PRIMARY STRUCTURE OF THE 16S rRNA BINDING PROTEIN S15 FROM *ESCHERICHIA COLI* RIBOSOMES

Tomonori MORINAGA, Gunki FUNATSU and Masaru FUNATSU
Laboratory of Biochemistry, Faculty of Agriculture, Kyushu University, Fukuoka, Japan

and

H. G. WITTMANN
Max-Planck-Institut für Molekulare Genetik, Berlin-Dahlem, Germany

Received 6 March 1976

1. Introduction

Protein S15 from the 30S ribosomal subunit of *E. coli* is one of the 16S rRNA binding proteins. It binds at about the same region of the 16S rRNA as protein S8, namely approximately 600–750 nucleotides from the 3′-end (for a review see [1]). Determination of the primary structure of protein S15 is necessary for a closer investigation of the molecular mechanism of the interaction between this protein and 16S rRNA.

Protein S15 which is a very basic protein [2], consists of 87 amino acid residues and its N-terminal sequence has been determined using a liquid phase sequenator [3]. In this paper, the complete determination of the primary structure of this protein is reported.

2. Materials and methods

Protein S15 was isolated from *E. coli* strain K as previously described [4]. Tryptic and chymotryptic digestions were carried out at pH 8 and 37°C for 4 h, 2 h or 45 min, and carboxypeptidase Y digestion [5] was conducted at pH 5.5 and 37°C for 2 h. Separation of the peptides was performed by SE-cellulose column chromatography and paper chromatography as previously described [6]. Cyanogen bromide cleavage of the protein S15 was performed in 70% formic acid at room temperature for 20 h and the resulting peptides were separated by gel filtration on Biogel P-30 in a 5% acetic acid solution. Amino acid compositions of the peptides were determined with an automatic amino acid analyzer (JEOL; type JLC-6AH) after hydrolysis with 5.7 N HCl containing 0.02% mercaptoethanol at 108°C for 20 h. The amino acid sequence of the peptides was determined by manual Edman degradation as modified by Iwanaga et al. [7] and the PTH-amino acids were identified by thin-layer chromatography on silica gel plates.

3. Results and discussion

The amino acid sequence of the protein S15 is shown in fig.1. Details of its determination will be described elsewhere.

Protein S15 has an amino acid composition of $Asp_5Asn_2Thr_6Ser_6Glu_5Gln_5Gly_5Ala_7Val_5Met_1Ile_3Leu_{12}Tyr_2Phe_2Lys_6His_4Arg_{11}$ which results in a mol. wt. of 10 000. Proline, cysteine and tryptophan are absent. The number of each amino acid residue from the sequence data agrees well with that obtained from the amino acid analysis of the whole protein. The 21 basic and 10 acidic amino acids are compatible with the high isoelectric point of this protein [2]

Eleven peptides as well as free arginine and lysine were isolated from the tryptic digest of the protein S15. The yields of peptide T8 (Arg–Lys) and free arginine were about twice that of the other peptides. Peptides

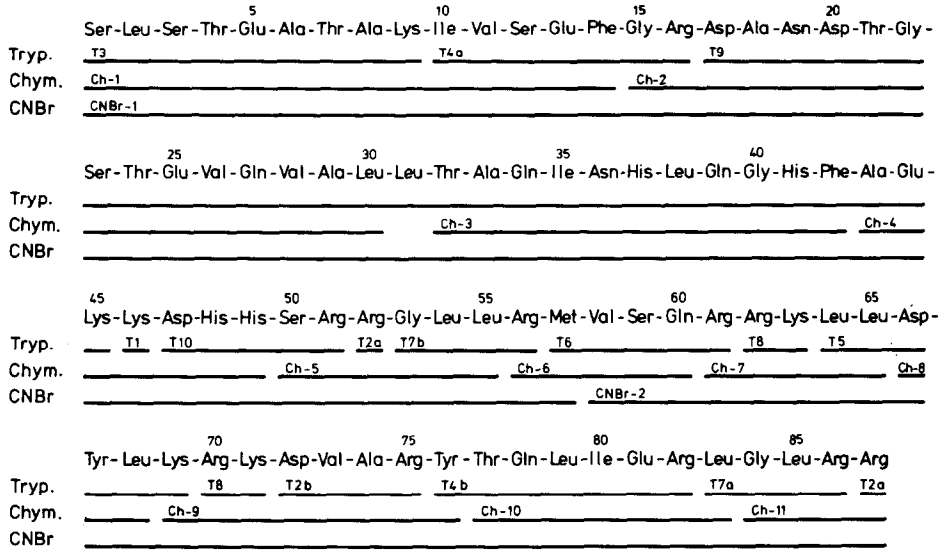

Fig.1. Primary structure of *E. coli* ribosomal protein S15. Tryp. = peptides from tryptic digestion. Chym. = peptides from chymotryptic digestion. CNBr = peptides resulting from CNBr cleavage.

T7a and T7b were separated on paper and identified by the yellow ninhydrin color of peptide T7b. The sequence of all tryptic peptides except peptide T9 which consists of 29 residues, were completely determined by manual Edman degradation.

The sequence of peptide T9 and the order of the tryptic peptides were determined using chymotryptic peptides. The digestion of protein S15 with chymotrypsin for four hours produced 11 peptides of which peptides Ch-2, -3, -4, -5, -9, -10, and -11 were sequenced. On the other hand, a shorter digestion (45 min) did not split the peptides bonds between Ch-1 and -2, Ch-4 and -5, Ch-8 and -9. Edman degradation was also performed with these 'bridge' peptides. Using the results from the chymotryptic peptides, the order of all tryptic peptides could be established as shown in fig.1.

In order to confirm this sequence, the protein was cleaved at the C-terminal site of the only methionine residue in S15 by treatment with cyanogen bromide and the resulting two CNBr-peptides were separated by gel filtration on Biogel P-30. The amino acid compositions of the two CNBr peptides and the tryptic peptides derived from the CNBr peptides agreed completely with the established sequence.

A noteworthy feature of protein S15 is that most of the basic amino acids occur in the C-terminal portion of the protein in a continuous sequence whereas the middle part of the protein contains many hydrophobic amino acids. This suggests that the C-terminal portion may be located on the surface of the molecule and may participate to the interaction with the 16S rRNA.

Two methods [8,9] for the prediction of secondary structure of proteins gave consistent results for the occurrence of α-helices in the following three regions of protein S15: positions 5–10, 29–35 and 41–47 (B. Wittmann-Liebold, personal communication).

Comparing the primary structure of protein S15 with the sequence of other *E. coli* ribosomal protein [10] gave the following: a region consisting of six amino acids (positions 31–36) of protein S15 has the same sequence as a hexapeptide in protein S20. Further, the sequence of the last five amino acids of S15 (positions 83–87) is identical to that in a pentapeptide in L30 (positions 26–30). Finally, three tetrapeptides in S15 (positions 6–9, 61–64 and 72–75) have the same sequence as positions 33–36 in L1, 15–18 in L18 and 55–58 in L29, respectively.

References

[1] Zimmermann, R. A. (1974) in: Ribosomes. Monograph Series. pp. 225–269. Cold Spring Harbor Laboratory, Long Island, N. Y.
[2] Kaltschmidt, E. (1971) Anal. Biochem. 43, 25.
[3] Wittmann-Liebold, B. (1973) FEBS Lett. 36, 247.
[4] Hindennach, I., Stöffler, G. and Wittmann, H. G. (1971) Eur. J. Biochem. 23, 7.
[5] Hayashi, R., Moore, S. and Stein, W. H. (1973) J. Biol. Chem. 248, 2296.
[6] Wittmann-Liebold, B. and Wittmann, H. G. (1971) Biochem. Biophys. Acta 251, 44.
[7] Iwanaga, S., Wallen, P., Gröndahl, N. J., Henschen, A. and Blombäck, B. (1969) Eur. J. Biochem. 8, 189.
[8] Chou, P. Y. and Fasman, G. D. (1974) Biochemistry 13, 222.
[9] Burgess, A. W., Ponnuswamy, P. K. and Scheraga, H. A. (1974) Israel J. Chem. 12, 239.
[10] Wittmann-Liebold, B. and Dzionara, M. (1976) FEBS Lett. 61, 14.