THE PRIMARY STRUCTURE OF PROTEIN L27 FROM THE PEPTIDYL-tRNA BINDING SITE OF *ESCHERICHIA COLI* RIBOSOMES

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1. Introduction

The ribosomal protein L27 is a constituent of the larger (50S) subunit of the *E. coli* ribosome. Studies with different affinity analogues of peptidyl-tRNA [1–3] led to the identification of protein L27 as one of the major products labelled with the analogue. Thus, protein L27 can be inferred to be very close to the peptidyl-tRNA binding site (P-site). In addition, antibodies against protein L27 inhibit the formation of 70S ribosomes [4]. This effect can be explained by the location of L27 at the interacting surface of the two subunits.

2. Materials and methods

2.1. *Protein and enzymes*

Protein L27 was prepared as previously described [5] and was provided by Dr H. G. Wittmann, TPCK-trypsin and TLCK-chymotrypsin were purchased from Merck (Darmstadt), *Staphylococcus* protease from Miles (Slough, UK), thermolysin from Serva (Heidelberg), citraconic anhydride from Pierce (Rotterdam), cellulose thin-layer plates, Polygram cel 300, from Machery and Nagel (Düren) and micro polyamide plates F 1700 from Schleicher and Schüll (Dassel).

2.2. *Peptide isolations*

5.7 mg of protein was digested with trypsin under standard conditions. The resulting peptides were then purified by passage through a 0.6 × 10 cm phosphocellulose microcolumn. In addition, 4.5 mg of protein was pretreated with citraconic anhydride [6] before being submitted to the tryptic digestion. A Sephadex G-25f column was used to remove the excess reagent. After the tryptic digestion the peptides were prefractionated through a Sephadex G-50f column which resulted in two main fractions. One of them was chromatographed through a micro Dowex 50W × 7 [7] column while the other was chromatographed through a micro phosphocellulose column. Furthermore 8 mg of protein was cleaved at the glutamic acid residues by means of the specific *Staphylococcus* protease [8]. The hydrolyzate was then passed through a Sephadex G-50f (1 × 150 cm) column which was equilibrated with 36% acetic acid.

The size of all fractions taken from the Sephadex and the ion exchange columns was 500 μl. Fractions eluting from the Sephadex columns were monitored at 280 nm. In addition, 40 μl aliquots of every other fraction were spotted onto thin-layer plates and chromatographed in a system containing pyridine, *n*-butanol, acetic acid and water in the ratio of 50:75:15:60 (v/v). The peptides were detected by the ninhydrin reaction. The cellulose thin-layer/ninhydrin procedure was also used to detect the peptides isolated from ion exchange columns [7].

Peptide mixtures obtained by chymotryptic and thermolytic hydrolyzation of peptide SP3 were analyzed by fingerprinting on cellulose thin layer plates. Before the samples (6 nmol each) were applied, the thin-layer plates were purified by chromatography in 5% pyridine followed by chromatography in 1.5% formic acid. The peptides isolated under these conditions were used for both amino acid analyses as well as sequence determinations by the Dansyl-Edman technique.

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2.3. *Amino acid analyses*

The analyses were performed on a Durrum D-500 analyzer (Palo Alto, California). The sensitivity range of 0.5 *A* was generally used.

2.4. *Sequence determinations*

The amino acid sequences of all the peptides were determined by the Dansyl-Edman technique [9]. With the exception of one large peptide (SP2) which was sequenced using a 50 nmol sample, all determinations were performed with less than 10 nmol samples. The technique for the identification of the amide side chains from aspartyl- and glutamyl residues will be published elsewhere.

3. Results and discussion

We have perfected the technique for the manual sequence determination on a micro scale during the

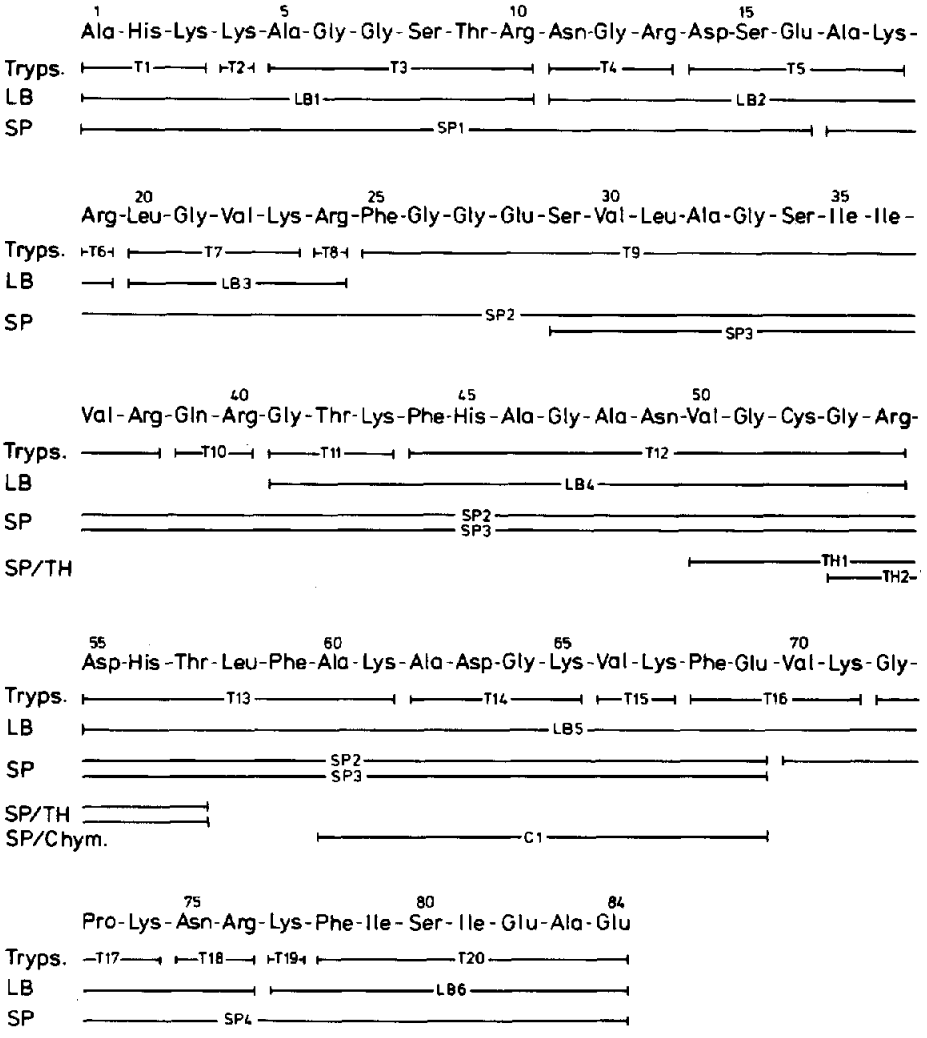

Fig.1. The amino acid sequence of protein L27 from the *E. coli* ribosome. Tryps. = tryptic peptides, LB = tryptic peptides obtained after the protein was modified with citraconic anhydride. SP = peptides from *Staphylococcus* protease digestion. SP/TH = thermolytic peptides from peptide SP3. SP/Chym = chymotryptic peptide from peptide SP3.

determination of the amino acid sequences of ribosomal proteins S9 [10]. S11 [11] and L18 [12]. This technique has enabled us to establish the primary structure of L27 using approximately 24 mg of protein.

After tryptic digestion, we have isolated 21 peptides by the phosphocellulose micro column procedure. However, tryptic peptide T9, which is 14 amino acids long, has not been detected either by ion exchange chromatography or by fingerprinting. A possible explanation is that peptide T9 adsorbs onto glass during the isolation procedure. The amino acid sequence of the protein region containing peptide T9 was determined as described below. All other tryptic peptides were completely sequenced by the Dansyl-Edman technique.

Six peptides were isolated from the tryptic hydrolyzate of a protein L27 sample in which the lysine residues were modified with citraconic anhydride. Five of these peptides were sequenced. The N-terminal region of peptide LB2 (fig.1) contains one arginine which was not cleaved by trypsin. The sixth peptide, LB5, which consist of 22 amino acids and which has an N-terminal aspartic acid, was inaccessible to the manual degradation. By contrast, peptide T13, which comprises the N-terminal part of LB5, could be sequenced without any difficulties. Table 1 gives the compositions of all lysine blocked peptides and of the tryptic peptides within them.

After the digestion of protein L27 with *Staphylococcal* protease, four peptides were isolated and sequenced. The amino acid sequence of peptide SP1 ensured the alignments of the tryptic peptides T1–T2–T3–T4. The alignment of peptides T1 to T10 was independently obtained by Dr B. Wittman-Liebold by the automatic Edman degradation of protein L27 [13]. Peptide SP2 was sequenced until its 27th amino acid residue by the Dansyl-Edman technique. In this case 50 nmol of peptide was necessary. The result gave us the order of peptides T5–T6–T7–T8–T9–T10–T11. Moreover, the sequence of peptide T9, which could not be isolated, was determined in this way since it is part of peptide SP2. The sequence analysis of peptide SP3 which extends from position 29 to position 69, confirmed the amino acid sequence of T9.

Table 1
Determination of the order of tryptic peptides

Lysine blocked peptides	Tryptic peptides
LB1	T1–T2–T3
LB2	T4–T5–T6
LB3	T7–T8
LB4	T11–T12
LB5	T13–(T14,T15,T16,T17)–T18
LB6	T19–T20

From the sequence analysis of peptide LB1 it follows that the order of tryptic peptides is T1–T2–T3. Analysis of peptides LB2–LB6 resulted in the order of the tryptic peptides as given in table 1. Further information about the alignment of the tryptic peptides was obtained by sequence analysis of peptides SP1–SP4 as described in the text.

Chymotryptic peptides obtained from the digestion of peptide SP3 were isolated by fingerprinting on cellulose thin layer plates. The peptide of interest, C1, was completely sequenced by the Dansyl-Edman technique resulting in the order of peptides T13–T14–T15–T16. The alignment of peptides T12–T13 was determined by peptides TH1 and TH2 which were obtained from thermolytic cleavage of peptide SP3. The alignment of peptides T16–T17–T18–T19–T20 was obtained by sequencing peptide SP4.

Further details of the experimental data will be published elsewhere. The complete amino acid sequence of protein L27 is presented in fig.1. It is fully consistent with the results from the amino acid analyses of the whole protein.

The mol. wt of protein L27 is 8993. According to the method of Chou and Fasman [14,15] 35% α-helical regions (positions 1–6, 55–71 and 78–84), and 11% β-sheet structure (positions 30–39) can be expected.

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