PRIMARY STRUCTURE OF AN ACIDIC RIBOSOMAL PROTEIN FROM *MICROCOCCUS LYSODEIKTICUS*

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1. Introduction

The acidic ribosomal protein L7/L12 of *Escherichia coli* is important in the function of elongation factors [1]. Ribosomes from other bacterial species have proteins homologous to L7/L12. The ubiquity of this protein as a ribosomal constituent is indicated by a high degree of conservation in their N-terminal amino acid sequences in prokaryotic or in eukaryotic organisms [2–4]. Comparative primary structural analyses of these proteins would give some insights into the evolutionary changes of this protein as well as the nature of active sites within the molecule.

The complete primary structure of the L7/L12-equivalent protein, BL9, from *Bacillus subtilis* has been determined [5] and its comparison with L12 from *E. coli* shows 50% sequence similarity; position 76–84 (Thr–Gly–Leu–Gly–Leu–Lys–Glu–Ala–Lys), which is a region having predictable β-turn or bend structure, is especially highly conserved within these molecules [6].

Micrococcus lysodeikticus is a Gram-positive bacterium having the highest GC-content of DNA among all the living organisms (72%). We now report the complete primary structure of an acidic ribosomal protein from this bacterium, which migrates in the two-dimensional gel to approximately the same position as that of *E. coli* L7/L12 or *B. subtilis* BL9. The amino acid sequence of this protein has been compared with the published primary structures of L12-equivalent proteins from other organisms such as *E. coli* [7], *B. subtilis* [5], *Halobacterium cutirubrum* [8], *Saccharomyces cerevisiae* [9] and *Artemia salina* [10].

2. Materials and methods

The acidic proteins were selectively extracted from 70 S ribosomes using the NH_4Cl/EtOH splitting technique [11] and concentrated by precipitation with 2 vol. acetone at −20°C. Separation and purification of the acidic proteins were carried out by DEAE-cellulose column chromatography (DE52 from Whatman) as in [12]. The purity of the protein samples was examined by two-dimensional polyacrylamide (2D) electrophoresis [13].

Enzymatic digestions of the protein were performed with TPCK-trypsin for 3 h in 0.1 M methylmorpholine–acetate buffer (pH 8.0) at an enzyme/substrate ratio of 1:50. Thermolysin digestion was done at pH 8.0 for 1.5 h at 52°C and *Staphylococcus aureus* protease digestion was at 37°C (pH 8.0) for 20 h at an enzyme/substrate ratio of 1:100 and 1:30, respectively. Digestion with pepsin was carried out in 0.05 M HCl at 37°C for 2 h at an enzyme/substrate ratio of 1:50, and with carboxypeptidase Y from yeast in 0.1 M pyridine–acetate buffer (pH 5.5) at 37°C [14].

Peptides were isolated by fingerprint technique [6] on thin-layer plates (Cel 300 from Macherey and Nagel, Düren). The first dimension was electrophoresis at pH 6.5 in pyridine/acetic acid/water (50:1.5:450), run at 400 V for 80 min. The chromatogram was developed for the second dimension in a system composed of pyridine/*n*-butanol/acetic acid (50:75:15:60). This fingerprint method was effective to isolate all the peptides except T4. This large T4 peptide was isolated with a good yield from the digested protein by Sephadex G-50 gel filtration with 15% acetic acid [6]. Amino acid analyses were performed after hydrolysis in 5.7 N HCl–0.02% β-mercaptoethanol with a

Beckman 121M analyzer. Determination of amino acid sequences of peptides or of the N-terminal region of intact protein was performed by double-coupling method using 4-*N,N*-dimethylaminoazobenzene 4′-isothiocyanate/phenylisothiocyanate (DABITC) [15]. The 4-*N,N*-dimethylaminoazobenzene 4′-thiohydantoin of leucine and isoleucine were separated on polyamide thin-layer sheets in 10% formic acid/ethanol (10:9) [16].

The nature of the amino-terminal blocking group in either the whole protein or respective peptide was determined with the aid of the hydrazine–dansyl method [17,18].

3. Results and discussion

Two L7/L12-type acidic ribosomal proteins (MA1 and MA2) were extracted from 70 S ribosomes of *M. lysodeikticus* IFO 3333 as in [11] and purified by DEAE chromatography as in section 2. The positions of these proteins on 2D electrophoretogram are shown in fig.1.

The amino acid compositions of MA1 and MA2 were very similar. The N-terminal amino acid sequence was established as:
Met–Asn–Lys–Glu–Gln–Ile–Leu–Glu–Ala
by DABITC-degradation of MA1-protein, while no degradation occurred with MA2-protein by the same technique. Therefore, MA2 must have a blocked amino acid at the N-terminus. Fig.2 shows the tryptic peptide maps of MA1 and MA2 where MA1 has a positively charged peptide (T1) while MA2 has not. In place of T1 of MA1, MA2 has an almost neutrally charged and strongly fluorescamine-stained peptide 'T13' at the position of T13 of MA1. 'T13' of MA2 gave a mixed amino acid composition of T1 (Asp_1 Met_1 and Lys_1) and T13 (Ser_1 Glu_1 Gly_1 Val_1 and Lys_1) of MA1. No significant difference in the peptide positions and amino acid compositions was observed between other peptides of MA1 and MA2. The carboxypeptidase digest of 'T13' of MA2 includes a spot that was stained with chlorine/starch/iodide [19] and co-migrated with *N*-acetyl-D,L-methionine to the anode on thin-layer chromatographic (TLC) electrophoresis at pH 3.5. The presence of an acetyl group in the N-terminal residue of 'T13' of MA2 and intact MA2 was also demonstrated by detecting 1-acetyl-2-dansyl hydrazide. These results indicate that the N-terminal amino acid sequence of MA1 is Met–Asn–Lys and that of MA2 *N*-acetyl-Met–Asn–Lys; the difference between these two proteins probably resides only in acetylation of methionine of the MA2 N-terminus.

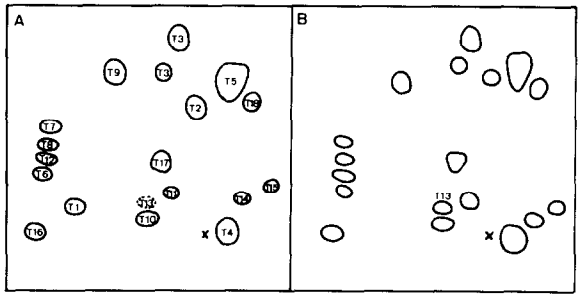

Fig 2. Thin-layer peptide map from tryptic digest of acidic ribosomal proteins from *M. lysodeikticus*: (A) MA1-protein; (B) MA2-protein. The direction of the horizontal electrophoresis is from the cathode (left) to the anode (right) and the direction of the vertical chromatography is from bottom to top. (X) Origin.

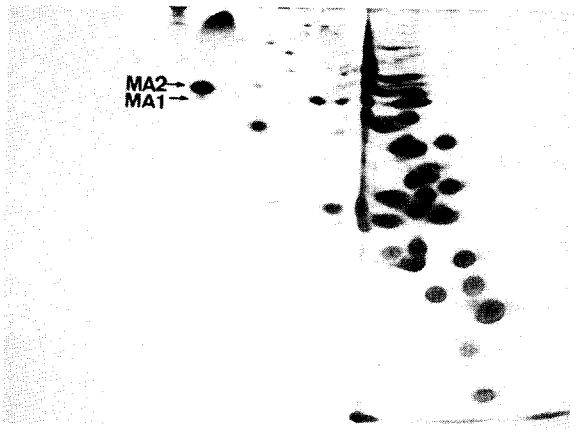

Fig.1. Two-dimensional polyacrylamide gel electrophoresis of 70 S ribosomal proteins from *Micrococcus lysodeikticus*.

The primary structure of MA1 was established by the required overlapping peptides produced by various enzyme digestions. A treatment of the protein with trypsin gave 18 peptides which, with the exception of T4, were completely sequenced with the DABITC method. The largest peptide T4, which had been purified through Sephadex G-50 in 15% acetic acid, produced by pepsin digestion nine peptides having various degrees of overlaps. The whole sequence of T4 was then constructed by aligning these peptic peptides each of which had been sequenced by the DABITC

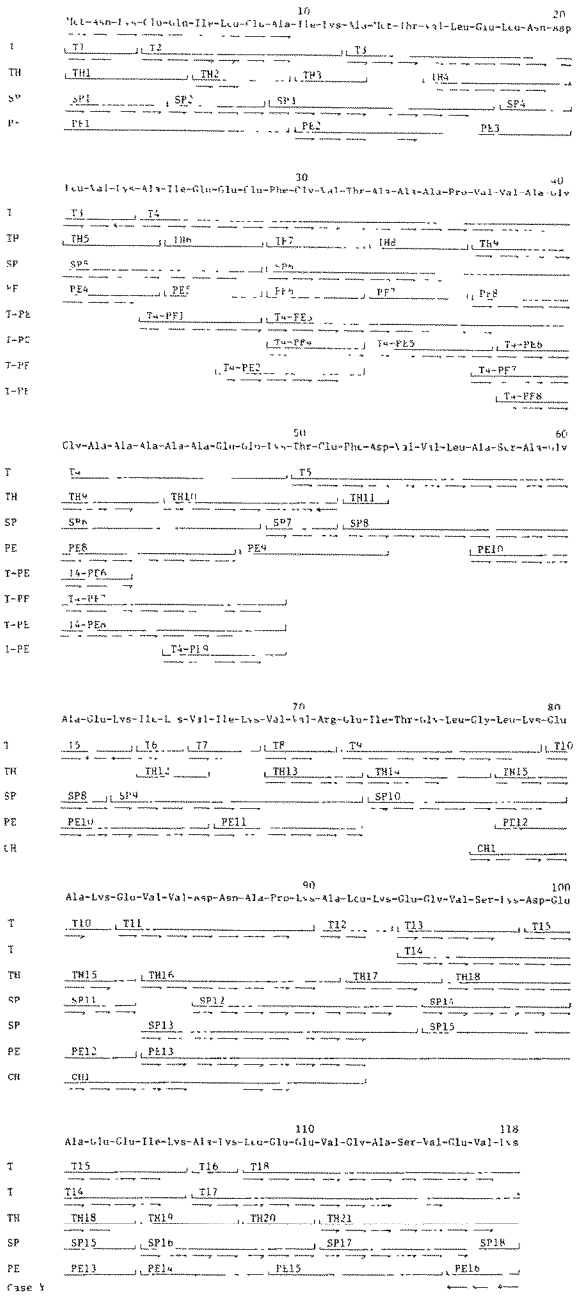

Fig.3. Primary structure of protein MA1 from *M. lysodeikticus*. *Abbreviations*: T, tryptic digestion; TH, digestion with thermolysin; SP, digestion with *Staphylococcus aureus* protease; PE, digestion with pepsin; CH, digestion with chymotrypsin; T-PE, peptic digestion of tryptic peptide T4; case Y, carboxypeptidase Y digestion. Amino acids in the region indicated by (—→) were identified by using the double-coupling method with DABITC/PITC; (—▽) identified as DABTH-Ile or Leu by using the formic acid-ethanol system; (←) identified as amino acids released after carboxypeptidase Y digestion.

method. Alignment of all the tryptic peptides was deduced from the results of DABITC-degradation of intact MA1 together with sequences of the various peptides obtained from thermolysin, pepsin, *Staphylococcus aureus* protease and chymotrypsin digestions of intact MA1. These peptides were separated by thin-layer fingerprinting and their amino acid sequences were determined. The results obtained in this way gave us all the required information for the alignment of the tryptic peptides and reconfirmation of their internal sequence. A treatment of the protein with carboxypeptidase Y released lysine, valine and glutamic acid from the C-terminal region. The combination of the results described so far makes it possible to align all the peptides and therefore to construct the entire amino acid sequence (fig.3). The amino acid composition of MA1 derived from its sequence is: Asp_4, Asn_3, Thr_4, Ser_3, Glu_{20}, Gln_1, Pro_2, Gly_8, Ala_{21}, Val_{16}, Met_2, Ile_7, Leu_9, Phe_2, Lys_{15} and Arg_1. This is in good agreement with the amino acid composition obtained from the complete hydrolysis of the protein. MA1 consists of 118 amino acid residues and has 12 372 M_r.

The primary structure of MA1 from *M. lysodeikticus* was compared with L12 from *E. coli* and BL9 from *B. subtilis* (fig.4). The sequence of a Gram-positive bacterium *M. lysodeikticus* is more similar to that of a Gram-positive bacterium *B. subtilis* (74% similarity) than to that of a Gram-negative bacterium *E. coli* (60%) when whole sequences are compared. Some regions resemble the corresponding regions of L12 (i.e., 47–56 and 95–103) more than those of BL9. On

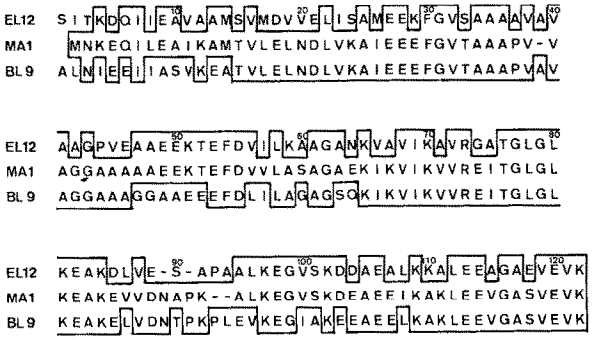

Fig.4. Similarity of amino acid sequences of acidic ribosomal proteins among *E. coli*, *M. lysodeikticus* and *B. subtilis*. *Abbreviations*: EL12, acidic protein from *E. coli*; BL9, acidic protein from *B. subtilis*, MA1, acidic protein from *M. lysodeikticus*. The regions identical to MA1 are boxed.

the other hand, some other regions are the same as those of BL9 (15–38, 65–75 and 104–122) where such a high similarity was not observed with L12 of *E. coli*. As mentioned before, *M. lysodeikticus* contains two-types of acidic proteins (acetylated MA2 and non-acetylated MA1) as *E. coli* does, whereas *B. subtilis* contains only one-type of acidic protein (non-acetylated form). Thus, *M. lysodeikticus* acidic proteins reveal a character somewhat intermediate between *E. coli* and *B. subtilis*. Such an intermediate nature was also recognized in the sequence of 5 S RNA from *M. lysodeikticus* [20].

The region from position 76–84 (Thr–Gly–Leu–Gly–Leu–Lys–Glu–Ala–Lys) is highly conserved in these 3 bacteria, and therefore very likely to be functionally important. It is noteworthy that this region is a positively charged part of the molecule having a predictable β-turn of bend structure [6]. Most of the phosphorylated residues were found to exist within the regions of β-turn in various phosphorylated proteins [21]. Anion-binding sites of *E. coli* L7/L12 were reported to be residues 61,62 and 65 [22]. A possibility then arises that the region close to the highly conserved region, is related to L7/L12 function such as binding of phosphates of GTP molecules in the factor-dependent GTP hydrolysis reaction.

As discussed [9,23], there is very little apparent sequence similarity between the eubacterial acidic ribosomal proteins (MA1, L12 and BL9) and the eukaryotic or metabacterial acidic ribosomal proteins YA1-yeast, eL12-*Artemia salina* and H20-*Halobacterium cutirubrum*).

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