THE PRIMARY STRUCTURE OF PROTEIN L10 FROM *ESCHERICHIA COLI* RIBOSOMES

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1. Introduction

Protein L10* of the *E. coli* 50 S ribosomal subunit is included in the group of proteins responsible for the formation of ribosome complexes with the elongation and initiation factors of protein biosynthesis. In particular protein L10 together with proteins L6 and L18 is necessary for the binding of protein L7 and L12 by the ribosome [1,2], at the same time the binding of protein L10 depends on the binding of protein L11 by the ribosome [3].

2. Materials and methods

Protein L10 was isolated from *E. coli* MRE-600 70 S ribosomes as described earlier [5] with a yield of about 60 mg per 10 g of the 70 S ribosome total protein. The peptides derived after cleavage of 1–2 μmol of carboxymethylated protein by trypsin or chymotrypsin were separated by chromatography on Aminex A5 resin, by paper chromatography in the system of solvents *n*-butanol–pyridine–acetic acid–water (15:10:3:12 by vol) and high-voltage electrophoresis at pH 1.9, 3.5 and 6.5. The mixture of peptides obtained after cleavage of 2 μmol of protein by cyanogen bromide was treated with maleic anhydride and then separated on Biogel P-10 in the presence of 6 M guanidine hydrochloride. Maleic protecting groups were removed by keeping the peptide in 50% acetic acid at 37°C [6]. Peptides obtained at cleavage of the cyanogen bromide fragment BC-4 as well as the mixture of fragments BC-3 and BC-5 by *N*-bromosuccinimide (at the tyrosine residues [7]) were separated on Sephadex G-25. The amino acid composition of the protein and separated peptides was determined on amino acid analyzers BC-201 (Bio-Cal, BRD) and D-500 (Durrum, USA). The amino acid sequence of the separated peptides was determined by Edman's phenylisothiocyanate method, by the dansyl Edman method [8] and by automated Edman degradation on a sequencer (model 890C, Beckman, USA) using fast and slow peptide programs. PTH-derivatives of amino acids were identified by gas-liquid chromatography on a Hewlett-Packard GC instrument (model 5710A, USA) and thin-layer chromatography, the DNS-derivatives by two-dimensional thin-layer chromatography on silica gel. The protein and peptide C-terminal amino acid residues were determined by carboxypeptidases A and B [9].

* Preliminary results of primary structure studies of protein L10 see also in [4].

3. Results and discussion

According to the data of electrophoresis in polyacrylamide gel in the presence of sodium dodecylsulphate, protein L10 has a mol. wt. of 19 000 [5,10], does not contain tryptophan residues and its amino acid composition was determined as follows in moles/%): Asp-7.2, Thr-6.0, Ser-4.7, Glu-11.8, Pro-2.8, Gly-6.9, Ala-17.6, Val-9.5, Met-2.8, Ile-3.4, Leu-8.1, Tyr-1.8, Phe-3.3, His-0.8, Lys-6.8, Arg-6.7 [11].

We have determined that the N-terminal residue is alanine and protein hydrolysis with carboxypeptidases A and B has shown that the C-terminal residue is also alanine.

Analysis of the protein L10 tryptic hydrolyzate by peptide mapping revealed 24 spots. Two μmol of the hydrolyzate were separated on Aminex A5 ion

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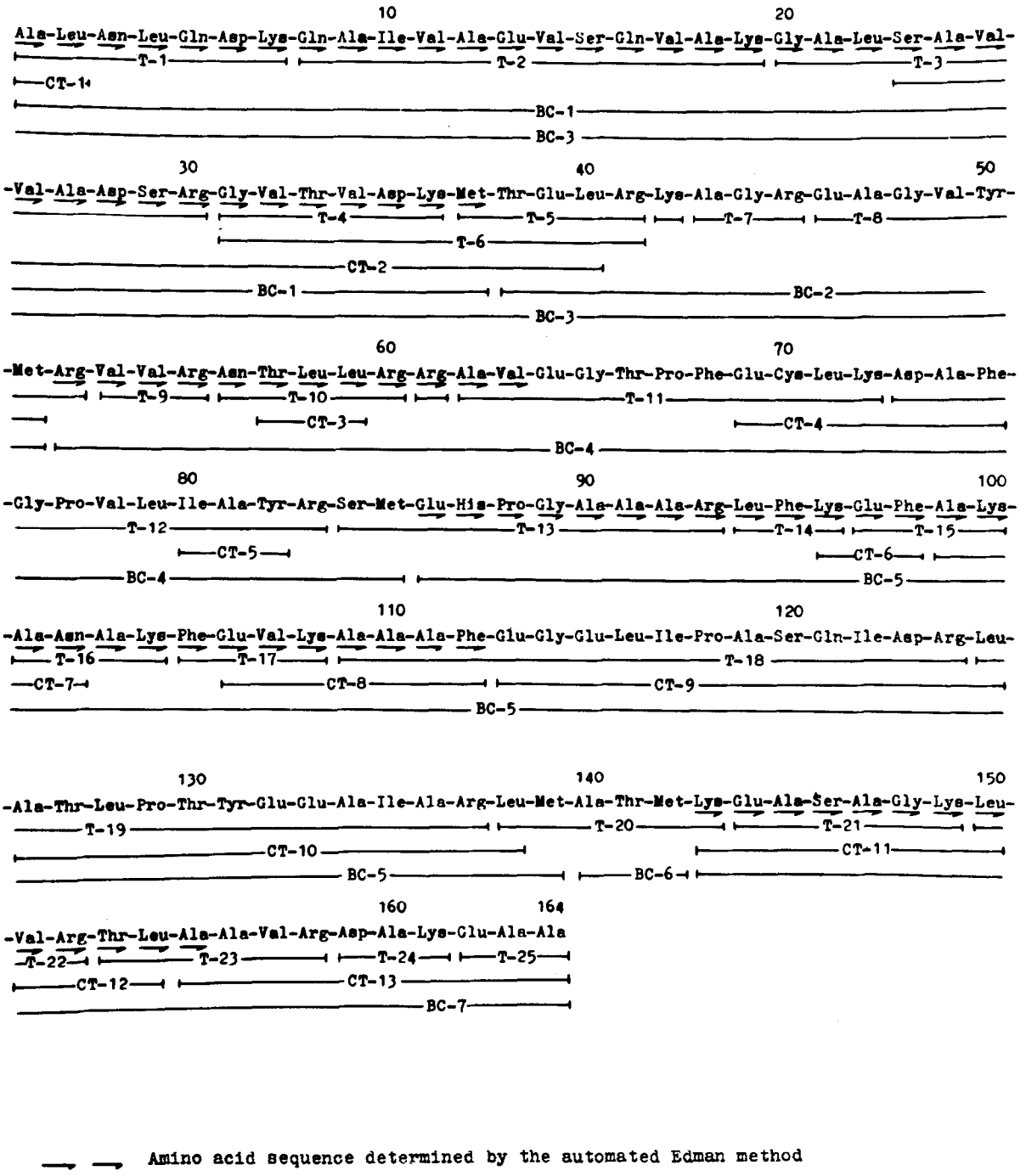

Scheme 1. Primary structure of protein L10.

exchange resin with a following fractionation of the obtained fractions by paper chromatography and paper high-voltage electrophoresis. As a result 25 peptides, T-1 to T-25, were isolated as well as free lysine and arginine (see scheme 1). Peptide T-2 was obtained with a closed N-terminal amino group due to transformation of the N-terminal glutamine residue into a pyroglutamic acid residue and its sequence was not determined. In peptide T-18 we determined the sequence of twelve of the sixteen amino acid residues. The structure of the remaining tryptic peptides was determined completely. It should be noted that peptide T-6 contained an uncleaved bond –Lys–Met– which permitted to join peptides T-4 and T-5.

To obtain overlapping peptides and a reconstitution of the polypeptide chain, the protein N-terminal sequence was determined by the Edman automated method (see scheme 1) and cleavage of the protein molecule by chymotrypsin and cyanogen bromide was also carried out.

The sequence of amino acid residues up to position 37 was determined on a whole molecule by the automated Edman method* which permitted to join the following tryptic peptides into one polypeptide chain: T-1, T-2, T-3, T-4 and T-5.

The protein L10 molecule contains 5 methionine residues, so we cleaved the protein at the methionine residues by cyanogen bromide to obtain large fragments. Seven fragments, BC-1 to BC-7, were obtained (see scheme 1). The peptide mixture obtained as a result of cleavage of 2 μmol of protein by cyanogen bromide was treated with maleic anhydride and then separated on a column with Biogel P-10 in the presence of 6 M guanidine hydrochloride. As a result fragments BC-1, BC-2, BC-4, BC-6 and BC-7 were separated as well as a mixture of peptides BC-3 and BC-5. Fragment BC-1 proved to be the N-terminal and fragment BC-7 the C-terminal regions of the molecule. Fragment BC-3 also proved to be the N-terminal region containing an uncleaved bond –Met–Thr–. The structure of fragments BC-2 and BC-6 was completely determined by the dansyl Edman method. The mixture of fragments BC-3 and BC-5 was subjected to Edman automated degradation which resulted in determination of the sequence of 27 amino acid residues in fragment BC-5. The sequence of 12 and 13 amino acid residues in fragments BC-4 and BC-7, respectively, was determined by automated Edman degradation, Thus, studies of the structure of cyanogen bromide fragments permitted to join the following tryptic peptides into one polypeptide chain: T-5, T-6, T-7, T-8, T-9, T-10, T-11 and T-13, T-14, T-15, T-16, T-17 and T-18 and, finally, peptides T-20, T-21, T-22 and T-23.

At hydrolysis of 1 μmole of protein by chymotrypsin 13 peptides, CT-1 to CT-13, were separated (see scheme 1), some of which were overlapping tryptic peptides in particular peptides T-11 and T-12 as well as T-19, T-20, T-21, T-22, T-23, T-24 and T-25.

To corroborate overlapping in positions 83–84 and 137–138 peptide BC-4 and the mixture of peptides BC-3 and BC-5 were cleaved by *N*-bromosuccinimide at the tyrosine residues. In the first case we separated the tripeptide Arg–Ser–Hse and in the second –Glu–Glu–Ala–Ile–Ala–Arg–Leu–Hse which confirmed the presumed protein structure.

Thus, structure studies of peptides isolated after cleavage of the protein molecule or its fragments by trypsin, chymotrypsin, cyanogen bromide and *N*-bromosuccinimide the complete amino acid sequence of protein L10 was determined (see scheme scheme 1).

The exact mol. wt. of the protein is 17 402, the polypeptide chain consists of 164 amino acid residues and has the following amino acid composition:

Asp_6, Asn_3, Thr_8, Ser_6, Glu_{14}, Gln_4, Pro_5, Gly_9, Ala_{33}, Cys_1, Val_{14}, Met_5, Ile_5, Leu_{14}, Tyr_3, Phe_6, His_1, Lys_{12}, Arg_{13}.

It must be noted that the protein L10 polypeptide chain contains about 60% of hydrophobic amino acid residues and the distribution of functional groups in the chain yields interesting information for understanding interactions of proteins and their functioning within the ribosome.

* Data on the structure of penta- and tripeptide from the N-terminal region of the molecule are also given in [12].

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