THE PRIMARY STRUCTURE OF PROTEIN L34 FROM THE LARGE RIBOSOMAL SUBUNIT OF *ESCHERICHIA COLI*

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1. Introduction

The determination of the primary structure of *E. coli* ribosomal proteins makes rapid progress. More than 40% of the approx. 8000 amino acids within the *E. coli* ribosome have so far been sequenced. Comparison of the primary structures revealed homologous regions among some ribosomal proteins [1].

In this paper we report the complete amino acid sequence of protein L34 which is the smallest and most basic protein within the large subunit of the *E. coli* ribosome. Its amino acid sequence shows some degree of sequence homology with at least four proteins from both the small and the large subunits.

2. Materials and methods

Protein L34 has been isolated according to the method described by Alakhov et al. [2]. From 2 g of total protein from *E. coli* K 50S subunits 4 mg of protein L34 was obtained. Its identity and purity were shown by two-dimensional polyacrylamide electrophoresis [3]. TPCK-trypsin and chymotrypsin were purchased from Merck (Darmstadt), thermolysin from Serva (Heidelberg), *N*-formyl-methionine from Sigma (St. Louis), *N*-acetyl-methionine from Serva (Heidelberg), carbamylmethionine was prepared by the cyanate procedure [4], cellulose thin-layer plates Polygram Cel 300 or 400 were purchased from Macherey and Nagel (Düren) and micro polyamide plates F1700 from Schleicher and Schüll (Dassel).

Protein L34 was digested with trypsin, thermolysin and chymotrypsin under conventional conditions. For one fingerprint 10 nmol of each digest was spotted onto a prepurified cellulose thin-layer plate and electrophoresed at pH 4.4 for 105 min [5]. The chromatography in the second dimension was in a system containing pyridine: *n*-butanol:acetic acid:water in the ratio of 50:75:15:60 (by vol). The peptides were localized on the thin-layer plate by the ninhydrin colour reaction. For amino acid analyses as well as N-terminal determinations the fingerprints were sprayed with 3% ninhydrin solution, while 1% was used, when the peptides were meant for sequence determinations [5].

All peptide isolations were done by fingerprinting on cellulose thin layer plates. The amino acid analyses were performed on a Durrum D-500 analyzer (Palo Alto, California). The sensitivity of 0.5 A was generally used. The amino acid sequences of all tryptic and thermolytic peptides as well as one chymotryptic peptide were determined completely by the micro Dansyl-Edman technique [6–8]. They were sequenced on 5–10 nmol per sample. The technique for the identification of aspartyl- and glutamyl residues has previously been used [5,9,10] and will be published elsewhere in detail [8].

3. Results and discussion

Approx. 3 mg of lyophilized material was used to establish the amino acid sequence of protein L34.

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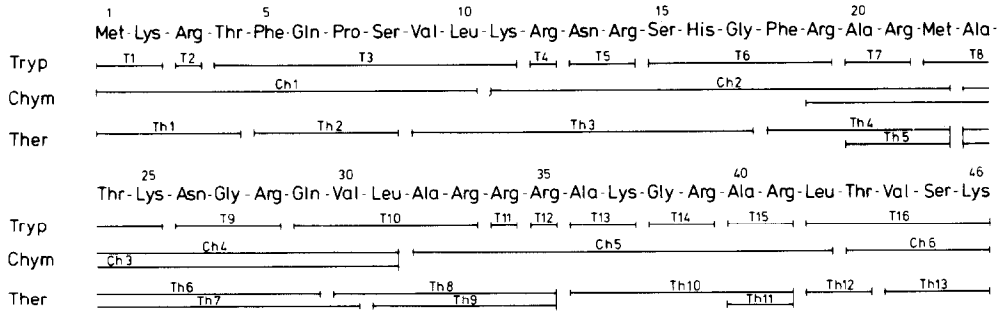

Fig.1. Amino acid sequence of protein L34 from *E. coli* ribosomes. Tryp = tryptic digestion; Chym = chymotryptic digestion; Ther = thermolytic digestion.

This was mainly due to refined techniques [5,8], which allowed us to work on small quantities. As a standard procedure, two fingerprints were developed on each enzymatic hydrolyzate of L34. One was used for amino acid analyses and N-terminal determinations, while the other was meant for preparative isolations of the peptides from which the amino acid sequences were determined. 14 ninhydrin positive spots were identified on the tryptic fingerprint of L34, of which 11 contained pure peptides, comprising T1,T3,T6,T7,T8,T10,T13,T15,T16. The amino acid sequence within these tryptic peptides was determined by the Dansyl-Edman technique. A carbamylated form of peptide T1 was found which was not accessible to Edman degradation.

The thermolytic peptides Th1–Th13 were isolated and all of them (except Th1) were completely sequenced. Fig.1 shows how the alignment of the tryptic peptides was deduced from the thermolytic peptides. However, no overlapping thermolytic peptide was isolated which gave the order of T12 to T13 and T15 to T16. Therefore, L34 was digested with chymotrypsin which gave rise to peptide Ch5 among others. This peptide was sequenced completely demonstrating the position of T13 to be behind T12 and T16 behind T15. The C-terminal amino acid of L34 was determined as lysine by carboxypeptidase B digestion. Carboxypeptidase A + B released Lys, Ser, Val from the protein and thus showed that peptide T16 was the C-terminal tryptic peptide.

The amino acid composition of L34 was determined as Asx_2, Thr_3, Ser_3, Glx_2, Pro_1, Gly_3, Ala_5, Val_3, Met_2, Leu_3, Phe_2, His_1, Lys_5, Arg_{11}. This result is in complete agreement with the amino acid sequence presented in fig.2. From the sequence the mol. wt. was calculated as 5380 which is lower than the data reported earlier [2,11]. A similar disagreement was found with other small ribosomal proteins [12–14] which is probably caused by the inaccuracy of the SDS gel method for very basic and small proteins.

When compared to other ribosomal proteins of which the sequences have been so far studied, sequence homologies are found between L34 and S21 [13], L4 [15], L18 [10], L27 [5]. The greatest degree of

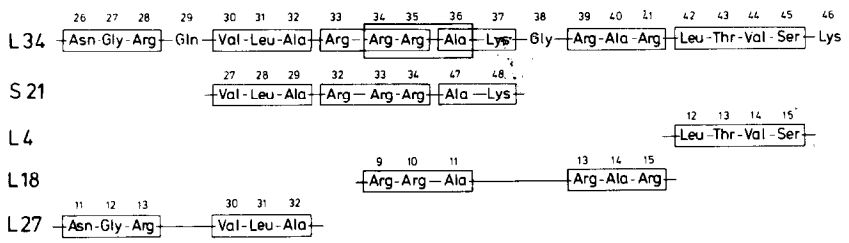

Fig.2. Homologous sequences between protein L34 and protein S21, L4, L18 and L27.

homology is observed with the C-terminal half of L34, comprising 20 amino acids from position 26 to 45: eight amino acids of S21, four amino acids of L4, six amino acids of L18 and six amino acids of L27 can be placed parallel to the respective sequence, indicating that there exists a family of ribosomal proteins with some identical regions.

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