THE PRIMARY STRUCTURE OF PROTEIN S16 FROM *ESCHERICHIA COLI* RIBOSOMES

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1. Introduction

Ribosomal protein S16 is involved in the binding to the 16 S RNA of the small subunit [1]. In the presence of proteins S4 and S20 it binds to the 5'-proximal fragment of the 16 S RNA [2,3]. According to immuno-electron microscopy the protein S16 is located on the right side of the 'body' of the 30 S subunit [4].

A prerequisite for a closer insight into the role which protein S16 plays in the structure and the function of the *E. coli* ribosome is the knowledge of its primary structure. This paper summarizes the experimental data which led to the establishement of the complete sequence of S16.

Protein S16 contains 82 amino acids. The molecular weight, calculated from the sequence, amounts to 9192. A cluster of 5 basic residues Arg–His–Gly–Ala–Lys–Lys–Arg was observed at the N-terminal end. The sequence of S16 was compared with the sequence of other *E. coli* ribosomal proteins; an extensive homology was found between proteins S16 and S20.

2. Materials and methods

Ribosomal protein S16 was isolated from *E. coli* K (strain A19) as described by Hindennach et al. [5]. The purity of the isolated protein S16 was judged by two-dimensional polyacrylamide gel electrophoresis [6]. The protein samples of S16 used for the sequence determination were provided by Dr H. G. Wittmann.

Enzymatic digestions employing trypsin treated with 1-chloro-4-phenyl-3-tosylamido-butan-2-one and α-chymotrypsin (both from Worthington, Freehold, N.J.) were performed at pH 8.0 in 0.5% ammonium bicarbonate for 3 h at 37°C. Digestion with thermolysin (from Daiwa Kasei, Osaka) was at pH 8.5 in 0.2 M ammonium acetate buffer containing $CaCl_2$ (5 mM) for 3 h at 37°C. The hydrolysis with *Staphylococcus aureus* protease [7] was performed at pH 7.8 in 0.5% ammonium bicarbonate for 18 h at 37°C.

The purification of the peptides was achieved by a 'three-dimensional' separation on paper similar to a method used recently [8] as follows:

(a) High-voltage electrophoresis was performed in the first dimension on Whatman 3 MM paper at pH 6.5 with 10% pyridine–0.5% acetic acid.

(b) Descending chromatography in the second dimension in 1-butanol/pyridine/acetic acid/water (15:10:3:12, by vol.).

(c) For the third dimension the paper strip containing the neutral peptides was sewed onto a sheet

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of paper and subjected to high-voltage electrophoresis at pH 3.5 in 0.5% pyridine–5% acetic acid.

The peptides were detected after staining with fluorescamine in dry acetone (1 mg/200 ml) [9] under ultraviolet light.

The amino acid sequence of the isolated peptides was obtained with the micro dansyl-Edman procedure described by Bruton and Hartley [10]. In addition, carboxypeptidase or leucine amino peptidase degradation were performed on some of the isolated peptides. Carboxypeptidase A and B (from Worthington, Freehold) treatment was carried out at pH 7.8 in 0.2% ammonium bicarbonate [11] and leucine amino peptidase digestion at pH 8.0 in 0.04 M borate buffer containing $MgCl_2$ (2.5 mM).

Protein S16 was treated with carboxypeptidase A as well as a mixture of A and B in 0.2 M Tris–HCl at pH 8.0 and 37°C and aliquots were analysed at several time intervals. Amides were assigned from the molécular weight and relative electrophoresis mobility at pH 6.5 according to Offord [12] or after extensive leucine amino peptidase digestion. Cysteine was determined after oxidation with performic acid [13]. Tryptophan was detected on fingerprints of the hydrolysates from trypsin, chymotrypsin and thermolysin by dipping with Ehrlich reagent.

3. Results and discussion

The sequence of the 56 amino-terminal amino acids of S16 has been determined by Edman degradation [14] using an improved Beckman sequenator [15] equipped with an automatic conversion device [16].

Fourteen tryptic peptides were isolated from the fingerprints and ten of them could be ordered in the amino-terminal sequence (fig.1) on the basis of amino acid composition and partial N-terminal dansyl-Edman degradation. Peptides T10 (positions 57–70), T11 (71–76), T12 (77–80) and T13 (81–82) provide the sequence information for the carboxyl-terminal sequence. They were completely sequenced by means of the dansyl-Edman degradation procedure. Peptide T10 showed a positive reaction after an Ehrlich reagent test indicating the presence of tryptophan. The sequence of T10 was obtained by a combination of dansyl-Edman degradation, amino acid analysis and charge determination at pH 3.5 after each successive Edman degradation (for histidine localisation), leucine amino peptidase digestion (for amide assignment), and by carboxypeptidase A digestion on the tryptophan containing chymotryptic fragment of T10 (Ala_1, Ile_1, His_1, Trp+).

Overlapping peptides were obtained by cleavage with chymotrypsin. The chymotryptic peptides which could not be ordered in the known amino-terminal sequence were fully sequenced by the dansyl-Edman procedure. The results are summarised in fig.1. Peptide CH-7 (positions 53–60) showed a positive Ehrlich reaction. The sequence of CH-7 was obtained by the dansyl-Edman degradation. The position of tryptophan was unambiguously identified by carboxypeptidase A digestion on the tryptophan containing tryptic fragment of CH-7 (residues 57–60).

The sequences of CH-7, CH-8 and CH-9 allowed the determination of the order of the tryptic peptides T9——T10 (overlapping peptide CH-7), T10——T11 (overlapping peptide CH-8) and T11——T12——T13 (overlapping peptide CH-9). With this information the complete amino acid sequence of protein S16 was established as shown in fig.1.

A third type of digestion was performed with the *Staphylococcus aureus* protease in conditions where cleavage occurs at the aspartate and glutamate residues [7]. Each of these peptides could be ordered in the S16 sequence deduced from the alignment of tryptic and chymotryptic peptides.

In these various digests (trypsin, chymotrypsin, Staphylococcal protease) no peptides were isolated which could not be placed in the S16 sequence. In addition, there were only minor differences between the amino acid composition as determined from the sequence and from acid hydrolysis (results not shown and [17]).

The complete experimental details for the elucidation of the primary structure of protein S16 will be presented elsewhere. Ribosomal protein S16 from *E. coli* contains 82 amino acid residues: Asp_4, Asn_4, Thr_3, Ser_3, Glu_5, Gln_2, Pro_2, Gly_6, Ala_{11}, Val_8, Met_1, Ile_6, Leu_4, Tyr_1, Phe_4, His_2, Lys_5, Arg_{10}, Trp_1. The content of basic amino acids, especially arginine, is very high; however, with the exception of region 8–14 (Arg–His–Gly–Ala–Lys–Lys–Arg) no clustering of basic residues was observed. On the other hand 5 out of 9 acidic residues are clustered in positions 45–55.

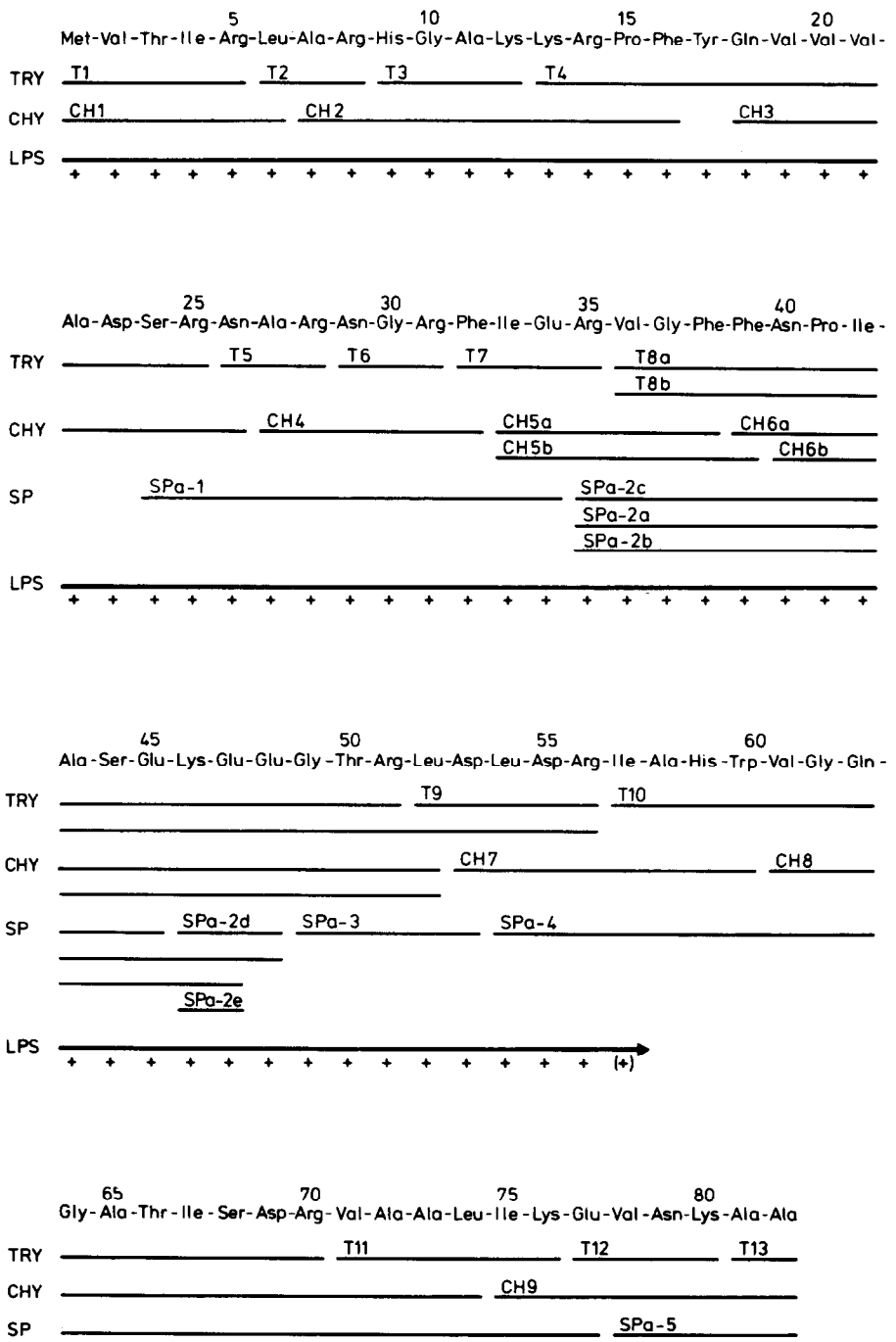

Fig.1. Complete amino acid sequence of protein S16 from *E. coli* ribosomes. Placement of the tryptic (TRY), chymotryptic (CHY), and staphylococcal protease (SP) peptides is shown below the sequence. LPS means liquid-phase Edman degradation of protein S16. (+) Means positive identification by thin-layer chromatography of the phenylthiohydantoin derivative after automatic conversion reaction.

Compared with other *E. coli* ribosomal proteins, the following identical stretches could be observed: Val–Val–Ala–Asp–Ser–Arg in S16 (residues 20–25) and in protein L10 [18] (residues 25–30) as well as Lys–Glu–Glu–Gly in S16 (residues 46–49) and in S8 [19] (residues 40–43). Several identical peptides were found for protein S16 and S20 [20], namely Ala–Lys–Lys–Arg in S16 (residues 11–14) and in S20 (residues 6–9), Ser–Glu–Lys in S16 (residues 44–46) and in S20 (residues 13–15), Asn–Lys–Ala–Ala in S16 (residues 79–82) and in S20 (residues 69–72).

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References

[1] Mizushima, S. and Nomura, M. (1970) Nature (London) 226, 1214–1218.
[2] Muto, A., Ehresmann, C., Fellner, P. and Zimmermann, R. A. (1974) J. Mol. Biol. 86, 411–432.
[3] Zimmermann, R. A. (1974) in: Ribosomes pp. 225–269, Cold Spring Harbor Laboratory Monograph Series, Cold Spring Harbor, N.Y.
[4] Tischendorf, G. W., Zeichhardt, H. and Stöffler, G. (1975) Proc. Natl. Acad. Sci. USA 72, 4820–4824.
[5] Hindennach, I., Stöffler, G. and Wittmann, H. G. (1971) Eur. J. Biochem. 23, 7–11.
[6] Kaltschmidt, E. and Wittmann, H. G. (1970) Proc. Natl. Acad. Sci. USA 67, 1276–1282.
[7] Houmard, J. and Drapeau, G. R. (1972) Proc. Natl. Acad. Sci. USA 69, 3506–3509.
[8] Chen, K. C. S. and Krause, R. M. (1975) Anal. Biochem. 69, 180–186.
[9] Vandekerckhove, J. and Van Montagu, M. (1974) Eur. J. Biochem. 44, 279–288.
[10] Bruton, C. and Hartley, B. S. (1970) J. Mol. Biol. 52, 165–178.
[11] Ambler, R. P. (1967) Methods Enzym. 11, 155–166.
[12] Offord, R. E. (1966) Nature (London) 211, 591–593.
[13] Hirs, C. H. W. (1956) J. Biol. Chem. 219, 611–621.
[14] Edman, P. and Begg, G. (1967) Eur. J. Biochm. 1, 80–91.
[15] Wittmann-Liebold, B. (1973) FEBS Lett. 36, 247–249.
[16] Wittmann-Liebold, B., Graffunder, H. and Kohls, H. (1976) Anal. Biochem. 75, 621–633.
[17] Kaltschmidt, E., Dzionara, M. and Wittmann, H. G. (1970) Molec. Gen. Genetics 109, 292–297.
[18] Heiland, I., Brauer, D. and Wittmann-Liebold, B. (1976) Hoppe-Seyler's Z. Physiol. Chem., in press.
[19] Stadler, H. and Wittmann-Liebold, B. (1976) Eur. J. Biochem. 66, 49–56.
[20] Wittmann-Liebold, B., Marzinzig, E. and Lehmann, A. (1976) FEBS Lett. 68, 110–114.