THE PRIMARY STRUCTURE OF PROTEIN L15 LOCATED AT THE PEPTIDYL-TRANSFERASE CENTER OF *ESCHERICHIA COLI* RIBOSOMES

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1. Introduction

Protein L15 is a constituent of the large subparticle of the *Escherichia coli* ribosome [1]. Affinity labelling experiments using *p*-nitrophenylcarbamyl-phenylalanyl-tRNA as affinity analogue of the peptidyl-tRNA revealed that L15 is located at or close at the peptidyl-tRNA binding site of the peptidyl-transferase center [2]. When isolated by gentle procedures which avoided denaturing conditions protein L15 was found to form a specific protein–RNA complex with 23 S rRNA [3]. In experiments with the antibiotic erythromycin it was found that the drug binds to the isolated protein L15 in contrast to all other ribosomal components and that its binding to ribosomes is absolutely dependent on the presence of this protein [4].

In this communication the amino acid sequence of ribosomal protein L15 is reported. This knowledge will contribute to a deeper understanding of the experimental results mentioned above.

2. Materials and methods

Protein L15 was isolated from *E. coli* K (strain A19) as described [5] and was provided by Dr H. G. Wittmann. Its identity and purity were judged by one-dimensional dodecylsulphate gel electrophoresis [6] and by two-dimensional polyacrylamide gel electrophoresis [7].

2.1. *Enzymic digestions*

Protein L15 was digested with trypsin (substrate : enzyme ratio, 50 : 1, w/w) in 0.2 M *N*-methylmorpholine buffer, pH 8.1, for 4 h at 37°C. Digestion with chymotrypsin (50 : 1) was for 30 min at 37°C and with thermolysin (50 : 1) for 2 h at 50°C using 0.2 M *N*-methyl-morpholine buffer, pH 8.1, in both cases. Digestion with *Staphylococcus aureus* protease (50 : 1) proceeded for 16 h at 37°C in 0.05 M NH_4-acetate, pH 4 [8]. In some experiments the ϵ-amino groups of lysines were acylated with citraconic anhydride before L15 was digested with trypsin [9]. By protecting these residues against tryptic attack larger 'lysine-blocked' tryptic peptides were obtained.

2.2. *Isolation of peptides*

In order to obtain information on the number, size and charge of the peptides, 10 nmol of each L15 digest were fingerprinted on cellulose thin-layer plates (Polygram cell 300 or 400; Macherey and Nagel, Düren, FRG) [10]. In the first dimension electrophoresis was performed in pyridine/acetic acid/acetone/water (1 : 2 : 8 : 40), pH 4.4, at 480 V for 105 min. In the second dimension the chromatogram was developed in pyridine/*n*-butanol/acetic acid/water in the ratio of 50 : 75 : 15 : 60. After drying the plate thoroughly, it was sprayed with 0.3% ninhydrin solution to make the peptides visible. For preparative purposes amounts corresponding to approx. 20 nmol/peptide mixture were fingerprinted under the same conditions using purified cellulose thin-layer plates [10,11]. In addition, the plates were sprayed with diluted ninhydrin solution (0.1%) and stored overnight in the dark at room temperature in order to develop the stain.

Peptides used for amino acid analyses were extracted

from the cellulose by mixing vigorously in 5.7 N HCl which contained 0.02% β-mercaptoethanol. After removing the cellulose by centrifugation the peptides were hydrolysed for 20 h at 110°C. Peptides used for N-terminus determinations as well as sequence analyses were extracted from the cellulose in 50% acetic acid. They were centrifuged and dried before they were submitted to further analyses.

Peptides generated by *Staphylococcus aureus* protease treatment were purified on a Sephadex G-50f column (150 × 1 cm) in 5% acetic acid. 'Lysine-blocked' tryptic peptides of L15 were prefractionated on the same column and then repurified by fingerprinting on cellulose thin-layer plates. Fractions of 0.5 ml vol. were collected from the column. Every second fraction was examined for peptides by micro N-terminus determination [10,12] and by reacting the peptides with ninhydrin after aliquots had been chromatographed on cellulose thin-layer plates.

2.3. *Amino acid analyses*

Amino acid analyses of the peptides were performed on a Durrum D-500 amino acid analyser (Palo Alto, CA) in the sensitivity range 1–2 nmol according to [13]. The presence of tryptophan was investigated by spraying the tryptic peptide map with Ehrlich's reagent [14] and amino acid analyses of the oxidized protein were performed in order to determine cystein residues [15].

2.4. *Sequence determinations*

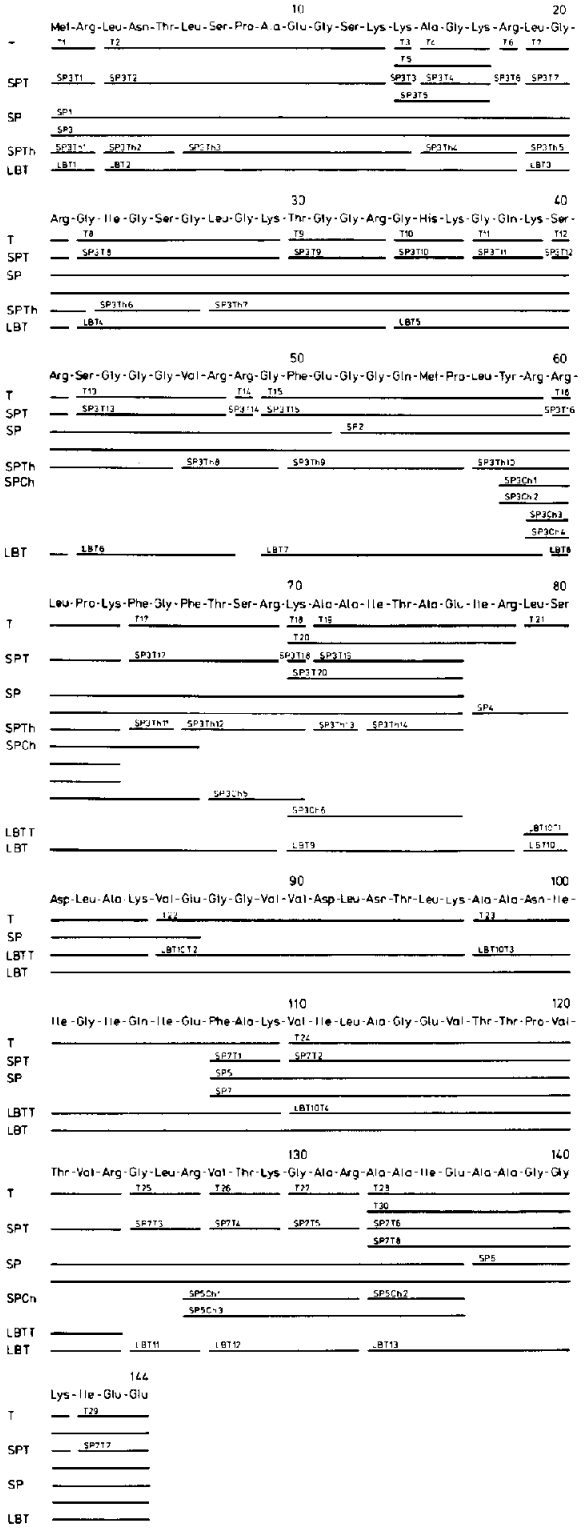
Sequence determinations of the peptides were done by the refined micro dansyl-Edman technique reported [10]. Before the degradation was initiated the peptides were transferred into 0.4 × 5 cm glass tubes, dried, redissolved in 50 μl glass-distilled water and dried again in order to remove traces of acetic acid. After each step of the analysis the new N-terminal residue was dansylated and identified on micro polyamide plates (F1700; Schleicher and Schüll, Dassel, FRG). Asparaginyl and glutaminyl residues were distinguished from their amides by the different mobilities of their phenylthiohydantoins on silica gel thin-layer plates and by their colour reaction with ninhydrin [10]. Edman degradation of the intact protein was performed in a modified Beckman sequencer as described [16].

3. Results and discussion

From the tryptic digest of the protein 21 peptides (T2, T3, T4, T5, T6, T8, T10, T11, T14, T16, T17, T18, T19, T20, T21, T22, T24, T26, T28, T29 and T30) were directly isolated in pure form from the thin-layer fingerprint. The remainder of the tryptic peptides were obtained from second digestions of 'lysine-blocked' tryptic peptides (LBT-peptides) and from peptides that were obtained from a *Staphylococcus aureus* protease treatment of L15 (SP-peptides). From the seven peptides originated by digestion of L15 with *Staphylococcus aureus* protease five of them (SP1, SP3, SP4, SP5 and SP7) were isolated in high purity from the Sephadex column. The other two peptides (SP2 and SP6) were obtained almost pure from the column.

The alignment of the tryptic peptides T1–T13 and information on their amino acid sequences were obtained from liquid-phase Edman degradation of the intact protein performed by Dr B. Wittmann-Liebold (personal communication). In addition, the order of eight tryptic peptides (T1–T8) was derived from peptide SP1 which could be sequenced for 23 cycles. Peptide SP2 was also sequenced up to residue 23 (i.e., position 73 of the protein sequence) giving the alignment of peptides T15–T16–T17–T18–T19.

Peptides SP3 and SP7 were digested with trypsin in order to obtain the missing tryptic peptides T1, T7, T9, T12, T13, T15, T25 and T27. Digesting peptide SP3 with thermolysin generated peptide SP3Th7 which was sequenced completely revealing the order to peptides T8–T9–T10–T11–T12–T13. The alignment of T13–T14–T15 resulted from the sequence of peptide SP3Th8. Peptides SP4 and SP6 were sequenced completely. Their sequences gave the order of peptides T20–T21–T22 and of peptides T28–T29. Peptide SP7 was sequenced for 22 cycles (up to position 128 of the protein sequence) revealing the alignment of peptides T23–T24–T25–T26. From peptide SP5 the chymotryptic peptide SP5Ch3 was isolated. This peptide covered the sequence of peptides T26–T27–T28. From the partial tryptic digest of L15 the 'lysine-blocked' tryptic peptide LBT10 was isolated in pure form from the Sephadex column. The remaining LBT-peptides were further purified by fingerprinting on cellulose thin-layer plates. LBT10 was sequenced for 29 cycles determining the order



of peptides T21–T22–T23. For confirmation of the sequence of peptide T23, which had not been isolated from the tryptic digest of L15, peptide LBT10 was digested with trypsin. From the hydrolysate peptide T23 was isolated by fingerprinting and then sequenced by the refined micro dansyl-Edman technique.

In fig.1 the complete amino acid sequence of ribosomal protein L15 is presented. The amino acid composition as derived from the sequence is Asn_3, Asp_2, Thr_9, Ser_7, Gln_3, Glu_9, Pro_4, Gly_{26}, Ala_{15}, Val_9, Met_2, Ile_{10}, Leu_{12}, Tyr_1, Phe_4, His_1, Lys_{13}, Arg_{14}. This is in excellent agreement with the amino acid analysis of the hydrolysed protein. Protein L15 has no tryptophan and no cysteine residues. Its molecular weight as calculated from the amino acid composition is 14 981.

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Fig.1. Amino acid sequence of ribosomal protein L15. T, tryptic peptides; LBT, 'lysine-blocked' tryptic peptides; LBTT, tryptic digestion of LBT-peptides; SP, peptides obtained from the *Staphylococcus aureus* protease digestion; SPCh, chymotryptic digestion of SP-peptides; SPT, tryptic digestion of SP-peptides; SPTh, thermolytic digestion of SP-peptides.

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