# PRIMARY STRUCTURE OF PROTEIN S19 FROM THE SMALL RIBOSOMAL SUBUNIT OF *ESCHERICHIA COLI*

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## 1. Introduction

Protein S19 is located on the 'head' of the *E. coli* small ribosomal subunit as found by immunoelectron microscopy [1,2]. It has been crosslinked to protein S13 [3,4] and to S14 [5]. Protein S19 alone binds weakly to the 16 S RNA but a complex between S13 and S19 binds strongly [6,7]. Ribonucleoprotein complexes were isolated which, besides other proteins, contained protein S19 and RNA regions from the 3'-terminal portion of the 16 S RNA [8]. The gene for protein S19 has been mapped in the *spc–str* region of the *E. coli* chromosome [9] and mutants with an altered protein S19 have been isolated [10,11].

The amino acid sequence of the N-terminal region of S19 is in [12,13]. We describe here the determination of the complete primary structure of protein S19 which consists of 91 amino acids and has mol. wt 10 299.

## 2. Materials and methods

Protein S19 was isolated from *E. coli* K as in [14]. The identity and purity of the protein were checked by two-dimensional polyacrylamide gel electrophoresis [15]. Tryptic and chymotryptic digestion was at 37°C, pH 8.0, for 4 h or 24 h. Thermolytic digestion was at 55°C, pH 8.0, for 4 h. Digestion with *Staphylococcus aureus* protease, kindly supplied by Dr. G. R. Drapeau, University of Montreal, was in 50 mM acetic acid, pH 4.0, for 16 h [16]. Large fragments were produced by cyanogen bromide [17].

The isolation of the large peptides was achieved by gel filtration of various digests (about 5 mg each) on Sephadex G-50 and G-75 (superfine) columns (250 × 1.5 cm); 5% acetic acid or 0.01 N HCl was used for the elution. Smaller peptides in the various fractions eluted from the Sephadex columns were further separated by fingerprinting on cellulose thin-layer plates [18]. Amino acid analyses were performed with a Durrum D-500 amino acid analyzer. The presence of tryptophan in the peptides was established by finger-printing on cellulose thin-layer plates and spraying with Ehrlich's reagent.

Automatic Edman degradation [19] of the larger peptides was made in a Beckman model 890C sequenator. The amino acid sequence of smaller peptides was determined by a manual micro Edman technique [20] without dansylation. The thiazolinone or PTH derivatives were hydrolysed with 6 N HCl in the presence or absence of 0.1% $SnCl_2$ [21] at 130°C for 20 h, and the amino acids formed were analysed with the Durrum analyzer. The identification of PTH derivatives was made by thin-layer chromatography on silica gel plates [22,23].

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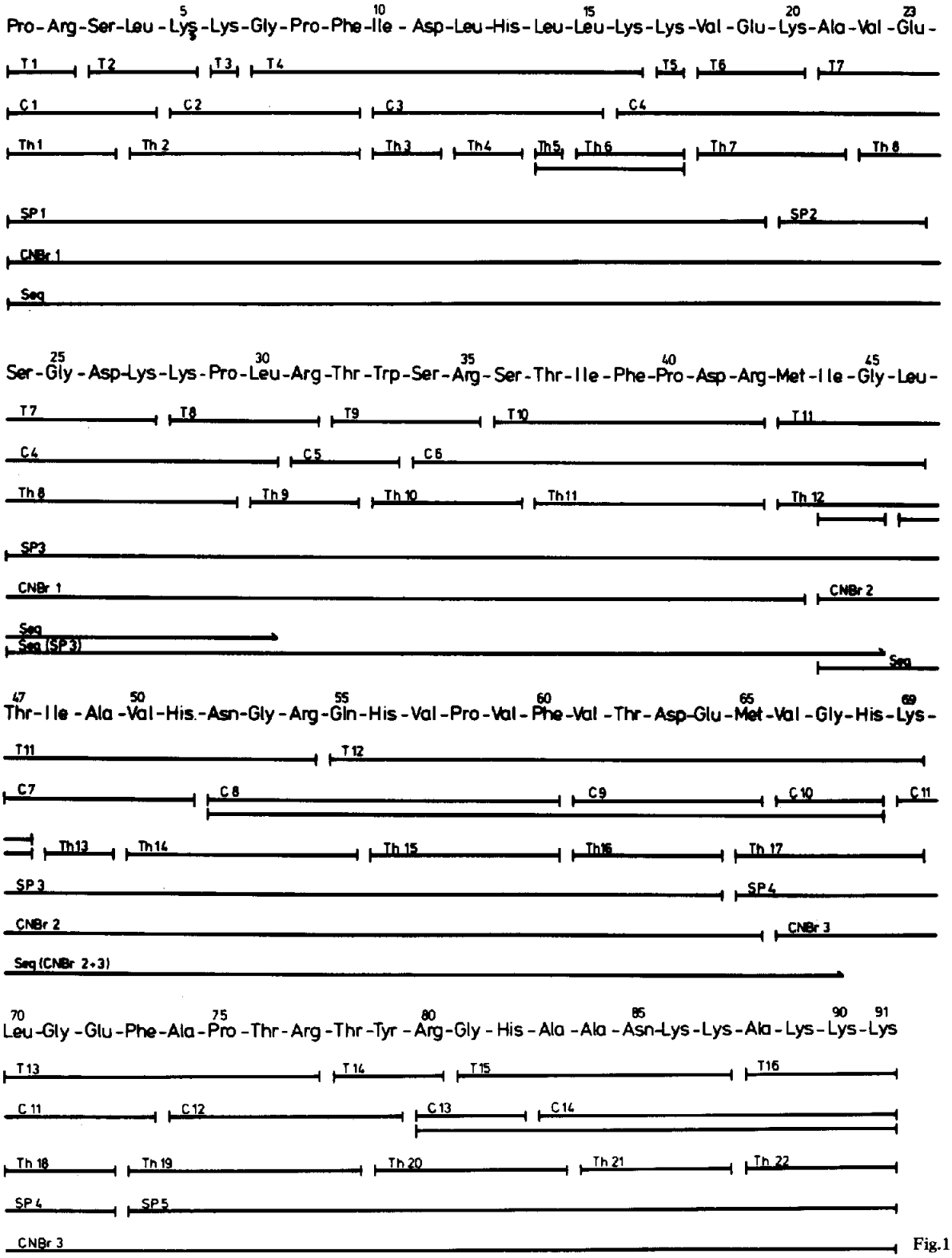
Pro-Arg-Ser-Leu-Lys-Lys-Gly-Pro-Phe-Ile-Asp-Leu-His-Leu-Leu-Lys-Lys-Val-Glu-Lys-Ala-Val-Glu-
T1 T2 T3 T4 T5 T6 T7
C1 C2 C3 C4
Th1 Th2 Th3 Th4 Th5 Th6 Th7 Th8
SP1 SP2
CNBr 1
Seq
Ser-Gly-Asp-Lys-Lys-Pro-Leu-Arg-Thr-Trp-Ser-Arg-Ser-Thr-Ile-Phe-Pro-Asp-Arg-Met-Ile-Gly-Leu-
T7 T8 T9 T10 T11
C4 C5 C6
Th8 Th9 Th10 Th11 Th12
SP3
CNBr 1 CNBr 2
Seq
Seq (SP3)
Seq
Thr-Ile-Ala-Val-His-Asn-Gly-Arg-Gln-His-Val-Pro-Val-Phe-Val-Thr-Asp-Glu-Met-Val-Gly-His-Lys-
T11 T12
C7 C8 C9 C10 C11
Th13 Th14 Th15 Th16 Th17
SP3 SP4
CNBr 2 CNBr 3
Seq (CNBr 2+3)
Leu-Gly-Glu-Phe-Ala-Pro-Thr-Arg-Thr-Tyr-Arg-Gly-His-Ala-Ala-Asn-Lys-Lys-Ala-Lys-Lys-Lys
T13 T14 T15 T16
C11 C12 C13 C14
Th18 Th19 Th20 Th21 Th22
SP4 SP5
CNBr 3

Fig.1

## 3. Results and discussion

The automatic sequence determination of protein S19 [12], Sp3 and CNBr2+3 established the first 67 residues of S19 as shown in fig.1. Treatment of protein S19 with trypsin resulted in 16 peptides which, except T11 and T12, were sequenced manually.

Digestion of protein S19 with chymotrypsin and thermolysin gave 14 and 22 peptides, respectively. Some peptides, namely Th12, C8+C9+C10, C13+C14, are joint peptides due to incomplete hydrolysis. Only those from the C-terminal region (C11, C12, C13, C14, Th18, Th19, Th20, Th21, Th22) were sequenced manually and established the alignment T13–T14–T15–T16.

*Staphylococcus aureus* protease specifically produced 5 peptides and cyanogen bromide 3 peptides. CNBr2 (positions 44–65) and SP4 (65–72) were completely sequenced manually, and the results confirmed the sequence of T11 and T12 and the alignment. Carboxypeptidase A and B released lysine as C-terminal residue.

The combination of these results gave the alignment of all peptides and the complete amino acid sequence as in fig.1. The amino acid composition derived from the sequence of S19 is:

$Asp_4$, $Asn_2$, $Thr_6$, $Ser_4$, $Glu_4$, $Gln_1$, $Pro_6$, $Gly_7$, $Ala_6$, $Val_7$, $Met_2$, $Ile_4$, $Leu_7$, $Tyr_1$, $Phe_4$, $His_5$, $Lys_{13}$, $Arg_7$, $Trp_1$.

Protein S19 is 10 299.

Comparison of the sequence of S19 with that of other ribosomal proteins of known structure [24–26] revealed several identical regions which are shown in table 1. In addition, 8 out of 12 residues between positions 16–27 of S19 and positions 32–43 of S20 were found to be identical [27].

Based on the amino acid sequence of protein S19, predictions for the possible secondary structure of this protein were made in a similar way as was done for other ribosomal proteins [28–31]. Using 4 predictions [32–35] the following structural features of S19 are predicted: helix regions for positions 11–22 and 82/87–88/90; extended structure for positions 55/57–61/62 and possibly also for 43–50; turns or loops for positions 6–8, 24–29, 34–36, 40–42, 52–54, 74–75, 78–80.

A ribosomal protein homologous to S19 has also been isolated from *Bacillus stearothermophilus* [36–38] and *Bacillus subtilis* [39]. There is a high degree of sequence homology among the N-terminal sequence of proteins S19 from *E. coli* and the two bacilli [13,39,40].

Table 1
Region of protein S19 identical with regions of other *E. coli* ribosomal proteins

| Peptide | Protein | Positions | Protein | Positions |
|---|---|---|---|---|
| Val–Glu–Lys–Ala–Val | S19 | 18–22 | S17 | 78– 82 |
| Val–Glu–Lys–Ala | S19 | 18–21 | L18 | 54– 57 |
| His–Leu–Leu–Lys | S19 | 13–16 | L29 | 41– 44 |
| Leu–Leu–Lys–Lys | S19 | 14–17 | L11 | 78– 81 |
| Met–Ile–Gly–Leu | S19 | 43–46 | L 3 | 1– 4 |
| Thr–Ile–Ala–Val | S19 | 47–50 | L 5 | 104–107 |
| Asn–Gly–Arg–Gln | S19 | 52–55 | L34 | 26– 29 |
| Arg–Gln–His–Val | S19 | 54–57 | L33 | 43– 46 |
| Ala–Ala–Asn–Lys | S19 | 83–86 | S 8 | 18– 21 |
| Ala–Ala–Asn–Lys | S19 | 83–86 | L25 | 22– 25 |
| Lys–Ala–Lys–Lys | S19 | 87–90 | S 4 | 145–148 |
| Ala–Lys–Lys–Lys | S19 | 88–91 | L 6 | 173–176 |

Fig.1. Amino acid sequences of protein S19 from *E. coli* ribosomes. T, tryptic peptide; C, chymotryptic peptide; Th, thermolytic peptide; SP, peptide from digestion with *Staphylococcus aureus* protease; CNBr, peptide cleaved with cyanogen bromide; Seq, automatic sequencing by liquid-phase degradation in a sequenator.

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