THE PRIMARY STRUCTURE OF RIBOSOMAL PROTEIN S7 FROM
ESCHERICHIA COLI STRAINS K AND B

Sequence of the C-terminal region of S7K and S7B

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1. Introduction

Protein S7 from the small subunit of Escherichia coli ribosomes interacts specifically with the 16 S RNA (reviewed in reference [1]) and it has been shown that binding occurs near the 3'-end of the 16 S RNA [2–4]. Protein S7 can be cross-linked to 16 S RNA by ultraviolet irradiation of the small subunit [5]. From studies in which an in vitro complex between protein S7 and 16 S RNA was irradiated and digested with trypsin, it was suggested that four peptides were cross-linked to the RNA [6].

Knowledge of the primary structure of protein S7 is necessary both for detailed studies of the molecular mechanisms of protein–RNA interaction as well as the elucidation of the alterations of protein S7 recently found in various mutants of E. coli [7,8].

It has long been known that E. coli strains differ in their S7 proteins [9–13]. Ribosomes from strains B, C and MRE 600 contain a protein S7 which differs extensively in size, charge, amino acid composition and immunological properties from the protein S7 in strain K [13].

In this paper, we describe the sequence analysis of the C-terminal regions of proteins S7 isolated from E. coli strains B and K. We show that protein S7 K is longer by 24 amino acids than protein S7 B and that this difference occurs at the C-terminal end of the protein chain.

2. Materials and methods

Protein S7 was provided by Dr H. G. Wittmann and was isolated from 30 S ribosomal subunits of E. coli K and B as described elsewhere [14]. Its purity was determined by two-dimensional gel electrophoresis [15].

Tryptic digestion was performed at 37°C for 3 h. The tryptic peptides and the peptides obtained by means of other endoproteases were separated on cellulose thin-layer plates and eluted [16]. Alternatively, they were isolated on a micro-column (0.3 × 10 cm) of Dowex M71 at 50°C with pyridine-formate gradients [17,18] followed, when necessary, by chromatography on cellulose thin-layer sheets.

Protein S7 was also digested with pepsin and a Staphylococcus aureus protease which is specific for glutamoyl bonds [19]. Larger tryptic peptides were produced by first blocking the ε-amino groups of the lysine residues by exo-cis-3,6-endoxo-Δ4-tetrahydropthalic acid anhydride so that trypsin would split only the arginyl bonds [20]. Carboxypeptidases A as well as B were used to establish or to confirm partial
sequences. In order to obtain large fragments, the protein was also cleaved by treatment with cyanogen bromide [21].

Alignment of the tryptic peptides was facilitated by isolation and analysis of peptides obtained after specific cleavage of the tryptophanyl bonds of S7 with BNPS-skatole, 2-(2-nitrophenylsulphenyl)-3-methyl-3' bromoindo1ene [22]. For the determination of tryptophan residues, proteins S7 K and S7 B were hydrolysed with 4 N methane sulphonic acid containing 0.2% 3-(2-aminoethyl) indole [23]. The presence of tryptophan in the peptides was established by fingerprints on cellulose thin-layer plates, spraying with Ehrlich's reagent.

The sequence determination of proteins S7 B and S7 K were performed using the following three methods:

(i) Automatic Edman degradation in a solid-phase sequenator according to the method of Laursen [24] with attachment of the C-terminal carboxyl group to amino-polystyrene resin [25,26];
(ii) Automatic Edman degradation [27] in an improved Beckman sequenator [28];
(iii) Manual Edman degradation employing the dansyl-Edman procedure [29,30].

3. Results and discussion

It is known that the molecular weight of S7 K is higher than that of S7 B by approximately 15% [13]. Thus the question arose as to whether the two proteins differ in their N-terminal or C-terminal regions. We therefore determined the amino acid sequences of the tryptic peptides in the N-terminal regions of both proteins and no differences were detected. On the other hand, we found that proteins S7 B and S7 K differed strongly in the amino acids released by treatment with carboxypeptidases A and B. The results show that both proteins differ in their C-terminal, rather than in their N-terminal region.

The next question was to differentiate between the following two possibilities:

(i) The sequence of protein S7 B is identical with that of S7 K, with the exception that approximately 25 amino acids at the C-terminal of S7 K are absent in S7 B. This can be caused by a mutation from a sense codon to a termination codon, leading to the shorter S7 B protein chain, or vice versa, by a mutation in the termination codon of S7 B to a sense codon, resulting in the longer chain.

(ii) The different lengths of the two S7 proteins can be caused by a frame-shift mutation in a similar way to that found in E. coli mutants with altered S4 proteins [31].

The consequences of these possibilities are that, in the first case, all peptides of the shorter protein (S7 B) should also be present in the longer protein (S7 K), whereas, in the second case, the shorter protein should also contain peptides which are different from that of the longer protein.

In order to distinguish between these possibilities, proteins S7 K and S7 B were digested with trypsin and the tryptic peptides were separated by the fingerprint method. These were eluted and analysed after hydrolysis. The finding that all the peptides present in S7 B were also found in S7 K and that no new peptide was detected can be easily explained by the first but not by the second possibility mentioned above.

Further proof was obtained by treatment of the two protein chains with the reagent BNPS-skatole which cleaves after tryptophan residues. It was found that treatment of protein S7 K resulted in one peptide more than treatment of S7 B. This extra peptide (BNPS-KII) from S7 K was isolated by gel-filtration on Sephadex G-100 and sequenced in a solid-phase sequenator. It was also digested with trypsin and, after isolation of the resulting three tryptic peptides by the fingerprint method, their sequences were determined by the manual dansyl-Edman method and by the solid-phase sequenator. The results from all methods were consistent and led to the sequence of peptide BNPS-KII as shown in fig.1. The location of the peptide BNPS-KII at the C-terminal end of protein S7 K is also in full agreement with the experiments in which peptide BNPS-KII and intact protein S7 K were treated with carboxypeptidases A and B. The same amino acids were liberated in both cases. This result shows that BNPS-KII is situated at the C-terminal end of protein S7 K.

The peptide at the C-terminus of protein S7 B was identified in the following manner. After treatment of S7 B with BNPS-skatole, a peptide, BNPS-BI, was isolated by gel-filtration and was sequenced by a combination of chemical and enzymatic methods as illustrated in fig.1. Treatment of this peptide and of the intact S7 B protein with carboxypeptidases A
and B resulted in the release of the same amino acids. This finding demonstrates the location of peptide BNPS-BI at the C-terminus of protein S7 B.

Comparison of the amino acid sequence of peptide BNPS-BI isolated from protein S7 B with that of BNPS-KI from S7 K showed that both peptides have almost identical primary structures. They differ only in one tryptophan residue which is located at the C-terminus of BNPS-KI but is absent in BNPS-BI.

As shown in fig. 1, which represents the terminal part of proteins S7 B and S7 K, both protein-chains are identical in this region with the exception that S7 K is longer by 24 amino acids than S7 B. All peptides found in S7 B are also present in S7 K, and no other peptide was found which differs in the two proteins. Hence, a frame-shift is improbable as the mutational
event which leads to the different lengths of the two proteins. Whether protein S7 B derived from S7 K by a mutation from a sense- to a termination-codon, or whether the reverse mechanism occurred cannot be decided with certainty. However, it should be borne in mind that ribosomes from all *E. coli* strains and from all other Enterobacteriaceae species studied so far [13,32,33] have a S7 protein similar to S7 B and not to S7 K. This finding would suggest that S7 K is derived from S7 B and not vice versa.

3.1. Secondary structure

Using the method of Chou and Fasman [34,35] for predicting secondary structure in proteins, the following results were obtained for the C-terminal region of protein S7 K:

(i) α-Helices in positions 1–6, 13–27, 31–50 and 59–64.


Following the method of Burgess et al. [36], α-helical regions were predicted for positions 4–8, 15–25, 32–37 and 40–49; bends for 9–13, 38–39, 52–53, 55–61, 66–69 and 72–73.

Both methods gave a very high α-helix content for the C-terminal region (positions 1–53) which is identical in proteins S7 B and S7 K: 89% according to Chou and Fasman [34,35] and 57% according to Burgess et al. [36]. Both methods predict the region from positions 15–50 to contain mainly α-helices.

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References


