THE PRIMARY STRUCTURE OF PROTEIN BL17 ISOLATED FROM THE LARGE SUBUNIT OF THE *BACILLUS STEAROTHERMOPHILUS* RIBOSOME

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Received 6 October 1980

1. Introduction

To understand, at the molecular level, the function of the ribosome which plays a central role in the biosynthesis of proteins, it is essential to know the structure of the particle in great detail. So far the primary structures of almost all of the 53 proteins [1] and the 3 RNA molecules [2] present in the *Escherichia coli* ribosome are known. Progress has been made towards the elucidation of secondary structures of both the proteins [1] and the RNAs [3–5] also.

The most direct way of determining the tertiary structure of a protein is by its crystallization followed by X-ray analysis. This approach can now be applied to ribosomal proteins. The C-terminal fragment of *E. coli* ribosomal proteins L7/L12 has been crystallized [6] and analyzed at a resolution of 2.6 Å (A. Liljas, personal communication). The first intact ribosomal protein which could be crystallized [7] is protein BL17 isolated from the large subunit of the *Bacillus stearothermophilus* ribosome. The determination of the tertiary structure of this protein by X-ray analysis is now in progress (O. Epp and R. Reinhardt, unpublished).

Here, we describe the complete primary structure of protein BL17 with 147 amino acids and present its secondary structure based on 4 prediction programmes. The amino acid sequence of BL17 is compared to that of other ribosomal proteins.

2. Materials and methods

Protein BL17 was isolated as in [8] avoiding urea and acetic acid extraction. Enzymic digestion was done as follows:

(i) Trypsin digestion at pH 8.1 (in 0.2 M *N*-methylmorpholine acetate buffer at 37°C for 3 h);
(ii) *Armillaria mellea* protease digestion at pH 8.1 (same buffer as above at 37°C for 6 h);
(iii) Pepsin digestion at pH 2 (in 5% formic acid at 37°C for 6 h);
(iv) *Staphylococcus aureus* protease digestion at pH 4.0 (in 0.1 M ammonium acetate buffer at 37°C for 48 h).

The resulting peptides were isolated by:

(i) One-dimensional preparative thin-layer chromatography (*A. mellea* protease peptides);
(ii) Thin-layer fingerprints (tryptic peptides);
(iii) Gel-filtration on Sephadex G50, superfine (1 × 140 cm) in 10% acetic acid followed by fingerprints on thin-layer plates (peptic and *Staph. aureus* peptides).

Sequencing of the various peptides was done with the DABITC/PITC double-coupling method [9]. DABITH-Ile and -Leu were determined by one-dimensional thin-layer chromatography on polyamide sheets [10]. Amino acid analysis was done on Durrum D-500 analysers as in [11].

3. Results and discussion

3.1. *Sequence determination*

Twenty-four peptides were isolated from the tryptic digestion of BL17 by thin-layer fingerprinting, and the complete sequences of these peptides (except T7, T10 and T15) were determined. The sequences of the peptides T7 (pos. 32–44), T10 (pos. 56–64/65) and T15 (pos. 95–107) and the order of the tryptic peptides were obtained from the sequences of the peptic peptides. Sixteen peptides (P1–P16) were isolated

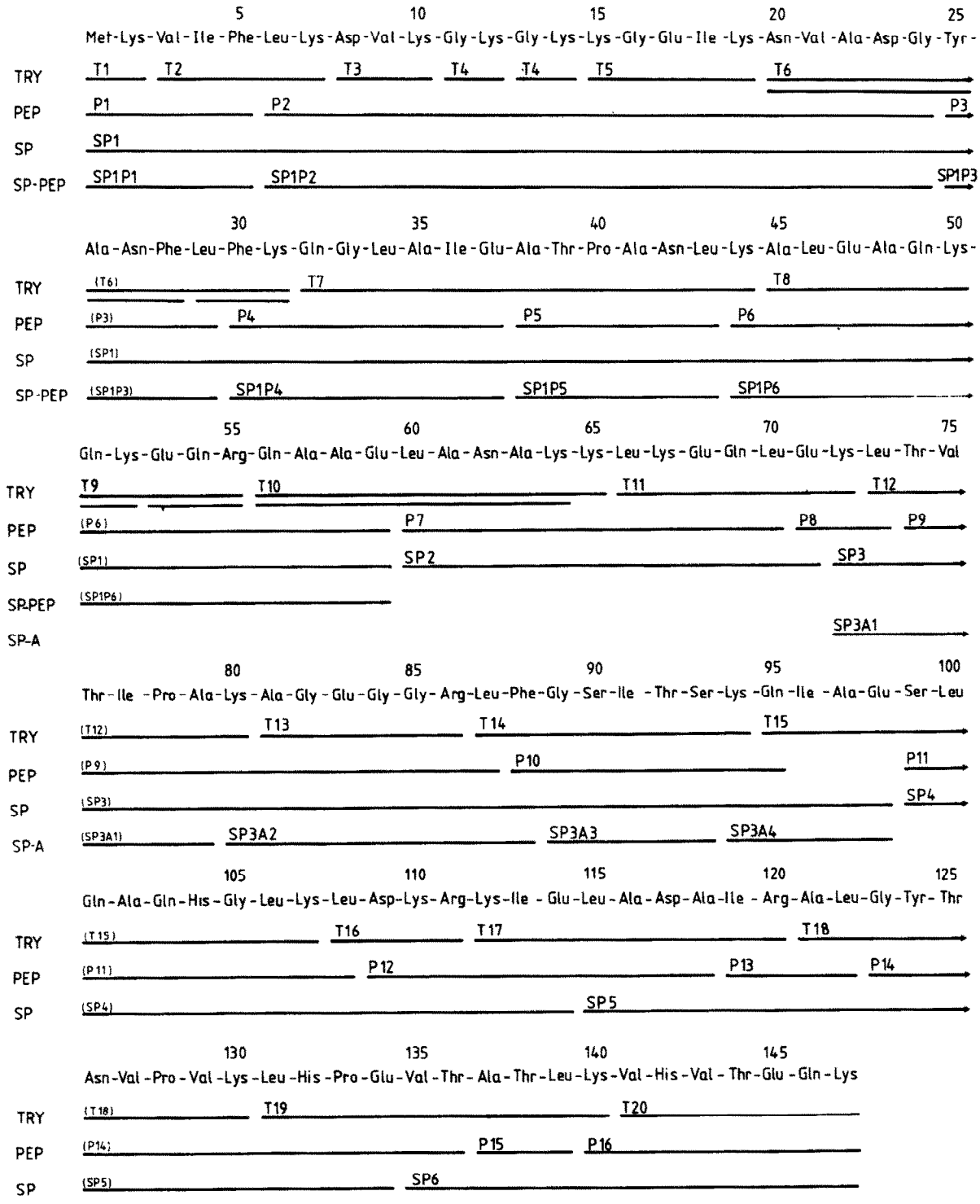

Fig.1. The primary structure of protein BL17. *Abbreviations:* TRY, trypsin digestion (peptides T); PEPS, digestion with pepsin (peptides P); SP, digestion with *Staphylococcus aureus* protease (peptides Sp); SP-PEPS, digestion of Sp peptides with pepsin (peptides Sp-P); SP-A, digestion of Sp-peptides with *Armillaria mellea* protease (peptides Sp-A).

from peptic digestion of BL17 and sequenced as far as possible. From the sequence of the peptides P5 (pos.38–43), P7 (pos.60–70) and P11 (pos.99–108) the sequences of the tryptic peptides T7, T10 and T15 could be deduced, as shown in fig.1.

Cleavage of BL17 with *Staph. aureus* protease occurred at positions 59, 71, 98, 114 and 134, thus yielding 6 peptides (Sp1–Sp6) which were isolated by gel filtration on Sephadex G50 followed by thin-layer fingerprinting. The small peptides (Sp2, Sp4, Sp5 and Sp6) were directly sequenced, and the large peptides Sp1 and Sp3 were digested with pepsin and *A. mellea* protease, and the resulting subfragments were isolated and sequenced. These results completely agreed with the sequences of the tryptic and peptic peptides and confirmed the sequence of protein BL17 as presented in fig.1.

3.2. *Characteristics of the sequence*

The amino acid composition derived from the sequence of protein BL17 is: Asp_4, Asn_5, Thr_8, Ser_3, Glu_{12}, Gln_{10}, Pro_4, Gly_{11}, Ala_{19}, Val_9, Met_1, Ile_8, Leu_{17}, Tyr_2, Phe_4, His_3, Lys_{23}, Arg_4. This composition is in good agreement with that from the total hydrolysis of protein BL17. The M_r based on the sequence is 16 068 which is in full agreement with the value (16 000) derived by sedimentation equilibrium centrifugation (Y. Georgalis, unpublished). Noteworthy features of BL17 are that tryptophan and cysteine are absent, a lysine-rich region is present in the N-terminal part (pos. 2–19), and the hydrophobic amino acids are not clustered but are evenly distributed along the protein chain.

3.3. *Secondary structure of protein BL17*

Based on the amino acid sequence, predictions of the secondary structure were made for protein BL17 employing 4 different methods as in [12]. The secondary structure of BL17 as deduced from this study is presented in fig.2. According to this prediction, protein BL17 contains 49% helix, 15% β-turn and 5% extended structure (average values). A helix region is strongly predicted for pos. 40–73, and turn areas are calculated for pos. 9–16 and 83–86/90.

3.4. *Comparison with other ribosomal proteins*

Using a computer programme the primary structure of BL17 was compared with the sequences of 48 *E. coli* ribosomal proteins [1] and with those of 5 ribosomal proteins from other organisms (yeast, *Bacillus*, *Artemia salina* and rat). Protein BL17 shares the pentapeptides Gly–Lys–Lys–Gly–Glu (pos. 13–17) and Ala–Lys–Lys–Leu–Lys (pos. 63–67) with the *E. coli* ribosomal protein S3 (pos. 77–81) and with the rat liver ribosomal protein L30 (pos. 3–7), respectively. When conservative replacements, such as glutamic acid for aspartic acid, isoleucine for valine, arginine for lysine and serine for threonine were allowed in the search programme, some structure similarities were observed between protein BL17 and several proteins of the *E. coli* ribosome, as shown in fig.3.

From this comparison it is difficult to correlate protein BL17 to an individual ribosomal protein of *E. coli*. Note that after washing of *B. stearothermophilus* ribosomes with sucrose and salt the intensity of the BL17 spot on two-dimensional gel electropherograms became weak (S. R. Fahnestock, personal communi-

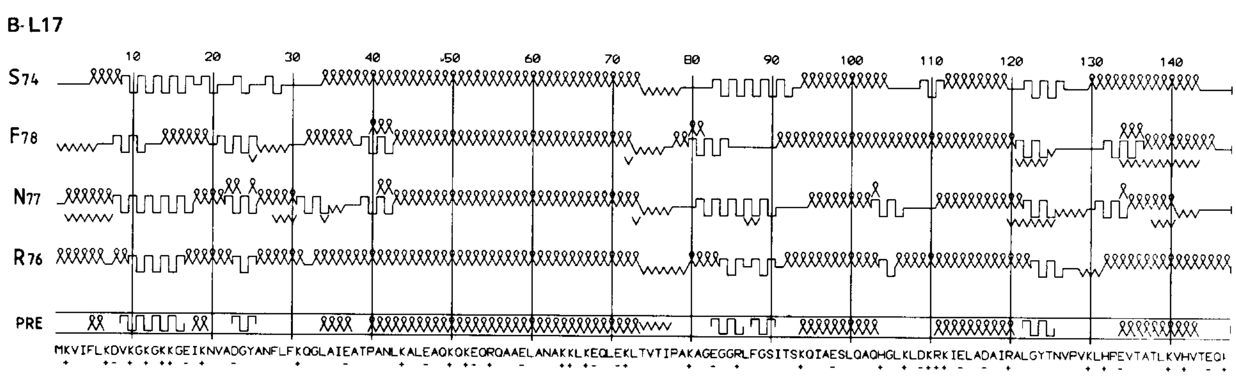

Fig.2. Secondary structure prediction of protein BL17 according to 4 methods (see [12] for details). The symbols represent residues in a helical region (ℛℛℛ), β-turns or loop (⊓⊔⊓⊔), extended structure (∿∿∿) and random coil conformation (——). The line 'PRE' summarizes the secondary structure obtained when at least 3 of the 4 predictions (S, F, N and R) are in agreement.

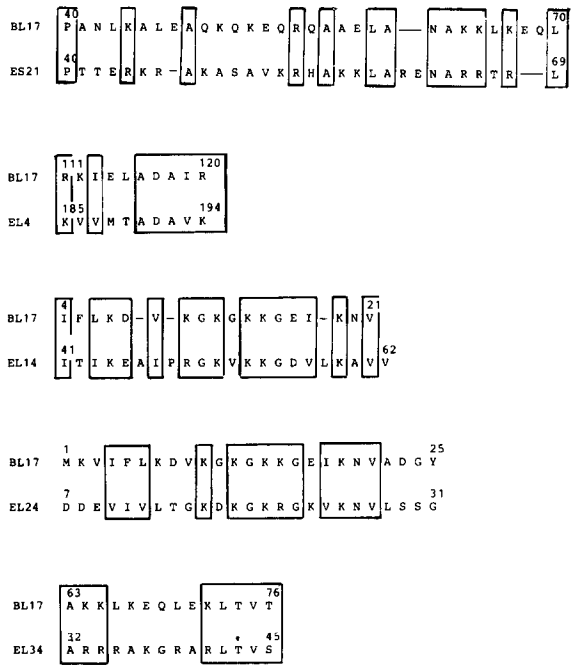

Fig.3. Comparison of the protein BL17 from *Bacillus stearothermophilus* with proteins ES21, EL4, EL14, EL24 and EL34 from the *E. coli* ribosome. Identical or similar amino acids are framed.

cation), and that reconstitution of 50 S subunits without BL17 (as well as other proteins) gave active particles [13]. However, one cannot conclude from these observations that BL17 is a non-ribosomal protein since washing of *E. coli* ribosomes with sucrose and salt reduces the intensity of many ribosomal proteins [14,15] and since ribosomes from *E. coli* mutants missing one (or even more) protein(s) are nevertheless active in protein biosynthesis [16–19]. Furthermore, the BL17 preparation used in these experiments was isolated from 70 S ribosomes which had been washed with sucrose and salt and was obtained in amounts comparable to those of the other ribosomal proteins. The weak BL17 spot on two-dimensional gels could be attributed to variable binding of the dye [7].

Acknowledgements

We thank Drs B. Wittmann-Liebold and H. G. Wittmann for many stimulating discussions and Mr K. Ashman and N. D. Rawlings for excellent technical assistance and computer analyses.

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