# THE PRIMARY STRUCTURE OF PROTEIN L16 LOCATED AT THE PEPTIDYLTRANSFERASE CENTER OF *ESCHERICHIA COLI* RIBOSOMES

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Received 28 June 1976

## 1. Introduction

Protein L16 from the large ribosomal subunit of *E. coli* binds directly to the 23S RNA [1]. Reconstitution experiments [2,3] and affinity labelling studies [4,5] demonstrated that L16 is involved in chloramphenicol binding and, thus, is located at the A-site of the peptidyltransferase center. Furthermore, affinity labelling experiments using modified Phe-tRNA [6,7] and inhibition experiments with antibodies [8] also showed that this protein is part of, or in the neighbourhood of, the peptidyltransferase center. Finally, from reconstitution experiments it was concluded that this protein may itself exert the peptidyltransferase activity [9]. The elucidation of the amino acid sequence of this functionally important protein is essential to obtain a further insight at the molecular level into those events which are associated with peptidyltransferase activity.

In determining the primary structure of L16 we have used refined micro techniques that have been developed in determining the primary structures of proteins S9, L18, L27 and L34 [10–13] and that have been described in detail elsewhere [14]. The analysis of protein L16 is a further example of the quality and feasibility of these micro techniques.

## 2. Materials and methods

Protein L16 was isolated from *E. coli* strain K as described previously [15] and was provided by Dr H. G. Wittmann. Enzymic digestions of the protein were performed as summarized in table 1. Hydrolyses of peptides with trypsin, chymotrypsin or thermolysin (Serva, Heidelberg) were carried out essentially as described for the whole protein (table 1) using 0.2 M *N*-methylmorpholine acetate buffer, pH 8.1. The thermolytic digestion lasted 45 min at 45°C.

Peptides obtained from different enzymic digestions were separated exclusively on cellulose thin layer plates (Polygram CEL 300, Macherey and Nagel, Dassel) by peptide mapping as described [14]. The locations of the peptides on the thin layer plates were detected by their reaction with ninhydrin solution or with fluorescamine (Roche, Basel) [18]. They were eluted from the cellulose plates with 5.7 N HCl containing 0.02% β-mercaptoethanol when used for amino acid analyses, or with 50% acetic acid for sequence determination by the micro dansyl-Edman technique. Peptides that had been protected from tryptic digestion by modification of their lysine residues [16] were prefractionated on a Sephadex G-50 superfine column (1 × 150 cm) equilibrated with 5% acetic acid before they were fingerprinted. Amino acid analyses of the peptides were generally performed in the 1–2 nmol range on a Durrum D-500 analyzer.

Sequence determination of the peptides was made exclusively using an improved micro-dansyl-Edman procedure [14] based on the techniques described by Gray and Hartley [19]. Polyamide thin layer plates (F 1700, Schleicher & Schüll, Dassel) were used to separate the dansyl amino acids. The amides of glutamic and aspartic acid were distinguished from the respective amino acids by converting the thiazolinones

Table 1
Enzymic digestion of protein L16

| Experiment | Enzyme and source | Amount of protein used | Enzyme/substrate (w/w) | Buffer | Time and temperature of cleavage |
|---|---|---|---|---|---|
| 1 | Trypsin TPCK treated | 2.0 mg | 1:100 | Water, adjusted with 0.05 $NH_4OH$ to pH 7.5 | 4 h; 37°C |
| 2 | Trypsin | 5.0 mg with lysine residues blocked by citraconic anhydride treatment (Pierce, Rotterdam) [16] | 1: 50 | 0.5% $NH_4HCO_3$ | 1 h; 37°C |
| 3 | Chymotrypsin TLCK treated (Merck, Darmstadt) | 0.35 mg | 1:100 | 0.2 M *N*-methylmorpholine acetate buffer pH 8.1 | 15 min; 37°C |
| 4 | *Staphylococcus aureus* protease (Miles, Slough) | 4.75 mg | 1: 30 | 0.05 M $CH_3COONH_4$ | 16 h; 37°C |
| 5 | Carboxypeptidase A (Boehringer, Mannheim) | 0.2 mg | 1: 20 | 0.2 M *N*-methylmorpholine acetate buffer, pH 8.1 | 10–120 min; 37°C |

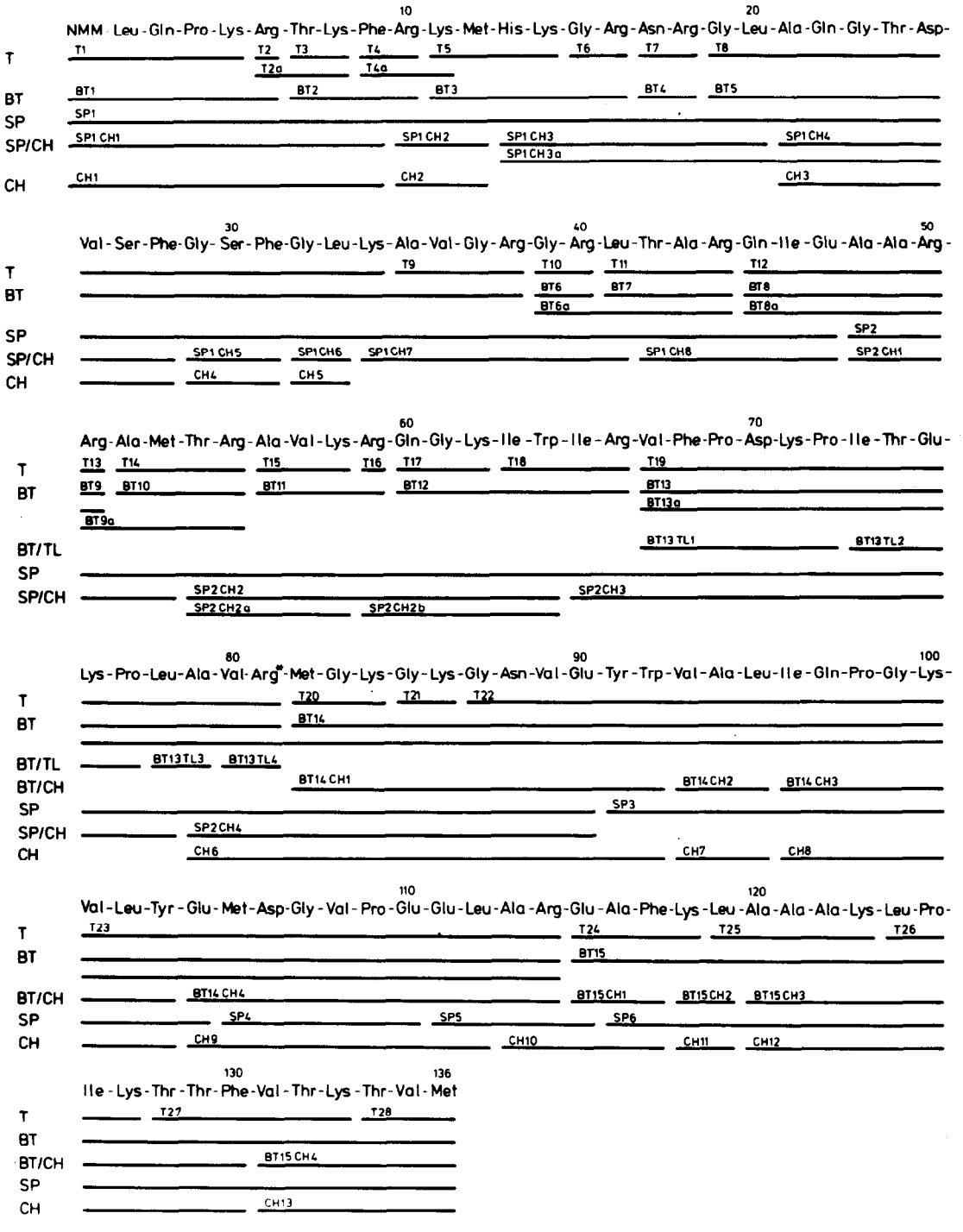


Fig.1. Amino acid sequence of ribosomal protein L16 (T) tryptic peptides, (BT) tryptic peptides obtained after blocking the lysine residues with citraconic anhydride, (BT/CH) BT peptides further digested with chymotrypsin, (BT/TL) BT-peptides further digested with thermolysine, (SP) peptides derived from cleavage with *Staphylococcus aureus* protease, (SP/CH) SP-peptides further digested with chymotrypsin, (CH) chymotryptic peptides, (Arg*) unusual amino acid related to arginine.

from the Edman degradation into the PTH-amino acids followed by chromatography as described elsewhere [14]. The same method was employed to determine tryptophan residues.

*N*-Methylmethionine was prepared by reductive methylation [20] of L-methionine (Calbiochem, Luzern). Arginine tests were carried out according to Yamada and Itano [21] and Sakaguchi [22].

## 3. Results and discussion

The tryptic peptides of L16 have been isolated and sequenced. Those peptides that could not be obtained pure enough directly from the tryptic hydrolysate of the protein were prepared from larger overlapping peptides isolated from a tryptic digest of L16 after modification of the lysine residues with citraconic anhydride [16]. Analysis of the lysine blocked peptides BT14 and BT15 revealed the alignment of the tryptic peptides T20–T23 and T24–28, respectively.

Carboxypeptidase A released Met, Val and Thr from the whole protein indicating that peptide BT15 was the C-terminal peptide. Peptides BT1–BT13 were sequenced in order to obtain further information about the order of the tryptic peptides and to confirm their sequences. Chymotryptic digestion of the whole protein was not very successful since it produced a large number of peptides of which only a few could be isolated in pure form and sufficient amount. Chymotryptic peptide CH10, however, was completely sequenced showing the position of BT15 behind BT14.

Digestion of protein L16 with *Staphylococcus aureus* protease produced only six peptides. The smaller ones, SP3, SP4, SP5 and SP6 were sequenced completely. In this way the amino acid sequence in the region of BT14–BT15 was confirmed. The determination of the amino acid sequences of the two larger peptides SP1 and SP2, however, was essential for the elucidation of the order of peptides BT1 to BT13. Due to their length, these peptides could only be partially sequenced. Therefore, peptide SP1 was digested with chymotrypsin and the resulting peptides isolated by fingerprinting. Sequence determinations and amino acid analyses finally revealed the amino acid sequence within SP1. This information enabled us to align peptides BT1–BT8. The order of peptides BT8–BT13 was determined by treating SP2 in the same way as SP1. Comparing the N-terminal residues of the protein with the SP-peptides it was clear that peptide SP1 was positioned at the N-terminus of the protein. Thus the complete amino acid sequence of L16 was established (fig.1). Additional peptides that had been isolated from different experiments confirmed the above sequence.

Recently N-monomethylated amino acids were observed at the N-termini of the two ribosomal proteins S11 (R. Chen and U. Arfsten, unpublished) and L33 [23]. Similarly we have identified *N*-monomethylmethionine as the N-terminal amino acid of L16. Further details will be reported elsewhere [25]. Besides *N*-monomethylmethionine a second unusual amino acid was observed in the amino acid analysis of protein L16. The compound was a constituent of peptide T19 and migrated in the amino acid analysis between ammonia and arginine. Its dansylated form migrated slightly above DNS-Arg on the micro polyamide plate in 0.05 M $Na_3PO_4$/ethanol (3:1). Trypsin digested the protein behind this unusual amino acid and both, Itano- and Sakaguchi-tests were positive, indicating that the amino acid was related to arginine. Further analyses are in progress and will be published elsewhere [24].

Table 2
Identical regions of protein L16 with other proteins from *E. coli* ribosomes

| Peptide | Protein | Positions | Protein | Positions |
|---|---|---|---|---|
| Glu–Ala–Ala–Arg | L16 | 47–50 | S4 | 72–75 |
| Leu–Ala–Ala–Ala | L16 | 119–122 | S13 | 33–36 |
| Leu–Ala–Arg–Glu | L16 | 112–115 | S21 | 59–62 |
| Asp–Val–Ser–Phe | L16 | 25–28 | L18 | 89–92 |
| Ala–Arg–Glu–Ala | L16 | 113–116 | L18 | 110–113 |
| Lys–Arg–Thr–Lys | L16 | 5–8 | L33 | 26–29 |

The amino acid composition derived from the sequence $Asn_2$, $Asp_3$, $Thr_9$, $Ser_2$, $Gln_5$, $Glu_7$, $Pro_7$, $Gly_{13}$, $Ala_{14}$, $Val_{11}$, $Met_5$, $Ile_6$, $Leu_{10}$, $Tyr_2$, $Phe_6$, $His_1$, $Lys_{16}$, $Trp_2$, $Arg_{13}$, $NMM_1$ and $Arg_1^*$ is in excellent agreement with the data determined from the total hydrolysis of the intact protein. The mol. wt. of 15 300 as calculated from the sequence is lower than the data obtained by SDS-gel electrophoresis [26,27], as has already been observed with other basic ribosomal proteins.

The secondary structure of protein L16 has been predicted using the methods of Burgess et al. [28] and Chou and Fasman [29,30]. The coincidence in the prediction of $\alpha$-helices in positions 45–56 and 110–124 by both methods strongly suggests that there are indeed $\alpha$-helical structures in the respective parts of the molecule. However, there is only little agreement in the prediction of $\beta$-sheet conformations. Tetrapeptides homologous to peptides in L16 are present within proteins S4, S13, S21, L18 and L33 (table 2), whereas identical tripeptides occur in almost every ribosomal protein [31].

## Acknowledgements

We would like to thank Dr H. G. Wittmann for his interest and support and Dr B. Wittmann-Liebold for provision of computer data including homologies and secondary structure.

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