THE PRIMARY STRUCTURE OF PROTEIN L13 FROM THE LARGE SUBUNIT OF *ESCHERICHIA COLI* RIBOSOMES

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1. Introduction

Protein L13, which is a constituent of the large *Escherichia coli* ribosomal subunit, seems to have contact points with widely separated regions of the 23 S RNA within the 50 S subunit. This conclusion follows from experiments in which protein L13 was found to be crosslinked to both the 13 S RNA and the 18 S RNA fragments of the 23 S RNA [1]. In agreement with this finding are results which were obtained when single proteins [2] or groups of proteins [3] were bound to each of the two RNA fragments. In the first case protein L13 was found to bind to the 18 S RNA [2] and in the second case to the 13 S RNA [3].

Studies on the 50 S assembly in vitro revealed that protein L13 is one of the early assembly proteins which are essential for the formation of the RI^{*}_{50} intermediate particle. Omission of L13 prevents the formation of this intermediate and of the 50 S particle [4].

Mutants with an altered L13 protein have been found by isolating revertants from an *E. coli* strain which shows a novel type of streptomycin dependence [5]. Hopefully, the mutants with altered L13 will facilitate the mapping of the structural gene for L13 whose location on the *E. coli* chromosome is not yet known.

In this paper the complete primary structure of protein L13 is presented, and it is compared to the amino acid sequences of *E. coli* ribosomal proteins of known primary structures. Furthermore, predictions based on four different computer programmes are made for the secondary structure of protein L13.

2. Materials and methods

Protein L13 was isolated from *E. coli* strain K as in [6]. The protein was provided by Dr H. G. Wittmann; its purity was tested by two-dimensional polyacrylamide gel electrophoresis [7].

Enzymatic digestions were performed with trypsin, treated with 1-chloro-4-phenyl-3-tosylamidobutan-2-one (Merck, Darmstadt or Worthington Corp., Freehold, NJ), at pH 8.1 for 4 h at 37°C; with thermolysin (Serva, Heidelberg) at pH 8.1 for 90 min at 50°C; or with *Staphylococcus aureus* protease (Miles Biochemicals, Elkhart) at pH 4 for 40 h or 26 h at 37°C [8]. The intact protein was treated with carboxypeptidase C (Boehringer, Mannheim) at pH 5.3 for up to 1 h at 50°C. The enzymatic methods are detailed in [9–11].

Peptides with C-terminal arginine residues were obtained by first modifying the lysine residues either with citraconic anhydride (Pierce, Rotterdam) or with ETPA (exo-*cis*-3,6-endoxo-Δ^4-tetrahydrophthalic acid anhydride) [12,13] and then digesting with trypsin. The thermolysin peptides and most of the tryptic peptides were isolated by peptide mapping on thin-layer Polygram Cel 300 and Cel 400 sheets (Macherey and Nagel, Düren). Peptides on the thin-layer plates were detected with ninhydrin or with fluorescamine solution (Roche, Basel). The peptides were extracted with 5.7 N HCl containing 0.02% mercaptoethanol for analysis of their amino acid composition or with 50% acetic acid for the sequence determination. In addition tryptic peptides were

separated on a 0.6 × 10 cm phosphocellulose ion-exchange column with pyridine–acetate gradients [14].

Peptides derived by tryptic digestion after blocking the lysine residues (LB peptides) and peptides derived by cleavage with *Staphylococcus aureus* protease (SP peptides) were separated on Sephadex G-50 (superfine) columns (1 × 150 cm) equilibrated with 5% or with 10% acetic acid.

Amino acid analyses of the protein and peptides were performed on a Durrum D-500 analyzer (Palo Alto, CA). Cysteine was determined as cysteic acid after performic acid oxidation of the protein. For the detection of tryptophan peptide maps were developed with Ehrlich's reagent (*p*-dimethylaminobenzaldehyde).

The amino acid sequences of the peptides were determined by the dansyl-Edman procedure based on the techniques in [15] as modified [15a] and by an analogous method using 4-*NN*-dimethylaminoazobenzene-4′-isothiocyanate (DABITC) in combination with phenylisothiocyanate based on the techniques in [16]. The DABITC reagent was purchased from Fluka AG, Buchs CH.

Polyamide thin-layer plates (F 1700, Schleicher and Schüll, Dassel) were used to detect both the dansyl amino acids and the coloured 4-*NN*-dimethylaminoazobenzene-4-thiohydantoins.

The identification of aspartic acid, glutamic acid and their amides as well as tryptophan was achieved by converting the thiazolinones from the Edman degradation to the PTH-amino acids followed by thin-layer chromatography [15a]. To distinguish isoleucine from leucine the phenylthiohydantoins derived from DABITC degradation were applied to silica-gel thin-layers and detected after chromatography [17]. If the identification of the dansylated amino acids was impossible the free amino acid liberated from the thiazolinone derivative by back hydrolysis was determined on an amino acid analyzer. In addition the N-terminal sequence was determined by automatic liquid-phase Edman degradation of the intact protein [18].

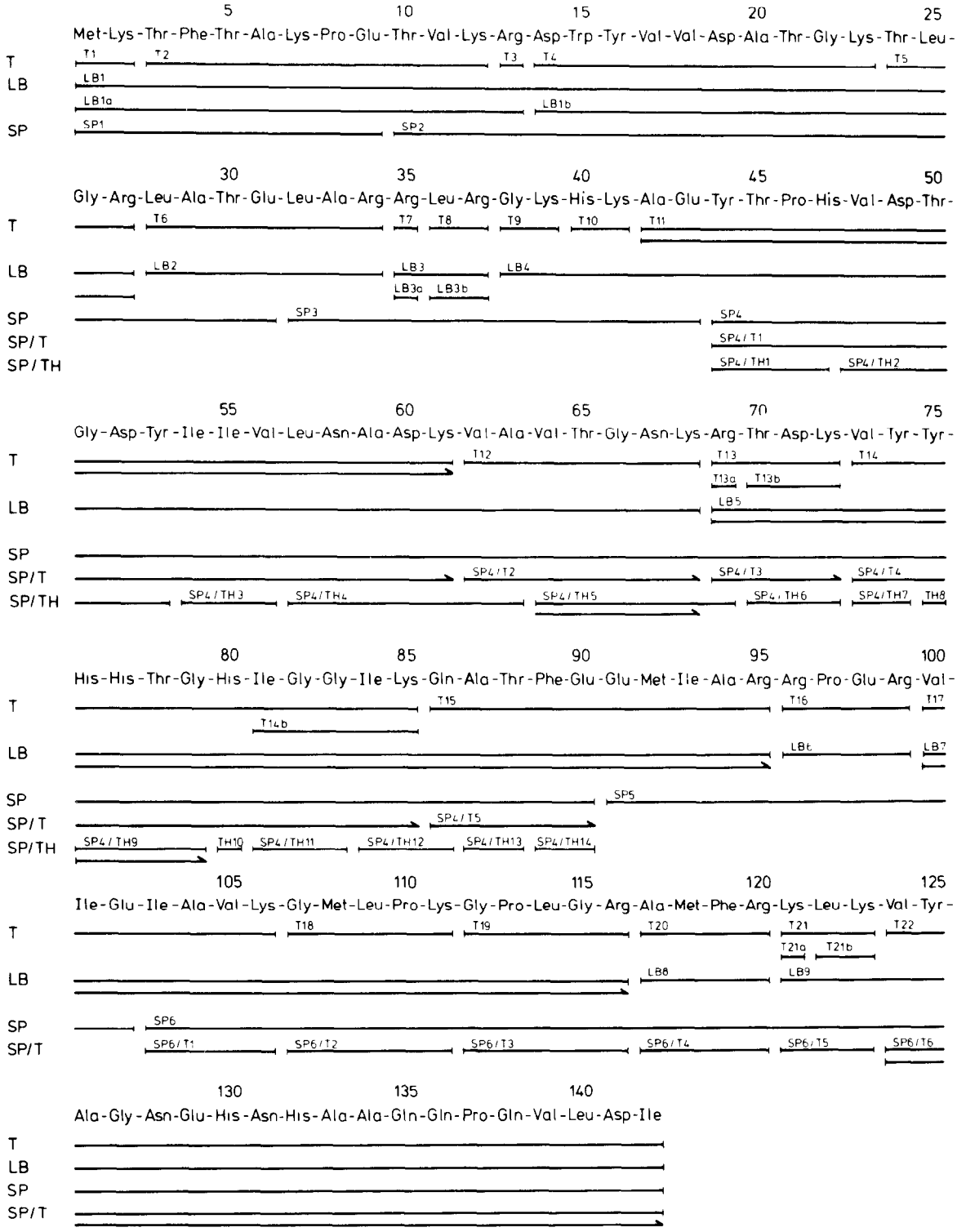
3. Results and discussion

Three main digestions of protein L13 were made:

1. Tryptic digestion: Twenty-two tryptic peptides (T1–T22) were isolated either from the phosphocellulose micro column or from peptide maps. The sequence of all peptides was determined. Non-specific partial cleavage appeared reproducibly in peptide T14 between residue 80 and 81. The very hydrophobic part Ile–Ile–Val–Leu in peptide T11 could only be sequenced with the DABITC method. Peptides T14 and T22 were also sequenced with the DABITC method to confirm the histidine, aspartic acid, glutamic acid, asparagine and glutamine positions.
2. Tryptic digestion after modifying the lysine residues either with citraconic anhydride or with ETPA: Nine LB-peptides (lysine-blocked peptides) were isolated from Sephadex G-50 (superfine) columns. Only partial tryptic cleavage occurred in peptide LB1, between residues 13 and 14. Peptide LB4 was sequenced up to the 29th step (residue 38–66), except that the very hydrophobic part, residue 54–57, was not determined. Peptides LB6 and LB8 were sequenced with the DABITC method as well as with the dansyl-Edman method.
3. Digestion with *Staphylococcus aureus* protease: Six SP-peptides were obtained after chromatography on Sephadex G-50 (superfine) columns. Cleavage did not occur between Glu–Arg, residues 98–99, and Glu–His, residues 129–130. Owing to its length, peptide SP4 (44–91) was digested with trypsin and with thermolysin for the determination of its structure. Another long peptide (SP6: 103–142) was also digested with trypsin. The alignment of the tryptic peptides was given through the sequences of the LB and SP peptides (see fig.1).

Carboxypeptidase C treatment of L13 liberated Ile, Asx and Leu from the C-terminus of the protein. L13 consists of 142 amino acid residues with the amino acid composition Asp_7 Asn_4 Thr_{12} Glu_8 Gln_4 Pro_6 Gly_{12} Ala_{14} Val_{12} Met_4 Ile_8 Leu_9 Thr_6 Phe_3 His_7 Lys_{14} Arg_{11} Trp_1. The amino acid composition obtained

Fig.1. Amino acid sequence of ribosomal protein L13. (T) tryptic peptides, (LB) tryptic peptides obtained after blocking the lysine residues, (SP) peptides derived from cleavage with *Staphylococcus aureus* protease, (SP/T) SP peptides further digested with trypsin, (SP/TH) SP peptides further digested with thermolysin.


5 10 15 20 25
Met-Lys-Thr-Phe-Thr-Ala-Lys-Pro-Glu-Thr-Val-Lys-Arg-Asp-Trp-Tyr-Val-Val-Asp-Ala-Thr-Gly-Lys-Thr-Leu-
T
LB
SP
T1 T2 T3 T4 T5
LB1 LB1a LB1b
SP1 SP2
30 35 40 45 50
Gly-Arg-Leu-Ala-Thr-Glu-Leu-Ala-Arg-Arg-Leu-Arg-Gly-Lys-His-Lys-Ala-Glu-Tyr-Thr-Pro-His-Val-Asp-Thr-
T
LB
SP
SP/T
SP/TH
T6 T7 T8 T9 T10 T11
LB2 LB3 LB4 LB3a LB3b
SP3 SP4 SP4/T1 SP4/TH1 SP4/TH2
55 60 65 70 75
Gly-Asp-Tyr-Ile-Ile-Val-Leu-Asn-Ala-Asp-Lys-Val-Ala-Val-Thr-Gly-Asn-Lys-Arg-Thr-Asp-Lys-Val-Tyr-Tyr-
T
LB
SP
SP/T
SP/TH
T12 T13 T14 T13a T13b
LB5
SP4/T2 SP4/T3 SP4/T4
SP4/TH3 SP4/TH4 SP4/TH5 SP4/TH6 SP4/TH7 TH8
80 85 90 95 100
His-His-Thr-Gly-His-Ile-Gly-Gly-Ile-Lys-Gln-Ala-Thr-Phe-Glu-Glu-Met-Ile-Ala-Arg-Arg-Pro-Glu-Arg-Val-
T
LB
SP
SP/T
SP/TH
T15 T16 T17 T14b
LB6 LB7
SP5 SP4/T5
SP4/TH9 TH10 SP4/TH11 SP4/TH12 SP4/TH13 SP4/TH14
105 110 115 120 125
Ile-Glu-Ile-Ala-Val-Lys-Gly-Met-Leu-Pro-Lys-Gly-Pro-Leu-Gly-Arg-Ala-Met-Phe-Arg-Lys-Leu-Lys-Val-Tyr-
T
LB
SP
SP/T
T18 T19 T20 T21 T22 T21a T21b
LB8 LB9
SP6 SP6/T1 SP6/T2 SP6/T3 SP6/T4 SP6/T5 SP6/T6
130 135 140
Ala-Gly-Asn-Glu-His-Asn-His-Ala-Ala-Gln-Gln-Pro-Gln-Val-Leu-Asp-Ile
T
LB
SP
SP/T

	Protein	Positions	Protein	Positions
Thr-Gly-Asn-Lys	L13	65-68	L18	65-68
Glu-Leu-Ala-Arg	L13	31-34	L16	111-114
Ala-Thr-Gly-Lys	L13	20-23	L24	75-78
Arg-Arg-Pro-Glu	L13	95-98	L6	151-154
Val-Thr-Gly-Asn	L13	64-67	L28	12-15
Lys-Val-Ala-Val	L13	61-64	L7/L12	65-68
Lys-Val-Tyr-Ala	L13	123-126	S20	33-36
Lys-Leu-Lys-Val	L13	121-124	S8	30-33
Lys-His-Lys-Ala	L13	39-42	L30	18-21
Arg-Leu-Ala-Thr	L13	27-30	L10	125-128
Arg-Arg-Leu-Arg	L13	34-37	L25	18-21
Asn-Lys-Arg-Thr	L13	67-70	L33	25-28

Fig.2. Sequence correlations of ribosomal protein L13 with other proteins from *E. coli* ribosomes.

from the sequence is in good agreement with the results of the amino acid analysis obtained from the intact protein. The mol. wt 16 019 as calculated from the sequence is somewhat lower than that obtained by SDS–gel electrophoresis [19] as has also been observed with other ribosomal proteins. The complete primary structure of L13 is shown in fig.1.

The secondary structure of protein L13 was predicted by four different methods [20–22]. According to these calculations L13 contains 25.4% α-helical regions, 7.8% extended or β-sheet structure and 22.5% turns (or loops). The comparison of the sequence of L13 with other ribosomal proteins of *E. coli* revealed a series of eleven identical tetrapeptides, which are summarized in fig.2. One pentapeptide of L13 was identical with one peptide of protein S9. It was shown that the occurrence of identical peptides of this length can be explained on a random basis [23].

Most of the peptides were sequenced with manual dansyl-Edman technique; a great help for sequencing hydrophobic peptides and peptides containing histidine, tryptophan, aspartic acid, glutamic acid or their amides was the DABITC method, which is very efficient: Peptide T22 was sequenced to the 19th step with 8 nmol peptide, and peptide SP4 was sequenced up to the 26th step with 20 nmol peptide. It is striking that L13 has no serine but 7 histidines.

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