THE PRIMARY STRUCTURE OF *BACILLUS SUBTILIS* ACIDIC RIBOSOMAL PROTEIN B-L9 AND ITS COMPARISON WITH *ESCHERICHIA COLI* PROTEINS L7/L12

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1. Introduction

The amino acid sequences of the *Escherichia coli* ribosomal protein L7 differs from L12 only in that its N-terminal serine is acetylated [1]; the proteins are important for the structure and function of the particle (reviewed [2]). Ribosomes from other bacterial species have proteins homologous to L7/L12 from *E. coli* [3–7]. The most compelling evidence comes so far from a comparison of partial amino acid sequences of ribosomal proteins from halophilic bacteria with that of *E. coli* proteins L7/L12 [7].

We report here the primary structure of the *Bacillus subtilis* large ribosomal subunit protein B-L9. This is the first complete amino acid sequence of a ribosomal protein from a bacterial species other than *E. coli.* A comparison has also been made of the structure of *B. subtilis* B-L9 and *E. coli* L7/L12 proteins.

2. Materials and methods

Protein B-L9 was isolated from *Bacillus subtilis* by a procedure similar to that used for the preparation of L7/L12 from *E. coli* [8,9]. The identity and purity of the protein were verified by two-dimensional polyacrylamide gel electrophoresis [10]; the protein was designated according to [5]. Enzymatic digestion was for 4 h with TPCK-trypsin (from Merck, Darmstadt) or for 1 h with chymotrypsin at 37°C (pH 8.1). Thermolytic digestion was at 55°C (pH 8.1) for 2 h; treatment with *Staphylococcus aureus* protease was at 37°C (pH 8.1) for 20 h. Digestion with pepsin was in 0.05 M HCl at 37°C for 3 h.

Peptides were isolated by the fingerprint technique [11] on thin-layer plates (Cel 300 from Macherey and Nagel, Düren) or by gel filtration on Sephadex G-50 (superfine) columns (120 × 1 cm). Elution was with 10% acetic acid or 50% formic acid.

Amino acid analyses were performed with a Durrum D-500 analyser. Determination of the amino acid sequences within the peptides was by a manual micro-Edman-technique with dansylation [12]. The N-terminal region of the protein was sequenced by the automatic Edman degradation procedure [13] in an improved Beckman sequenator [14]. Aspartic acid, glutamic acid and their amides were determined by converting the released 2-anilino-5-thiazolinones into the phenyl-thiohydantoins and identifying them by chromatographic methods [14]. Where the identification after dansylation was uncertain, and also for quantitative determinations, the amino acids were liberated from their 2-anilino-5-thiazolinones by hydrolysis and identified with an amino acid analyser.

3. Results and discussion

Purified protein B-L9 was added to *E. coli* PI-cores prepared as in [15]; the reconstituted particles were

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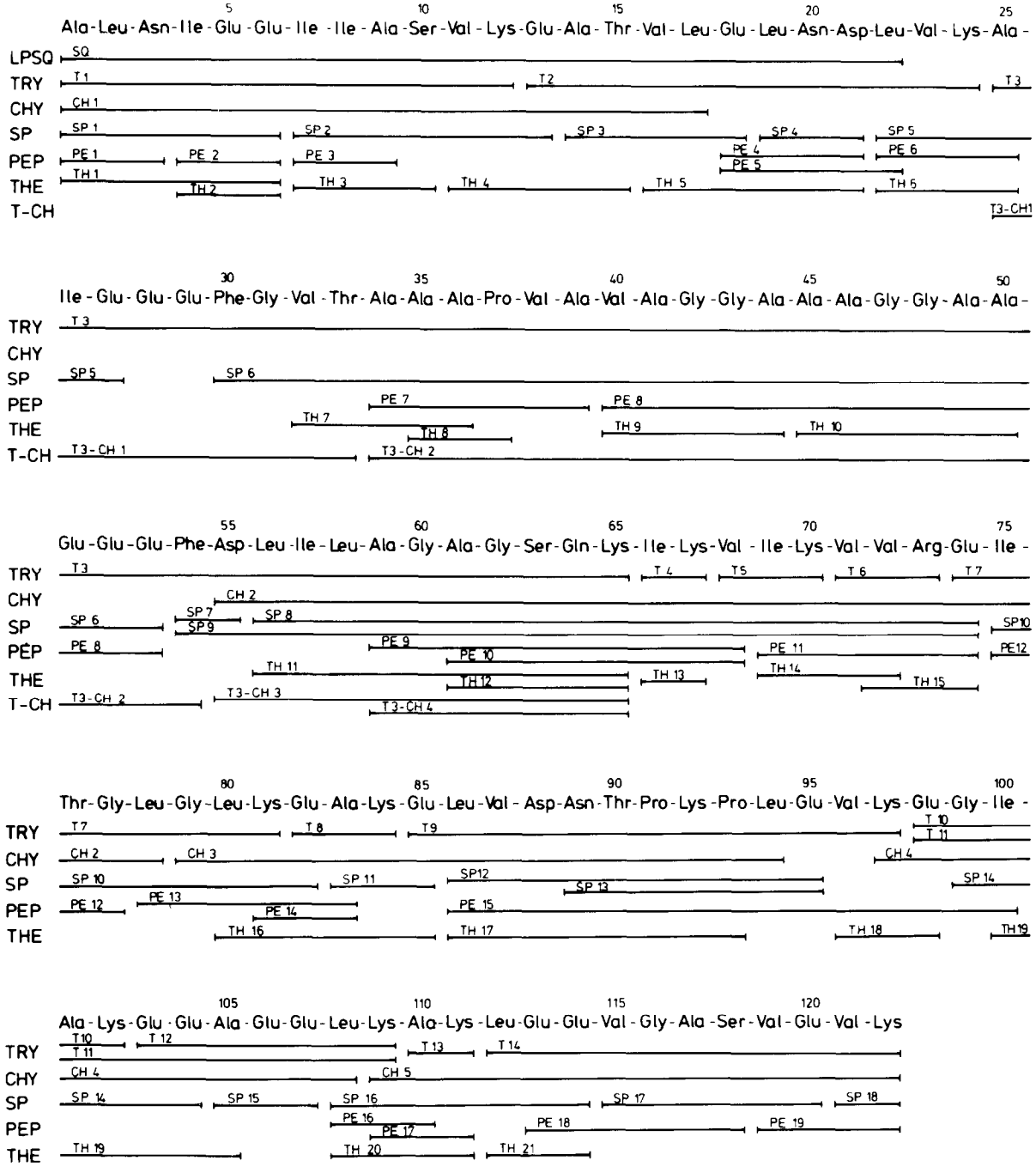

Fig.1. Primary structure of protein B-L9 from *Bacillus subtilis*. *Abbreviations:* LPSQ, degradation of intact protein with liquid-phase sequencer; TRY, tryptic digestion, peptides T; CHY, chymotryptic digestion, peptides CH; SP, peptides isolated after digestion with *Staphylococcus aureus* protease; PEP, pepsin digestion, peptides PE; THE, thermolysin digestion, peptides TH; T-CH, chymotryptic digestion of tryptic peptide T_3.

active in EF-G dependent binding of GDP (details will be published elsewhere). This result shows that protein B-L9 from *B. subtilis* is functionally equivalent to protein L7/L12 from *E. coli.*

The 22 N-terminal amino acid residues of B-L9 were identified with a liquid-phase sequenator. Treatment of the protein with trypsin gave 14 peptides which, with the exception of T3, were completely sequenced manually. Peptide T11 consists of T10 and T12 due to incomplete hydrolysis by trypsin. Peptide T3, which was purified on Sephadex G-50 in 10% acetic acid, was split into four peptides (T3-CH1, T3-CH2, T3-CH3, T3-CH4) by chymotryptic digestion, and the peptides were sequenced manually. Digestion of the protein with thermolysin, chymotrypsin, *Staphylococcus aureus* protease and pepsin resulted in 21, 5, 18 and 19 peptides, respectively. These peptides were sequenced manually. Treatment of the protein with carboxypeptidase A and B released the C-terminal residues lysine and valine. The combination of these results gave the alignment of all peptides and the entire amino acid sequence as shown in fig.1.

The amino acid composition of B-L9 derived from its sequence is: Asp_3, Asn_3, Thr_4, Ser_3, Glu_{22}, Gln_1, Pro_3, Gly_{11}, Ala_{21}, Val_{14}, Ile_9, Leu_{12}, Phe_2, Lys_{13}, Arg_1. This is in very good agreement with the data obtained from the hydrolysis of the protein. B-L9 consists of 122 amino acid residues and has mol. wt 12 633.

The comparison of protein B-L9 from *B. subtilis* and E-L12 from *E. coli* shows a relatively high degree of homology (fig.2): 65 of the 122 amino acid residues, i.e., 53%, are identical in both proteins. At ten additional residues chemically similar amino acids, e.g. Asp/Glu or Thr/Ser, are present in proteins B-L9 and E-L12. This result supports the notion that ribosomal proteins have been conserved within the bacterial kingdom.

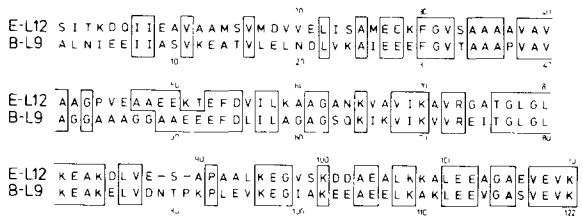

Fig.2. Comparison of the primary structures of *E. coli* protein E-L12 and *B subtilis* protein B-L9. Abbreviations for amino acids are according to the one-letter code.

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