THE PRIMARY STRUCTURE OF PROTEIN S9 FROM THE 30S SUBUNIT OF *ESCHERICHIA COLI* RIBOSOMES

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1. Introduction

Protein S9 has an important role in the 30S subunit assembly reaction. Without S9, reconstituted particles have lower sedimentation coefficients (25S) and greatly reduced ribosomal activities [1,2]. Protein S9 is an interface protein and is involved in the association of 30S and 50S subunits [3]. Furthermore, its presence is required for the ribosome dependent GTPase activity of elongation factor EF-G [4,5]. The elucidation of the amino acid sequence of protein S9 which is presented in this paper will facilitate a closer insight into the structure and function of this protein.

2. Materials and methods

Protein S9 was isolated from *E. coli* strain K as previously described [6] and provided by Dr H. G. Wittmann. The tryptic digestion was done on 1.5 μmol of protein. Tryptic peptides were purified on a Dowex 50 X 7 micro column (0.28 X 10 cm). Fractions of 0.5 ml were collected. 20 μl of each fraction was spotted on cellulose thin-layer plates, chromatographed and sprayed with ninhydrin in order to identify those fractions which contained the peptides. Cyanogen bromide cleavage [7] was performed on 1.5 μmol of protein. The fragments were isolated by gel filtration on a Sephadex G100 column. In addition, 0.5 μmol of protein were digested by Staphylococcal protease [8] and the resulting peptides were separated on a phosphocellulose column (0.6 X 13 cm).

Amino acid analyses were performed with a Durrum D-500 analyzer in the 1–2 nmol range. The determination of the amino acid sequences within the peptides was independently done by two methods: the Dansyl-Edman technique [9] and the automatic degradation in a sequenator [10]. All tryptic peptides with the exception of two in which the *N*-terminal glutaminyl residue was cyclized to an α-pyrrolidone-carboxyl residue were sequenced by the Dansyl-Edman method. The smallest cyanogen bromide peptide (CN_2) and the peptides obtained by Staphylococcal protease digestion were also sequenced by this technique. The sequences of the large CNBr fragments (CN_3, CN_4, CN_3+_4) as well as the *N*-terminal region of S9, were determined in an improved Beckman sequenator [11] according to the method of Edmam and Begg [10]. One of the fragments (CN_4) was reacted with Braunitzer's reagent IV [12] prior to the automatic degradation. Full details of the methods will be described elsewhere.

3. Results and discussion

The complete amino acid sequence of S9 is given in fig.1. The amino acid composition, calculated from the sequence, agrees very well with that determined experimentally. Furthermore the mol. wt calculated from the sequence, namely 14 569, is in excellent agreement with the value of 14 500 obtained by sedimentation equilibrium [13].

It was important during sequencing work to use as little material as possible, since ribosomal proteins are difficult to isolate in large amounts. Our strategy, therefore, was to isolate all tryptic peptides and to sequence them by the Dansyl-Edman technique at the 10–20 nmol range. Alignment of peptides was done by automatic degradation of the large fragments

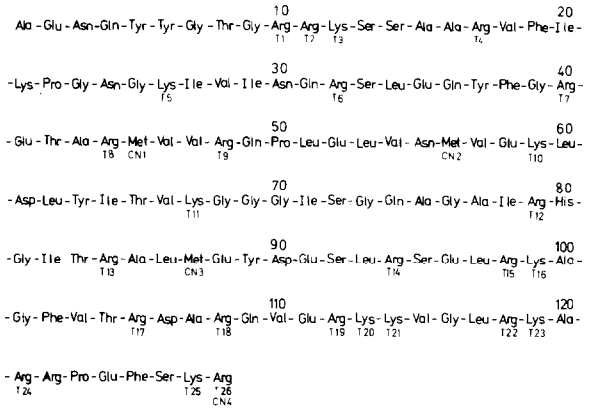

Fig.1. Sequence of ribosomal protein S9 of E. coli. T_1: tryptic peptides; CN_1: peptides obtained from cyanogen bromide cleavage.

(positions 1–41; 57–121) in a sequenator. The latter method also gave us the alignment of peptides T_1-T_7 and $T_{10}-T_{24}$ and independently confirmed the sequences obtained by the Dansyl-Edman technique. The alignment of the residual tryptic peptides was determined by manual sequencing of the cyanogen bromide peptide CN_2 and the overlapping peptides (positions 42–52; 46–56; 112–124; 125–128) obtained after digestion with Staphylococcal protease. Full details of the results will be described elsewhere.

About 60% of the basic residues occur in clusters of two or three basic amino acids (positions 10–12; 79–80; 98–99; 112–114; 118–119; 121–122 and 127–128) mainly in the C-terminal region. On the basis of the sequence the secondary structure of S9 was predicted. Following the method of Chou and Fasman [14,15] 37% α-helical regions and 23% β-sheet structures are postulated. The distribution of them is as follows: α-helix: positions 31–36; 51–61; 82–100; 105–115; β-sheet: positions 4–8, 15–20, 25–29, 42–49, 62–66.

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