THE PRIMARY STRUCTURE OF PROTEIN L30 FROM *ESCHERICHIA COLI* RIBOSOMES

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1. Introduction

Protein L30 from the 50S ribosomal subunit of *E. coli* is located near the GTPase-center of the particle, as shown by studies with a GDP photo-affinity label [1] and by the presence of L30, although in small amount, in the 5S RNA-protein complex [2], which has GTPase and ATPase activity [3]. A knowledge of the primary structure may allow further insight into the role of this protein.

2. Materials and methods

The protein L30 was isolated from *E. coli* according to procedures previously described [4]. About 2 μmol of protein were digested by trypsin. The trypsin peptides were separated either on a SE-cellulose column [5] followed by chromatography on a column (0.2 × 5 cm) of Dowex M71 [6] or on Sephadex G-15 followed by a small SE-cellulose column (0.3 × 30 cm). Amino acid analyses were performed using either a Beckman Multichrom 4255 or a Durrum D-500 analyzer. The intact protein was sequenced according to Edman and Begg [7] using an improved Beckman protein sequencer [8]. The sequences of the tryptic peptides were determined by the Dansyl-Edman technique [9]. It was possible to discriminate between Asp/Asn and Glu/Gln by electrophoresis of the tryptic peptides [10] or by thin-layer chromatography of the PTH-amino acids.

In order to obtain overlapping peptides the lysine residues were blocked by ETPA (exo-*cis*-3,6-endoxo-4-tetra-hydrophthalic acid anhydride) [11] and the modified protein was digested by trypsin, in which case cleavage occurred after the arginine residues only. After deblocking, these peptides were purified by chromatography on Dowex M71 or by the fingerprint technique. In addition, the protein was cleaved by thermolysin, chymotrypsin, Staphylococcus protease [12] and with CNBr [13]. The resulting peptides were separated by the fingerprint technique. Full details will be given later.

3. Results and discussion

The primary structure of protein L30 is shown in fig.1. It has the composition Asp_1 Asn_1 Thr_6 Ser_2 Glu_4 Gln_1 Pro_2 Gly_5 Ala_5 Val_4 Met_2 Ile_6 Leu_5 Phe_1 His_2 Lys_5 Arg_6. This is in very good agreement with the results of the total hydrolysis. Cysteine and tryptophan are absent, which was confirmed by the appropriate methods for their detection [14,15]. Protein L30 contains 58 amino acids and its mol. wt is 6410.

The sequence up to position 23 was determined by the sequenator and was completed by isolation and analysis of all tryptic peptides. Cleavage with trypsin resulted in 14 peptides, two of which (T1 and T2) contained no arginine or lysine. The determination of the sequences of the peptides T2 and T11 showed that they were part of the peptides T3. The cleavage after phenylalanine in position 52 was occasionally observed after digestion with trypsin or with Staphylococcus protease.

The tryptic peptides were aligned using the information obtained by the analyses of the peptides isolated after cleavage with other enzymes and with CNBr (fig.1). All the peptides found are in complete agreement with the given sequence. The sequence of the C-terminal region was confirmed by sequencing

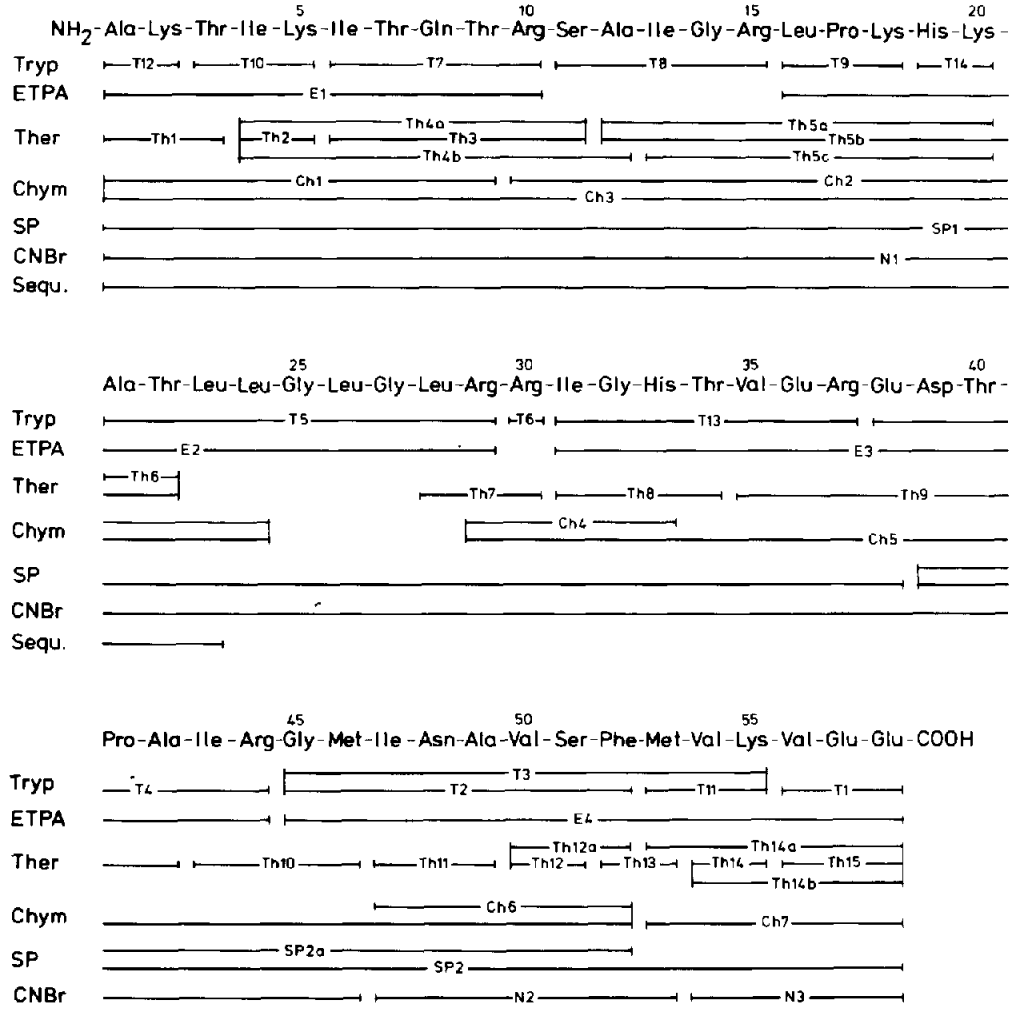

Fig.1. Amino acid sequence of protein L30 from *E. coli* ribosomes. Tryp = tryptic peptides. Ther = thermolysin peptides. Chym = chymotryptic peptides. ETPA = tryptic peptides after blocking the lysines with ETPA. SP = peptides from digestion with *Staphylococcus* protease. CNBr = peptides from cleavage with CNBr. Sequ = sequence region elucidated by the sequenator.

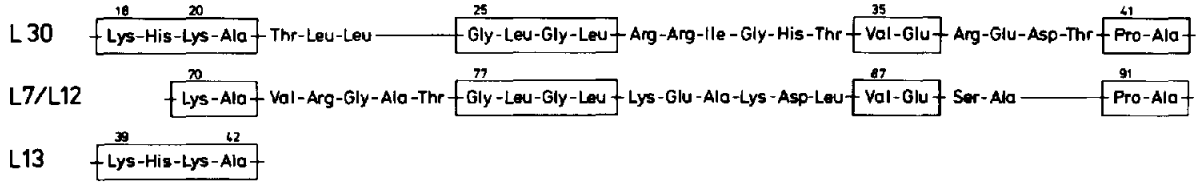

Fig.2. Comparison of homologous regions of the primary structures of proteins L7/L12, L13 and L30.

the overlapping peptide E4. After modification of the lysine residues by ETPA, only partial cleavage after arginine in position 37 with trypsin was observed, in contrast to tryptic digestion of the native protein in which cleavage at this point was complete.

There are some similarities in the primary structure of the proteins L7/L12, L13 en L30 from the 50S subunit as illustrated in fig.2. Several homologous regions among *E. coli* ribosomal proteins occur much more frequently than can be expected on a random basis [16].

Helical and β-sheet regions were predicted according to the method of Chou and Fasman [17,18]. The protein. is proposed to contain 31% α-helix (in the regions 17–26 and 33–40) and 43% β-sheet (from the N-terminus to position 10 and position 42–56. However, α-helix and β-sheet structure cannot be unequivocally assigned to certain regions of the protein, because they contain both high α-helix and β-sheet forming potential.

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