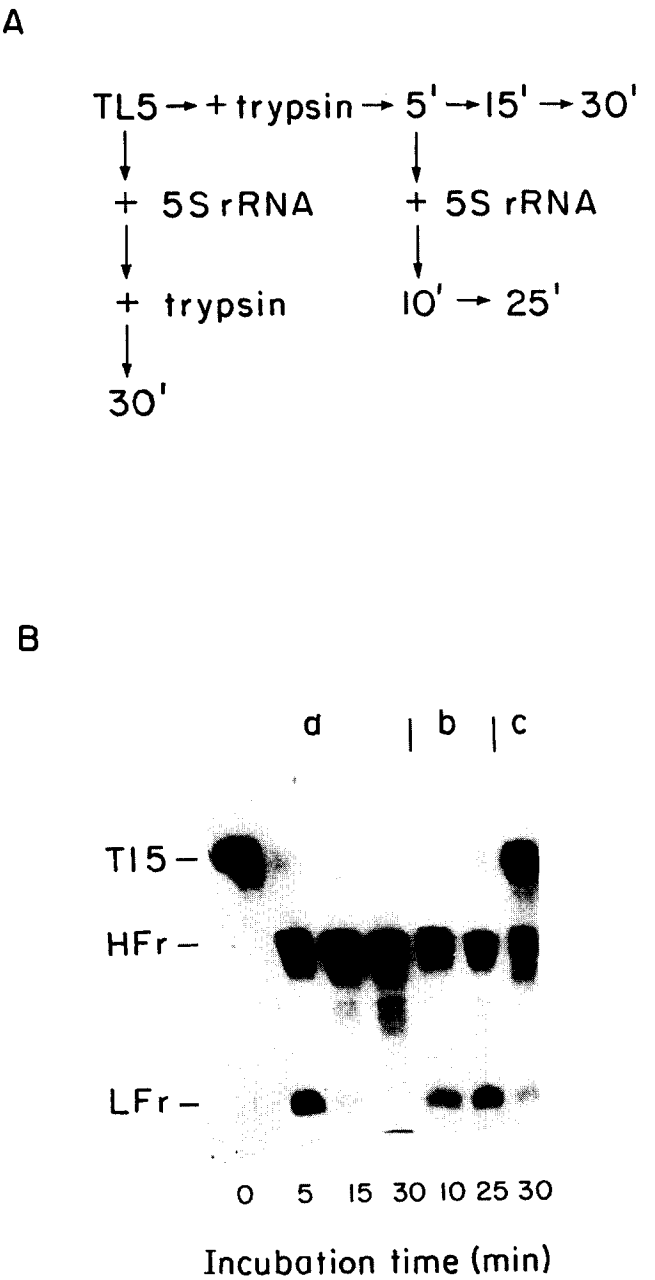

Fig. 2. Effect of the presence of 5S rRNA *B. stearothermophilus* on trypsinolysis of ribosomal protein TL5 from *T. thermophilus* (protein/trypsin ratio 100:1). (A) Scheme of experiment (sequence of components added and time of trypsinolysis are indicated). (B) SDS-PAGE of products of TL5 protein trypsinolysis. a: Free TL5 protein; b: fragments of TL5 protein after 5 min trypsinolysis mixed with the 5S rRNA; c, TL5-5S rRNA complex.

eluted at 0.5–0.8 M NaCl, and the native protein at higher NaCl concentration (more than 1 M).

HFr and LFr were isolated using reversed-phase HPLC. The N-terminal sequences for both fragments have been determined (Fig. 4), and it was established that the N-terminal region of LFr coincides with the N-terminal region of protein TL5. The N-terminal sequence of HFr is situated outside the determined N-terminal sequence of protein TL5 from *T. thermophilus*. The molecular mass of LFr is about 8 kDa (by SDS-PAGE analysis), this means that about 70 amino acid

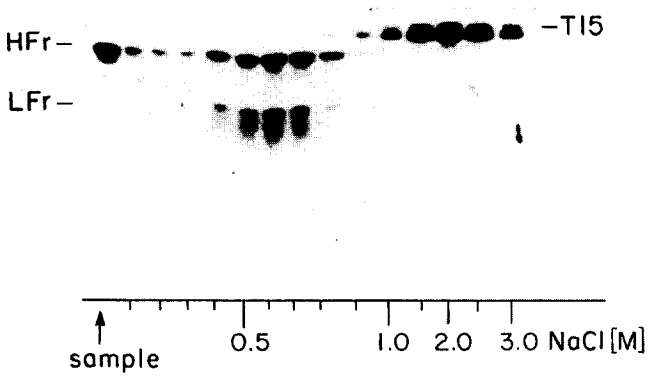

Fig. 3. Elution of trypsinolysis products of the ribosomal protein TL5 from *T. thermophilus* by buffer solution containing increasing concentrations of NaCl from the column with the 5S rRNA-Sepharose 6B.

residues are in this fragment. Therefore we can suggest that LFr covers the N-terminal region of TL5 from residue 1 to about residue 70.

Thus, 5S rRNA protects the N-terminal region of protein TL5 (about 70 amino acid residues) against trypsinolysis. The nick of TL5 does not influence the binding with the 5S rRNA. We suggest that this region of TL5 participates in the interaction of the protein with 5S rRNA.

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LFr-TL5.  MEYRLKAYYR
    TL5.  MEYRLKAYYREGEKPSALRRAGKLPGVMYNRHL...

          S
          R       F
HFr-TL5.  XRPEHVDXFV
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Fig. 4. The N-terminal sequences of LFr and HFr of protein TL5 from *T. thermophilus* (LFr-TL5 and HFr-TL5, respectively). The N-therminal sequence of protein TL5 is shown for comparison.

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