THE PRIMARY STRUCTURE OF THE 5S RNA BINDING PROTEIN L18 FROM *ESCHERICHIA COLI* RIBOSOMES

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1. Introduction

Protein L18 of the large subunit of *E. coli* ribosomes binds to 5S RNA [1,2] and together with proteins L5 and L25, forms a stable complex with 5S RNA [3] which has ATP- and GTPase activity [4]. A study on 50S subunit derived ribonucleoprotein fragments demonstrated that L18 is involved in the GTPase activity [5]. The elucidation of the amino acid sequence of protein L18 which is described in this communication will facilitate a closer insight into the mechanism of RNA-protein interaction and the function of this protein.

2. Materials and methods

Protein L18 was isolated from *E. coli* K using a new preparative procedure [6] with a yield of about 70 mg from 12 g 50S ribosomal subunits. 2 μmol of protein were digested by trypsin. The tryptic peptides were purified on sulfoethylcellulose followed by a micro Dowex 50W × 7 column [7] and by gel filtration on Sephadex G10 or G25. Digestion at the glutamic acid and aspartic acid residues was performed by *Staphylococcal* protease [8] on 275 nmol of protein. The derived peptides were separated on a phosphocellulose column (0.6 × 15 cm) and further purified on Sephadex G10 or Sephadex G25.

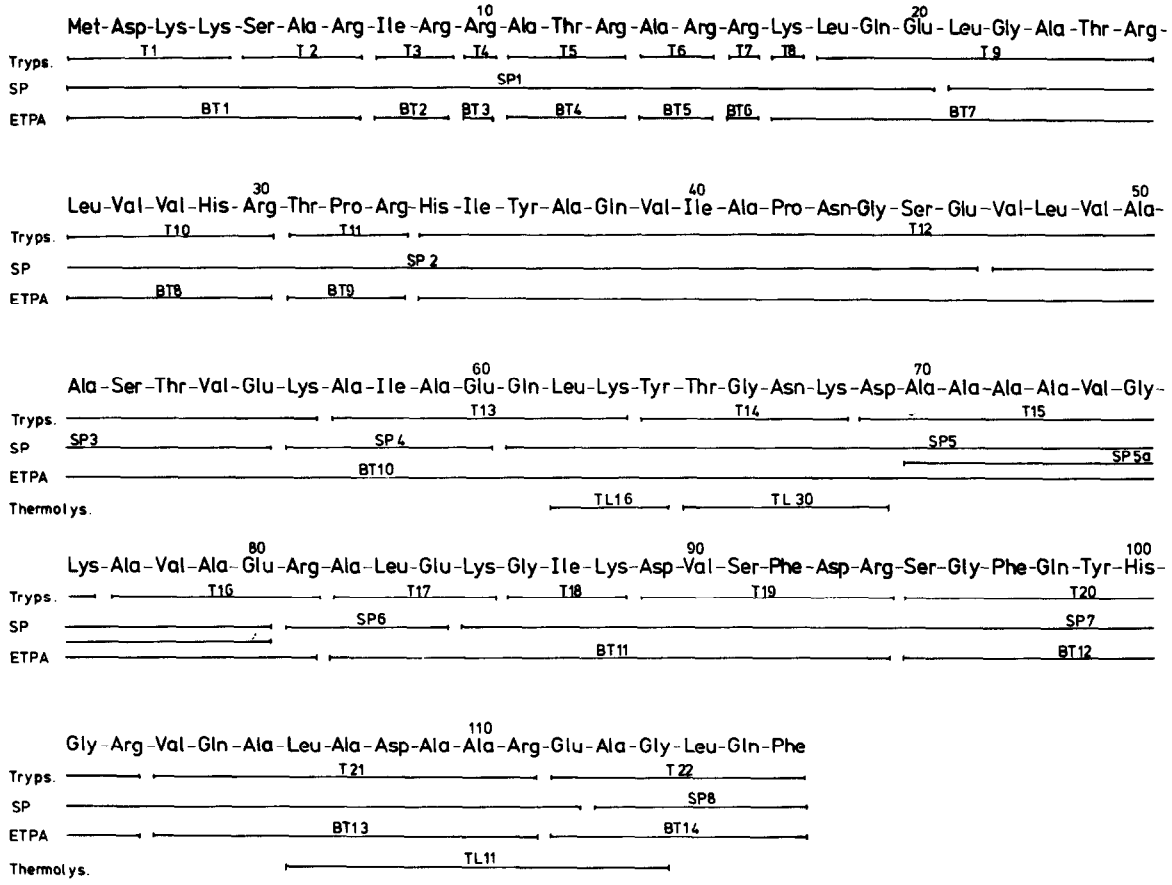
Peptides with C-terminal arginine were prepared by first modifying the lysine residues with ETPA (exo-cis-3, 6-endoxo-Δ^4-tetra-hydrophthalic acid anhydride) [9] and then digesting with trypsin. Furthermore peptide maps of thermolysin, lysine-blocked and *Staphylococcal* protease peptides were done on paper. The amino acid analyses were made with a Durrum D-500 analyzer in the 1–2 nmol range and with an LKB-Biocal 3201 analyzer. The sequences of all the tryptic peptides, except peptide T 12, were determined with a solid phase sequenator [10,11] and partially with the Dansyl-Edman technique [12]. Tryptic peptide T 12 and overlapping peptides were sequenced exclusively by Dansyl-Edman technique in the 10–100 nmol range. Full details of the methods will be given later [6].

3. Results and discussion

The tryptic cleavage revealed 19 peptides and two free amino acids, namely lysine and arginine. Peptides from *Staphylococcal* protease digestion were used to establish the alignment of the tryptic peptides and to confirm the sequence within them. Peptide SP 5 could not be sequenced because the N-terminus formed pyrrolidonecarboxylic acid during preparation but since cleavage occurred also at aspartic acid, the sequence could be determined partially from peptide SP 5a (position 70–80). Further peptides obtained by cleavage after aspartic acid were isolated, sequenced and found to be in agreement with all other peptides.

The lysine-blocked tryptic peptide BT 10 (position 34–81) was isolated on Sephadex G75 and sequenced in order to align the tryptic peptides T 12–T 15. The amino acid compositions of all other lysine-blocked peptides was in complete agreement with the sequence. The compositions of the thermolysin peptides obtained from peptide map also agreed with the above data. TL 11 with the amino acid composition (Asx, Glx,Gly, Ala_4,Leu,Arg) confirmed the alignment of the C-terminal tryptic peptides T 21 and T 22. TL 16 combined

Table 1



T 13–T 14 and TL 30 combined T 14–T 15. The complete sequence is given in table 1. The polypeptide chain with the mol. wt of 12 770 daltons consists of 117 residues. The amino acid composition derived from the sequence is in good agreement with the experimentally determined data. Ten basic amino acids are clustered between positions 3–17, and the region 70–79 is hydrophobic except for one lysine.

On the basis of the sequence the secondary structure of L18 was predicted using the method of Chou and Fasman [13,14]: 47% α-helical regions and 11% β-sheet structures are postulated. The distribution over the protein is: α-helix regions: positions 11–21, 46–63, 69–74, 77–85, 103–113; β-sheet regions: 23–28 and 35–41.

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