HOMOLOGIES IN PROCARYOTIC RIBOSOMAL PROTEINS: ALANINE RICH ACIDIC PROTEINS ASSOCIATED WITH POLYPEPTIDE TRANSLOCATION

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1. Introduction

Translocation of the growing peptide chain in the synthesis of proteins is mediated to some extent by certain novel acidic ribosomal proteins [1–16]. These proteins, designated L7 and L12 in *Escherichia coli*, are localized on the 50 S ribosomal subunit and differ only in the acetylation state of the N-terminal serine [2]. The existence of structural homologues to these alanine rich, acidic proteins in most procaryotic and eucaryotic cells has been suggested by recent immunological experiments by I. G. Wool and G. Stöffler (personal communication).

Their ubiquity as ribosomal constituents indicate a high degree of conservation in these molecules during the evolution of the ribosome. Hence, comparative structural and functional analyses of these unusual polypeptides could reflect both the genetic modifications possible in the evolution of the ribosomal unit as well as yield some insight into the nature of the 'active sites' within the molecules. As a first progression in this direction we report herein the isolation, chemical characterization and N-terminal sequence analysis of an L7–L12 equivalent from *B. stearothermophilus*. We compare the structural features with the *E. coli* polypeptides, a comparison that is of particular note in view of the considerable interest in the structure, function and evolution of the genetic translation apparatus.

2. Materials and methods

Ten grams of 50 S ribosomal subunits from *B. stearothermophilus* strain 10 were prepared as described previously [17] and the acidic proteins selectively extracted by the methods of Hamel, Koka and Nakamoto [13]. The three proteins liberated by this procedure were concentrated by precipitation with 2.25 vol. of −20°C acetone and the L7–L12 equivalents separated and purified as described for *E. coli* by Möller et al. [1]. Their purity was established by a 2-dimensional acrylamide electrophoresis method similar to that described by Kaltschmidt and Wittmann [18] except that the first dimension discs, run at pH 8.6, were 8% with respect to acrylamide. The homology and identity of the L12–L7 equivalents from *B. stearothermophilus* was confirmed by the immunochemical reactivity of the isolated pure proteins to the antisera produced in New Zealand red rabbits against purified *E. coli* L12 [15]. This was further evidenced by amino acid analysis in a Durrum D-500 amino acid analyser, following protein hydrolysis for 24 and 72 hr in 6 N HCl at 110°C, as well as by sequence analysis by automatic Edman degradation [19] in a Beckman Model 890 C Sequencer. For sequence runs the thiazolinone derivatives were hydrolyzed separately with 6 N HCl and HI [20] at 130°C for 22 hr, and the amino acids formed were analyzed with the amino acid analyzer. Molecular weights were determined by the dodecyl sulfate gel electrophoretic procedure of Weber and Osborn [21].

3. Results and discussion

Treatment of *B. stearothermophilus* 50 S ribosomal subunits with ethanol and ammonium chloride results

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in the release of 2 acidic proteins A_1 and A_2, the separation of which is depicted in fig. 1. The chemical similarity of the A_2 proteins to the L12 protein of *E. coli* is evidenced in table 1. While both sets are alanine rich and lack tyrosine, histidine and cysteine, the thermophilic proteins contain more lysine, isoleucine, glutamic acid and threonine and less serine, alanine and valine. Protein A_1 is very similar if not identical to A_2.

Structural homology to the *E. coli* proteins is further evidenced by the immunological reactivity of *B. stearothermophilus* proteins A_1 and A_2 to the antisera to *E. coli* L12 shown in fig. 2. Both A_1 and A_2 from *B. stearothermophilus* cross react with anti-*E. coli* L12. As shown below, A_2 corresponds to the equivalent *E. coli* protein L12, and preliminary results indicate that A_1 like L7 in *E. coli* may have a blocked N-terminus. Proteins A_2 and A_1 from the thermophile give molecular weights of 12 000 ± 500 on dodecyl sulphate electrophoresis which again agrees well with the published values for L7 and L12 in *E. coli* [1—3].

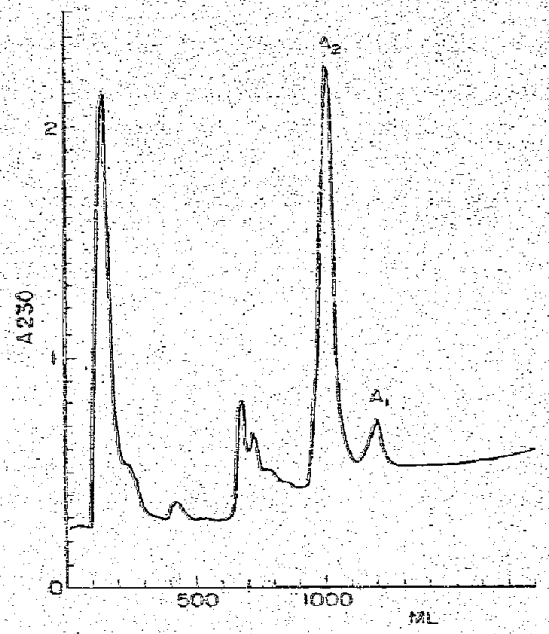

Fig. 1. DEAE-cellulose chromatography of proteins extracted from *Bacillus stearothermophilus* 50 S ribosomes by 1 M NH_4Cl–50% ethanol. See Methods for details.

Table 1
Amino acid composition of *Bacillus stearothermophilus* acidic protein (A_2)

Amino acids	Mole %[a, g]	Molar ratio[d]	Proposed residues	Residues of *E. coli* L12[e]
Aspartic acid	5.84	7.10	7	7
Threonine	4.82[b]	5.86	6	3
Serine	0.46[b]	0.56	0~1	6
Glutamic acid	19.23	23.38	23	17
Proline	2.15	2.61	2~3	2
Glycine	7.25	8.81	9	8
Alanine	18.18	22.11	22	28
Valine	9.88[c]	12.01	12	16
Methionine	1.44	1.75	2	3
Isoleucine	8.11[c]	9.86	10	6
Leucine	6.58	8.00	8	8
Tyrosine	0.00	0.00	0	0
Phenylalanine	1.44	1.75	2	2
Histidine	0.00	0.00	0	0
Lysine	13.95	16.96	17	13[f]
Arginine	0.66	0.80	1	1
Total	99.99	121.56	121~123	120

[a] Mean values of 24 and 72 hr hydrolysis.
[b] Extrapolated zero-time values.
[c] 72-hr value only.
[d] Each value is adjusted so that the value for leucine is 8.00.
[e] Taken from the sequence data of Terhorst et al. (Eur. J. Biochem. 34, 138 (1973)).
[f] 1 residue of monomethyllysine is included.
[g] No cysteic acid was detected after performic acid oxidation and hydrolysis.

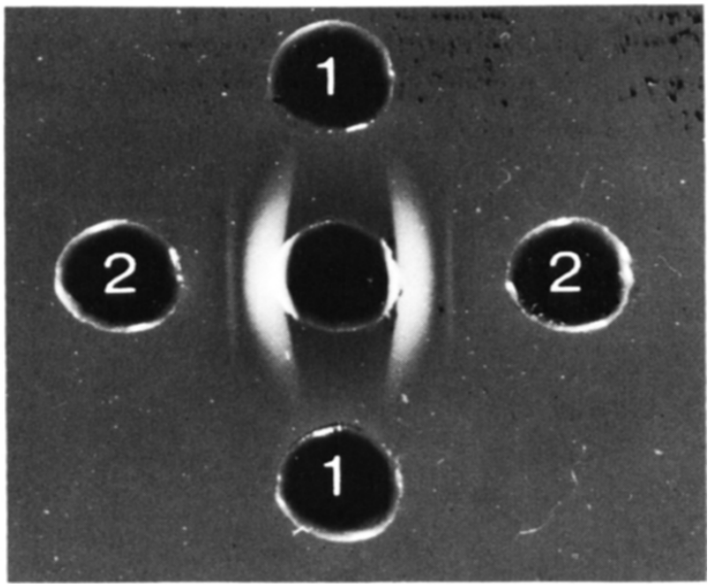

Fig. 2. Immunochemical reactivity of *B. stearothermophilus* proteins that correspond to *E. coli* proteins L7, L12. Centre well contained. Anti *E. coli* L12. (2) *B. stearothermophilus* A_2, 1 mg/ml in phosphate buffered saline pH 7.0 (1) 0.9% saline.

Furthermore, the molar ratios of A_1 and A_2 vary from one batch of cells to another depending apparently on the growth conditions. Similar variations have been reported for *E. coli* L7 and L12 [20]. Similar to the mobility of *E. coli* L7–L12, on two dimensional electrophoresis proteins A_1 and A_2 from *B. stearothermophilus* migrate to a position shown in fig. 3.

At a molecular level, the acetylation state of the N-terminal serine in *E. coli* L7 and L12 has been suggested as essential for the proper functioning of these proteins in EFG and EFT dependent GTPase reactions [1, 4, 10]. When the N-terminal region of the purified A_1 proteins from *B. stearothermophilus* was sequenced the N-terminal residue was found to be methionine rather than serine. The first 15 residues of the A_2 protein of *B. stearothermophilus* are: Met-Thr-Lys-Glu-Gln-Ile-Ile-Gln-Ala-Val-Lys-Asn-Met-Ser-Val-.

The absence of an N-terminal serine and the presence of *N*-methionine, at first surprising, it not unlike the data reported for the alcohol dehydrogenase from *B. stearothermophilus* [23] where the acetylated

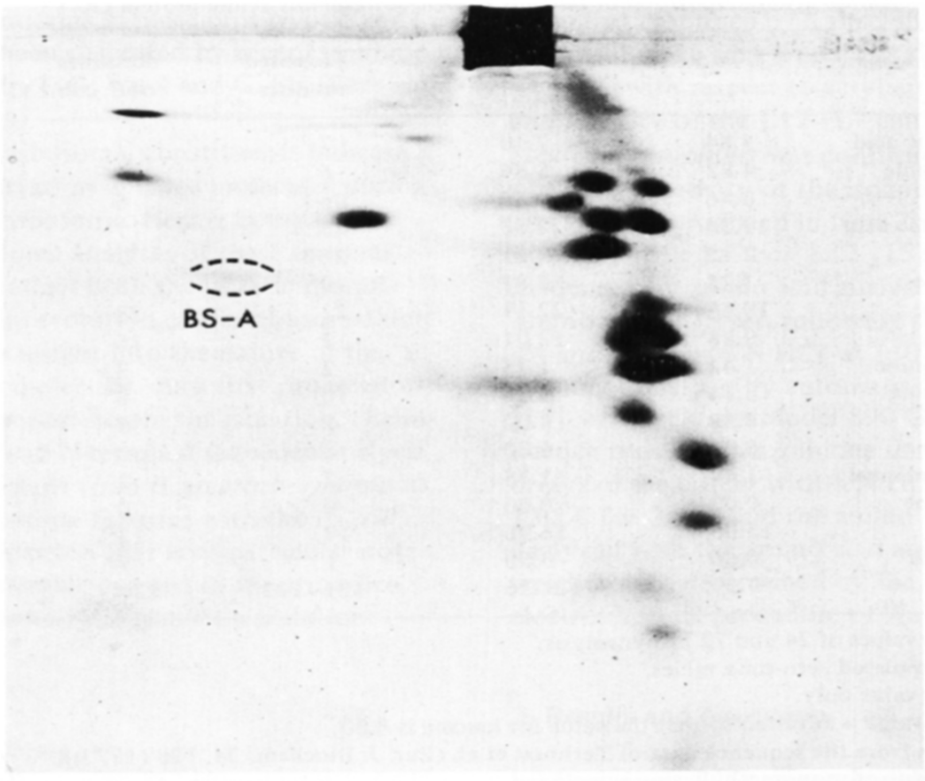

Fig. 3. Two dimensional electropherogram of *B. stearothermophilus* 50 S proteins indicating the position of the 'A' protein spot.

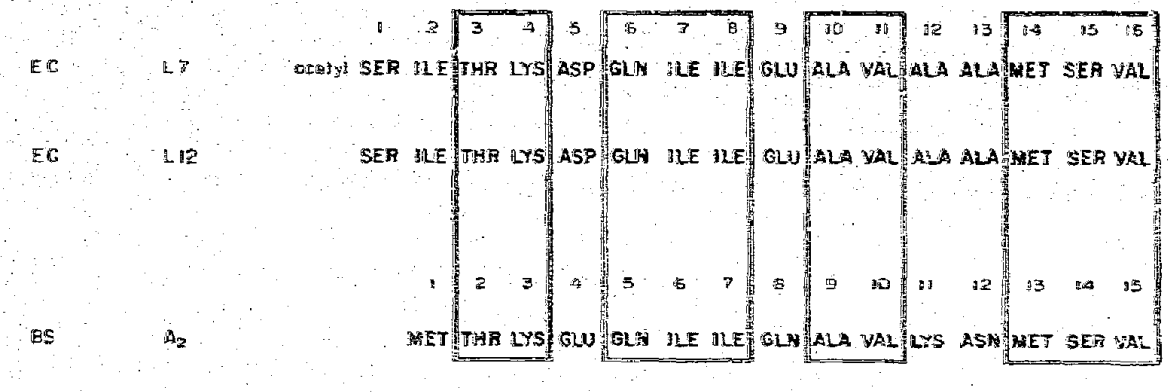

Fig. 4. Comparison of the N-terminal amino acid sequences in L7–L12 from *E. coli* and *B. stearothermophilus* A_2 protein. The sequences are aligned so that valine–16 (ref. [3]) corresponds to valine 15 in *B. stearothermophilus* A_2. Residues enclosed by double lines are identical.

serine is also replaced by a methionine. Furthermore, it is of interest that the alanine rich acidic proteins from the extreme halophilic bacterium, *Halobacterium cutinubrum*, also has a methionine *N*-terminal (Oda et al. unpublished observations).

For the A proteins from *E. coli* and *B. stearothermophilus*, it is possible to show homologies in the chains if the methionine *N*-terminal of the *B. stearothermophilus* protein is aligned with the isoleucine-2 in the *E. coli* L7 or L12 proteins [fig. 4]. If this is done then 10 out of the first 16 residues (62.5%) are identical in the two chains. In those that differ, met-1, glu-4 and gln-8, could have arisen by point mutation and the first two are as well conservative changes. Similar homologies have been shown for some 30 S proteins [24]. However, the degree to which this homology is continued in the primary structure of the A_1 and A_2 proteins awaits further sequence analyses of these proteins in *B. stearothermophilus*.

In view of the homologies in primary structure and N-substitution it is possible that the N-terminal region of these proteins has important functional roles. Therefore in order to elucidate the role of these polypeptides in total ribosomal function it is necessary that the chemical properties of the amino terminus be determined. Such studies have now been initiated in our laboratory.

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