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The complete amino acid sequence of the ribosomal 'A' protein (L12) from *Bacillus stearothermophilus*

W.G. Garland, K.A. Louie, A.T. Matheson and A. Liljas[+]

Department of Biochemistry and Microbiology, University of Victoria, Victoria, British Columbia V8W 2Y2, Canada and [+] *Institute of Molecular Biology, University of Uppsala, S-751 24 Uppsala, Sweden*

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The complete amino acid sequence of the ribosomal 'A' protein (Bst L12) has been determined from *Bacillus stearothermophilus*. The protein contains 122 amino acids and has a composition of Asp_4, Ans_3, Thr_6, Glu_{20}, Gln_2, Pro_3, Gly_9, Ala_{23}, Val_{13}, Met_2, Ile_{11}, Leu_8, Phe_2, Lys_{15}, Arg_1 and a molecular mass of 12737 Da.

Ribosomal protein L12; Amino acid sequence; (Eubacteria)

1. INTRODUCTION

In *Escherichia coli* (Eco) the ribosomal protein Eco L12 is present in the 50 S ribosomal subunit as a 4:1 complex with ribosomal protein Eco L10 [1,2]. Eco L12 forms part of the binding site for several of the factors involved in protein synthesis (see [3]) and is essential for the maximal rate of accuracy in translation [4]. This protein has been crystallized in two fragments and the structure of the C-terminal fragment (residues 47–120) has been determined to 1.7 Å resolution [5].

A similar 4:1 complex has been isolated from *Bacillus stearothermophilus* (Bst) ribosomes [6,7], consisting of ribosomal proteins Bst L12 (BL13) and Bst L10 (BL8). This complex has been crystallized [8]. In order to aid in the determination of the three dimensional structure of the complex we are determining the complete amino acid sequence of the proteins involved in the complex. In this paper we describe the structure of ribosomal protein Bst L12.

Correspondence address: A.T. Matheson, Department of Biochemistry and Microbiology, University of Victoria, Victoria, British Columbia V8W 2Y2, Canada

The ribosomal proteins from *B. stearothermophilus* (Bst) are designated by the equivalent protein in *E. coli* (Eco) as determined from their amino acid sequence similarity. This nomenclature replaces BL13 [6] and BL8 [6] by Bst L12 and Bst L10, respectively. The corresponding *E. coli* proteins are Eco L12 and Eco L10

2. MATERIALS AND METHODS

The Bst L12 was selectively extracted from *B. stearothermophilus* 50 S ribosomal subunits at 46°C with NH_4Cl/ethanol and purified on DEAE-Sephadex as described [8]. The protein was further purified using HPLC (RPSC C-3 column; 0.1% trifluoroacetic acid/acetonitrile, 0–60% gradient over 60 min). The purity of the protein was confirmed using 2D-PAGE [9]. The samples were hydrolyzed in constant boiling HCl for 24 h and analyzed on a Beckman 119CL amino acid analyzer [10].

Bst L12 was remarkably resistant to digestion by various proteases. Chemical modification of the protein by maleylation of lysine residues [10] or performing the proteolytic digestion in 4 M urea to make the protein more susceptible to hydrolysis, was often required to obtain the desired peptides.

The digestion of the protein by various proteolytic enzymes was carried out using the standard

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methods (see [10]). The following enzymes were used: (i) thermolysin (Sigma) using the native protein; (ii) trypsin (Sigma) using the native protein in 4 M urea or the maleylated protein; (iii) chymotrypsin (Sigma) using the maleylated protein; (iv) carboxypeptidase B (Boehringer Mannheim) using the native protein.

The peptides obtained were fractionated on an HPLC column (Beckman RPSC C-3 column, gradient 0.1% TFA/ACN) and further identified on a Swank-Munkres gel [11]. The peptides used for sequencing were chosen in part by their size and amino acid composition.

In the initial stages of this project the protein and peptides were sequenced using either the Beckman model 890 spinning-cup sequenator or the manual phase method [12]. An Applied Biosystems 470-A gas-phase sequenator with on-line HPLC was used in the latter part of the project.

3. RESULTS AND DISCUSSION

The complete amino acid sequence of Bst L12 is shown in fig.1. Using the gas-phase sequenator the first 71 residues were obtained from the intact protein. When the lysine groups were blocked by maleylation and the modified protein hydrolyzed by trypsin at the single arginyl residue, the peptide corresponding to the C-terminal portion was sequenced giving the sequence from residue 75 to 116. The two small segments remaining were sequenced in the following manner. The sequence of region 72–75 was obtained using a thermolysin peptide containing residues 49–82, while the se-

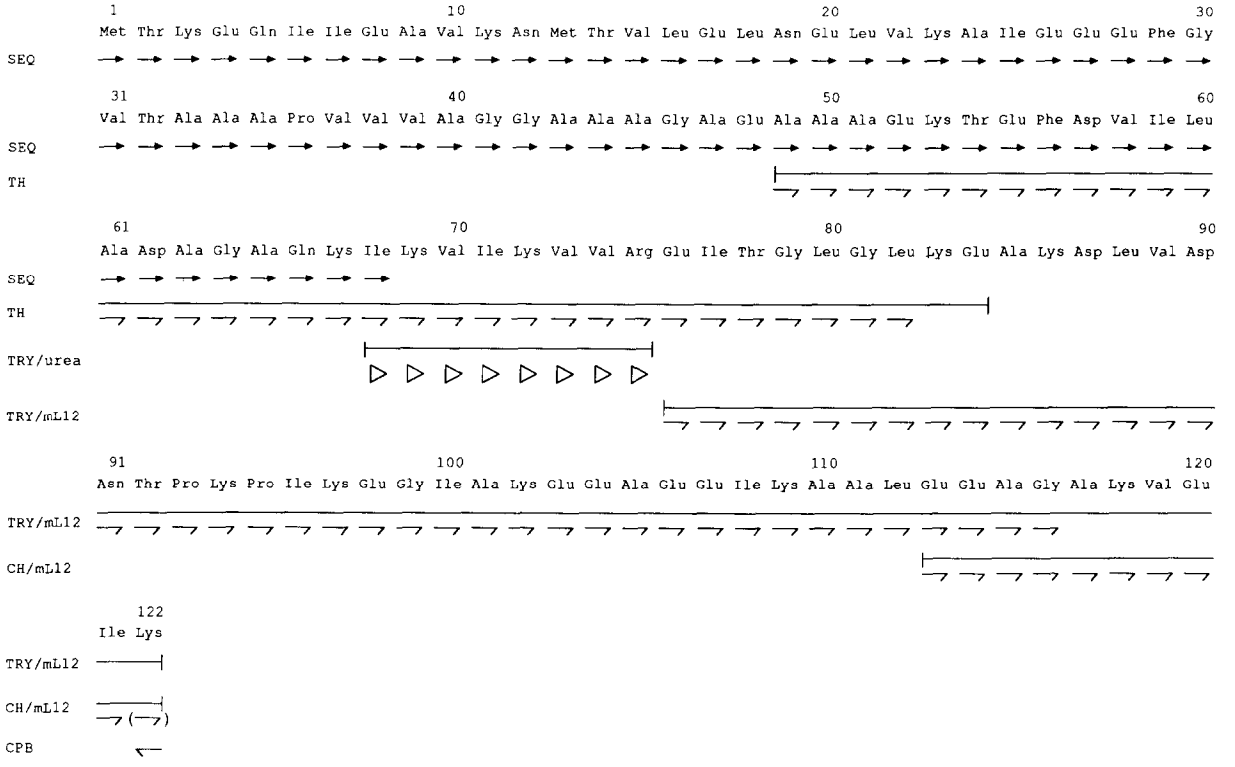

Fig.1. The amino acid sequence of ribosomal protein L12 from *B. stearothermophilus*. The methods used to determine the sequence of the peptides are indicated as follows: (→) automatic Edman degradation of intact protein (gas-phase sequenator); (⇀) automatic Edman degradation of peptides (gas-phase sequenator); (▷) DABITC/PITC double-coupling method [12]; (↽) carboxypeptidase hydrolysis; TH, TRY/mL12, TRY/urea, CHY/mL12, CPB indicates peptides obtained by digestion with thermolysin, trypsin (maleylated L12), trypsin (4 M urea), chymotrypsin (maleylated L12) and carboxypeptidase B, respectively.

quence of region 117–122 was obtained from a chymotryptic peptide containing residues 113–122.

Our earlier report [13] on the first 37 residues of this protein contained one error in that glutamine was incorrectly reported for residue 8. The C-terminal region of this protein was reported [14] to contain -Glx-(Ala-Gly-Ala-Glx)-Val-Glx-Ile-Lys-COOH. The correct sequence has now been determined as -Glu-Ala-Gly-Ala-Lys-Val-Glu-Ile-Lys-COOH.

The amino acid composition of Bst L12 is shown in table 1. The protein contains 122 residues and has an M_r of 12737.

The hydrophilicity profile of Bst L12 is shown in fig.2 and is compared to an equivalent profile for the L12 protein from *E. coli* (Eco L12). As might be expected from the large amount of sequence similarity between these two proteins, the hydrophilicity profile is very similar.

The Bst L12 protein shows a large amount of sequence similarity with other eubacterial L12 proteins. Earlier studies on the sequence similarity of eubacterial L12 proteins using a cluster analysis program [17] placed the *B. stearothermophilus*

Table 1

The amino acid composition of ribosomal protein Bst L12

Amino acid	Residues	Amino acid analysis
Asp	4	7.3
Asn	3	
Thr	6	6.0
Ser	0	0
Glu	20	22.2
Gln	2	
Pro	3	3.8
Gly	9	9.3
Ala	23	22.5
Val	13	11.3
Met	2	2.4
Ile	11	9.5
Leu	8	8.5
Tyr	0	0
Phe	2	2.3
His	0	0
Lys	15	14.1
Arg	1	1.1
Total	122	122

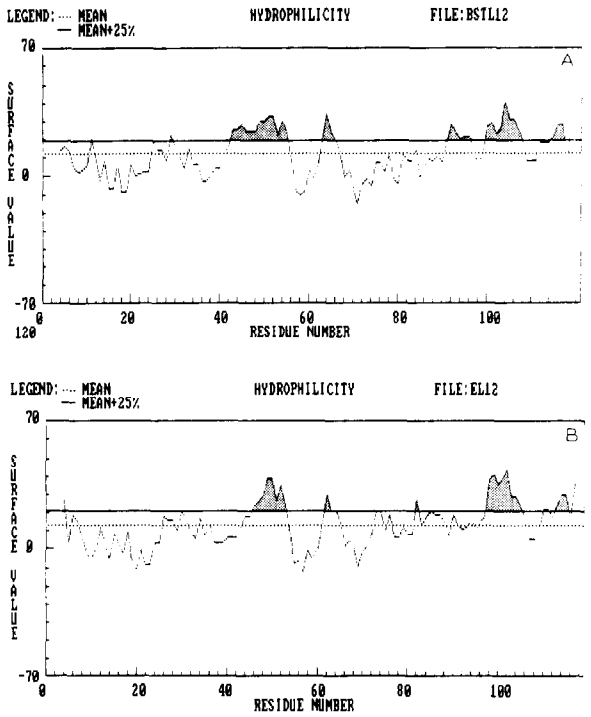

Fig.2. The hydrophilicity of ribosomal protein L12 from (A) *B. stearothermophilus* (Bst L12) and (B) *E. coli* (Eco L12) [15] using the SURFPLOT program of Parker et al. [16].

protein in a subgroup with *Clostridium pasteurianum, Micrococcus lysodeikticus* and *Bacillus subtilis*.

The sequence similarities in relation to structural features are discussed elsewhere [5,18].

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