The Complete Amino Acid Sequence of Ribosomal Protein S8 from *Thermus thermophilus*

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Protein S8 from *Thermus thermophilus* consists of 138 amino acids of M, 15,840. Its primary structure was established using peptide sequences from two different digests. Protein S8 from *T. thermophilus* shares a high percentage of identity with protein S8 from *Thermus aquaticus*. There are some consensus sequences between proteins S8 from eubacteria, archebacteria, chloroplasts, and cyanelles.

1. INTRODUCTION

Protein S8 acts as an important component to build up the complex structure of the 30S ribosomal subunit. S8 of *Escherichia coli* binds specifically to region C, near to the center of the 16S RNA (Ungewickell *et al.*, 1975). A slightly unwound helix containing three bulged adenines residues is a striking conserved element of the S8 binding site (Mougel *et al.*, 1987). During the assembly of 30S subunit, S8 interacts cooperatively with S6, S15, and S18 with the central domain of 16S RNA (Gregory *et al.*, 1984). S8 is also involved by playing a critical role in the translational regulation of spc operon expression (Dean *et al.*, 1981). X-ray crystallography would be the appropriate tool to characterize in atomic detail the S8-RNA complex. So far, bacterial ribosomes have proved difficult to crystallize for X-ray analysis. This study has been undertaken promisingly on eubacteria *Thermus thermophilus*. The knowledge of the primary structure of S8 from *T. thermophilus* is a necessary step in the elucidation of the fully understanding of the part that each amino acid of S8 plays in protein–RNA and protein–protein interaction within the ribosomal 30S subunit. The present paper deals with the determination of the complete amino acid sequence of S8 from *T. thermophilus*. This allowed a comparison with the known sequences of homologous proteins from eubacteria and other sources.

2. MATERIALS

Endoproteinase Glu-C and endoproteinase Lys-C were obtained from Boehringer (Mannheim, Germany). All chemicals used on HPLC columns were analytical grade products of Merck (Darmstadt, Germany) and of Applied Biosystems (Roissy, France) for the Edman degradation. Chromatography of peptides was performed on a HPLC apparatus (Millipore-Waters Associates, United States) using a Nucleosil 120-5, C4 column (Macherey-Nagel, Düren, Germany). Protein S8 was purified on a CM-Toyopearl 650S column (Toyo-Soda, Japan).
3. METHODS

3.1. Purification of Protein S8 from T. thermophilus

General procedure of purification of T. thermophilus strain VK1 ribosomal proteins from 30S ribosomal subunit will be described elsewhere. Extraction of proteins from small ribosomal subunit was carried out by two steps, with 3.5 M LiCl and subsequently 6 M LiCl. Each fraction consisted of 9–10 proteins. Protein S8 is core protein. It was extracted at second step by 6 M LiCl together with the other 8–9 proteins. Protein mixture has been loaded on CM-Toyopearl 650S in 0.025 M Na-cacodylate, pH 6.2, or in 0.05 M Tris-HCl, pH 7.5. After washing with the loading buffer, proteins were eluted with a linear NaCl gradient (200–700 mM) in the same buffer. The first peak of elution profile contained protein S8. Purity of protein S8, estimated by SDS-electrophoresis and two-dimensional electrophoresis, was about 95–98%.

3.2. Alkylation of Cys residues (Okazaki et al., 1985)

Before enzymatic cleavage, protein S8 was alkylated. Samples of S8 (0.2 mg in 50 μl Tris-HCl 1 M, EDTA 4 mM, pH 8) were denaturated in 75 μl guanidium-HCl 8 M, and 2.5 μl 2-mercaptoethanol 10% (v/v) during 2 hr at 25°C under N₂. S-pyridyl-ethylation of cysteine residues was performed by adding 4 μl of vinylpyridine during 2 hr at 25°C under N₂.

3.3. Enzymatic Cleavage

3.3.1. Digestion with Endoproteinase Lys-C

Samples of S8 (0.2 mg) were hydrolyzed in 100 μl Tris-HCl 0.1 M, pH 8, at 37°C overnight with endoproteinase Lys-C (0.5 U) added at 0 and 6 hr.

3.3.2. Endoproteinase Glu-C Digestion

Digestion with endoproteinase Glu-C was performed with 0.15 mg S8 dissolved in 50 μl ammonium bicarbonate 25 mM, pH 7.8. The protease/protein ratio was 1/30 (w/w); Glu-C was added at 0 and 6 hr and the reaction was allowed to proceed overnight at 37°C.

3.4. Isolation of Peptides, Amino Acid Analysis, and Sequencing

Chromatography on HPLC column was used to separate the peptides obtained from the enzymatic cleavages. The peptides were purified with linear gradients of volatile buffers (TFA 0.1% and acetonitrile).

Amino acid analyses were performed on a 420A–130A Derivatization and Analysis system (Applied Biosystems). Protein and peptides were sequenced by automated Edman’s degradation, using an Applied Biosystems 470A Protein Sequencer equipped with a PTH 120A Analyser (Hewick et al., 1981).

4. RESULTS AND DISCUSSION

The results of our study are summarized in Fig. 1, which shows (i) the complete amino acid sequence

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Fig. 1. The complete primary structure of protein S8 from T. thermophilus. GSQ, sequencing of the N-terminal region of S8 from T. thermophilus in an Applied Biosystems gas-phase sequencer. Lys-C and Glu-C, peptides arising, respectively, from Lys-C and Glu-C endoproteinases digestion.