# RIBOSOMAL PROTEIN S1/S1A IN BACTERIA

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## 1. Introduction

Recently, *Escherichia coli* ribosomal protein S1 has been shown to have important and diverse functions in the logistics of mRNA processing during in vitro protein synthesis [1–9] and Qβ replication [10–12]. In the course of an RNA–protein interaction study with S1, we discovered that there are two distinct large molecular weight ribosomal proteins, both of which have been designated as S1 by different laboratories. The two proteins have been purified, characterized, and designated as S1 and S1A.

In view of the functions ascribed to S1 in the interaction with mRNA [1–9], 16 S RNA [13] and other proteins (Qβ replicase) [11,14], the question naturally arises as to the universality of the S1 type protein in various organisms. Indeed, it has been stated that certain specialized bacterial types lack structural and functional S1 homologues [15]. This would be surprising in light of the translational control properties to this moiety in *E. coli* cells.

We have demonstrated the presence of S1 and/or S1A homologues in a variety of taxonomically and physiologically distinct bacteria by immunological criteria already used to study ribosomal protein homologies [16].

## 2. Materials and methods

*E. coli* Q13 was purchased from General Biochemicals. *E. coli* MRE 600 was grown in the medium of Evans, Herbert and Tempest [17]. *Bacillus stearothermophilus* [18], *Halobacterium cutirubrum* [19], and *Neisseria perflava* [20] were grown as described previously. *Arthrobacter glacilis* was grown at 4°C in a medium consisting of 1 g/liter yeast extract, 1 g/liter tryptone and 1 g/liter dextrose, at pH 7.0.

Ribosomes were prepared from these cells as previously described [21,22].

The ribosomes were washed with either 1 M or 2 M $NH_4Cl$ in Tris–HCl buffer (0.02 M Tris, 0.01 M $MgCl_2$, 0.006 M BME, pH 7.6). The supernatant

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from this wash was then fractionated by $(NH_4)_2SO_4$ precipitation [1], and the appropriate fraction was used to purify the S1 type protein by one of the methods of Miller [1], Inouye [3], or Hindennach [23]. Protein S1A was then extracted from the $NH_4Cl$ washed ribosomes by treating them with 2 M LiCl and 2 M urea. Further purification of protein S1A was accomplished by procedures similar to those used for the purification of protein S1. Proteins from *H. volcani, H, 'X'* and *Vibrio costicola* were the kind gift of Dr A. T. Matheson. *E. coli* protein S1, when not purified in this laboratory, was generously donated by Dr A. Wahba.

Purity of the protein samples was established by polyacrylamide gel electrophoresis in 8 M urea, at pH 4.5 and pH 8.7. Molecular weight determinations were performed by SDS–polyacrylamide gel electrophoresis [24].

Immunological cross-reactivity of pure proteins and crude protein fractions was demonstrated using the Ouchterlony double diffusion method [25] with rabbit antisera raised against purified *E. coli* S1 and S1A and *H. cutirubrum* S1.

Amino acid compositions and N-terminal sequences were determined as previously described [26].

## 3. Results and discussion

A simple procedure was established for the separation of the two large molecular weight proteins, S1 and S1A, from *E. coli* ribosomes. The 2 M ammonium chloride wash removed protein S1 but not S1A from the ribosomes. Protein S1A was then extracted from the S1 stripped ribosomes by washing in 2 M lithium chloride and 2 M urea. The two proteins were purified to homogeneity by the methods already described. Recently, Subramanian et al. [27] described the purification of what seems to be protein S1A. We are in agreement that the molecular weight of S1A is smaller than that of S1. They quoted a molecular weight of 80 000 for S1 and 70 000 for S1A [27]. However, we find that the molecular weights of the two proteins are 72 000 and 68 000, respectively (fig.1). The generally accepted values for S1 are between 65 000 and 68 000 [28]. Table 1 lists the molecular weights of the large proteins prepared from ribosomes of a variety of bacteria. The molecular

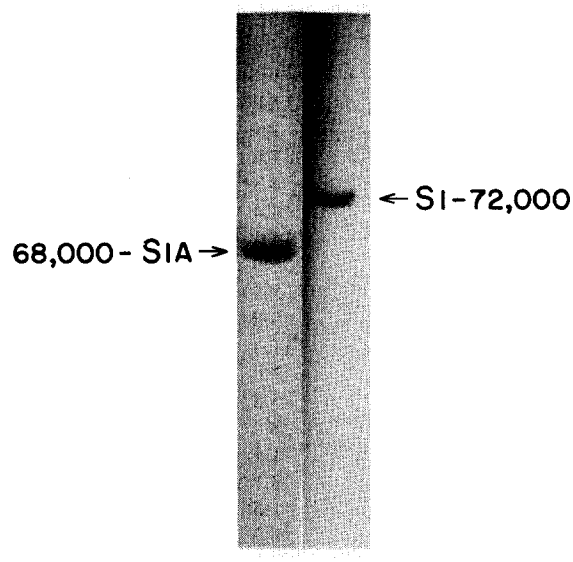


Fig.1. Molecular weight determinations of ribosomal proteins S1 and S1A from *E. coli*. The analysis was done by the SDS–polyacrylamide gel electrophoresis method [24] using the protein standards bovine serum albumin, ovalbumin, myoglobin and ribonuclease.

weights of these proteins were estimated to vary from 64 000 for the *B. stearothermophilus* protein to 81 000 for the *H. cutirubrum* protein.

The amino acid compositions of the ribosomal large molecular weight proteins are compared in table 2. The two *E. coli* ribosomal proteins, S1 and S1A, are quite similar in their amino acid content except for small variations in the residues glycine, alanine, phenylalanine and histidine. The amino acid

Table 1
Molecular weights of S1 type ribosomal proteins from different bacterial sources

| Organism | Molecular weight |
|---|---|
| *E. coli* MRE 600 (S1) | 72 000 |
| *E. coli* MRE 600 (S1A) | 68 000 |
| *B. stearothermophilus* | 64 000 |
| *H. cutirubrum* | 81 000 |
| *Halobacterium 'X'* | 73 000 |
| *A. glacilis* | 67 000 |

Molecular weight determinations of 'S1' type proteins from ribosomes of a variety of bacteria. Conditions are as stated in fig.1.

Table 2
Amino acid compositions of ribosomal protein 'S1' et al.

| Residue | ES1 | ES1 | ES1 | ES1 | ES1A | BS1 | HCS1 | A-Protein |
|---|---|---|---|---|---|---|---|---|
| Asp | 11.2 | 11.6 | 11.8 | 11.1 | 9.7 | 10.0 | 15.2 | 10.2 |
| Thr | 4.6 | 4.3 | 4.6 | 5.1 | 6.5 | 5.7 | 5.8 | 6.2 |
| Ser | 4.7 | 4.4 | 4.7 | 4.2 | 3.4 | 4.5 | 5.2 | 3.5 |
| Glu | 12.9 | 14.1 | 14.2 | 14.2 | 12.1 | 13.4 | 14.5 | 12.4 |
| Pro | 2.0 | 1.9 | 2.0 | 3.5 | 2.0 | 2.9 | 2.1 | 2.7 |
| Gly | 8.5 | 8.9 | 9.7 | 8.7 | 11.5 | 9.0 | 10.0 | 10.9 |
| Ala | 9.1 | 9.3 | 8.7 | 9.1 | 13.0 | 10.8 | 12.0 | 12.7 |
| Val | 11.3 | 11.3 | 12.0 | 9.9 | 12.8 | 7.5 | 10.1 | 9.9 |
| Met | 2.0 | 1.0 | 0.6 | 2.1 | 2.5 | 2.3 | 1.8 | 2.9 |
| Ile | 5.7 | 5.3 | 5.1 | 5.4 | 4.7 | 5.0 | 4.7 | 5.7 |
| Leu | 8.6 | 8.2 | 8.3 | 7.1 | 7.3 | 8.8 | 8.3 | 8.0 |
| Tyr | 1.7 | 1.1 | 0.6 | 1.7 | 1.2 | 2.5 | 0.7 | 1.0 |
| Phe | 2.7 | 3.1 | 3.1 | 3.2 | 1.3 | 2.6 | 1.3 | 1.6 |
| His | 1.9 | 1.5 | 1.7 | 1.7 | 0.3 | 1.5 | 1.5 | 0.5 |
| Lys | 7.8 | 8.2 | 7.5 | 7.1 | 7.2 | 7.5 | 3.5 | 7.4 |
| Arg | 5.4 | 5.5 | 5.5 | 5.8 | 4.3 | 5.5 | 3.6 | 4.5 |
| | [a]Kaltschmidt | [a]Kurland | [a]Moore | [a]Tal | Ottawa | Ottawa | Ottawa | Subramanian [27] |

[a] Data from Tal et al. [4]

The amino acid compositions of the 'S1' type proteins of ribosomes from *E. coli, B. Stearothermophilus* and *H. cutirubrum*. The analyses were done as described in the text.

composition of the protein from *B. stearothermophilus* showed a striking similarity to *E. coli* S1. The two proteins varied only in their valine content. The protein from *H. cutirubrum* was also considered to be similar to the proteins from *E. coli*, taking into account the usual alterations that have been observed in the acidic and basic amino acid residues in most proteins of the extreme halophile when compared to the mesophile [29]. The recent publication of the amino acid composition of *E. coli* A-protein [27] (table 2) showed that it is identical to protein S1A except for a small variation in the content of the amino acid residue valine.

The N-terminal sequence of the two *E. coli* proteins, S1 and S1A, show no homologies up to residue 5 (fig.2). This fact correlates with the immunological data presented in fig.3 and table 3. No cross-reaction was observed for the two proteins against antisera prepared from either protein. Therefore little or no sequence homology was expected for the two proteins.

Antisera prepared from *E. coli* ribosomal proteins S1 and S1A and from the *H. cutirubrum* ribosomal protein HcS1 were used to test for the presence of homologous proteins in ribosomes from several different bacterial sources (table 3). Antiserum to *E. coli* S1 cross-reacted with ribosomal proteins from *H. cutirubrum, N. gonorrhoeae, N. perflava,* and *A. glacilis.* The cross-reaction with *B. stearothermophilus* protein and S1 antisera was questionable since only occasionally a very faint band was observed. Antiserum to protein S1A cross-reacted with proteins from *B. stearothermophilus, Halobacterium 'X', N. gonorrhoeae* and *N. perflava.* Finally, the antiserum to HcS1 cross-reacted with the ribosomal proteins from *E. coli* (S1), *B. stearothermophilus, N. perflava, N. gonorrhoeae, H. volcanii* and *A. glacilis.* These data clearly show the widespread distribution of large

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          1   2   3   4   5
S1        Met-Thr-Glu-Ser-Phe-

          1   2   3   4   5   6   7   8   9   10  11  12
S1A       Ala-Ala-Lys-Asp-Val-Lys-Phe-Gly-Asn-Asp-Ala-Arg-
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Fig.2. The N-terminal amino acid sequence of *E. coli* ribosomal proteins S1 [10] and S1A [30].

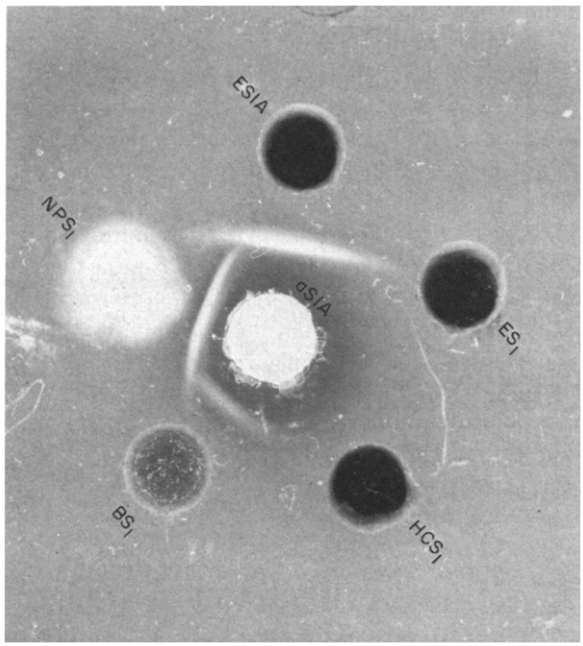


Fig.3. Ouchterlony double-diffusion of antibody against *E. coli* S1A. The centre well contains antibody to *E. coli* S1A (aS1A). The peripheral wells contain *E. coli* S1A (ESIA), *E. coli* S1 (ES1), *E. cutirubrum* S1 (HCS1), *B. stearothermophilus* S1 (BS1), *N. perflava* S1 (NPS1). Each S1 sample was at a concentration of 10 μg.

molecular weight 'S1' type proteins in both gram negative and gram positive bacteria. The conservation of the structure of these proteins in different bacterial ribosomes suggests important functional relationships in either protein biosynthesis or in the control of this process. The importance of protein S1 in protein biosynthesis has been studied [1–9], although its exact function is still unknown. Recently the presence of protein S1A on the ribosome was documented to be related to cell growth [27]. The amount of protein S1A associated with the ribosome was directly proportional to increasing growth phase. Regardless of its absolute cellular function, protein S1A can be considered an integral ribosomal protein for the following reasons:

(1) It is not washed off the ribosome with 1 M ammonium chloride and can only be successfully removed with either lithium–urea or acetic acid methods of ribosomal protein extraction.

(2) It is not a membrane associated ribosomal contaminant since it is not present in the 12 000 × *g* or the 40 000 × *g* pellets, nor in the 160 000 × *g* supernatant.

(3) Its wide distribution in most bacterial 30 S ribosome subunits.

Table 3
Immunological cross-reactivity of ribosomal 'S1' type proteins from different bacterial sources to *E. coli* anti-S1, *E. coli* anti-S1A, and *H. cutirubrum* anti-S1

| Organism | Anti-S1 *E. coli* MRE 600 | Anti-S1A *E. coli* MRE 600 | Anti-S1 *H. cutirubrum* |
|---|---|---|---|
| *E. coli* MRE 600 S1 | + | – | + |
| *E. coli* MRE 600 S1A | – | + | – |
| *E. coli* Q13 S1 | + | + | + |
| *B. stearothermophilus* | ? | + | + |
| *H. cutirubrum* | + | – | + |
| *Halobaterium 'X'* | – | + | – |
| *N. gonorrhoeae* | + | + | + |
| *N. volcanii* | – | – | + |
| *A. glacilis* | + | – | + |
| *T. aquaticus* | -- | + | – |

The immunological cross-reactivity of ribosomal 'S1' type proteins from a variety of bacteria against antisera to *E. coli* S1 and S1A and *H. cutirubrum* S1. Antisera were concentrated five-fold prior to use.

It is of interest to note that in the first report on the N-terminal sequences for ribosomal proteins, we reported the sequence of S1A for S1 [30]. It was (S1A) the only high molecular weight acidic protein that we found on highly purified 30 S ribosome subunits prepared and purified as described in the literature [23]. Indeed it possessed the electrophoretic and chromatographic properties of the S1 alluded to in the literature as a protein homologous to subunit 1 of Qβ replicase [3]. Unfortunately the protein which we designate as S1 herein does not migrate in the standard 2-dimensional electrophoretic system used to define the nomenclature for *E. coli* ribosomal proteins (at least not in our laboratory). In order to keep the nomenclature as simple as possible we suggest that the protein that is recognized by all as having a mRNA binding function be designated as S1 and the other tightly bound acidic protein, S1A.

In view of the results presented herein it appears that a careful reassessment of the function of S1 and S1A is necessary especially since many groups might be utilizing a mixture of the two moieties and in view of the multiple functions suggested for S1 (table 4). We suggest a rigorous sequence-based definition be applied to the protein one chooses to us in the analysis of S1/S1A function. As a further caution it should be taken into account that our very recent results indicate that there may exist some further heterogeneity in the S1/S1A proteins. We have detected immunologically similar proteins that differ considerably in their chromatographic behaviour (Hasnain et al., unpublished results and M. Grunberg-

Table 4
A compendium of 'S1' properties

1. S1 is not a ribosomal protein [31].
2. S1 is interference factor *i* [3].
3. S1 is subunit 1 of Qβ replicase [32].
4. S1 stimulates protein synthesis [33].
5. S1 inhibits protein synthesis directed by polypyrimidines [3].
6. S1 has no effect on protein synthesis [34].
7. S1 stimulates the binding of specific messenger RNA to the ribosome [35].
8. S1 binds the 3'-end of 16 S RNA and stabilizes the interaction of mRNA to the 16 S rRNA [13].
9. S1 has two binding sites for polynucleotides, one site binds single stranded DNA or RNA, another binds only RNA [36].
10. S1 has a specific binding site on Qβ RNA [12].
11. S1 is a melting protein and converts helical poly(U), poly(C,U), and acidic or neutral poly(C) to forms undistinguished from their denatured forms [31,38].
12. S1 is involved in the interaction of 30 S–50 S subunits [39].
13. S1 is strain specific in *E. coli* [3].
14. S1 does not exist in all bacteria, i.e., *B. stearothermophilus* [5].
15. S1 cross-reacts immunologically with anti-sera to L12 [40].
16. S1 is 50 000 or 60 000 or 67 000 or 69 000 or 72 000 or 73 000 or 81 000 mol. wt.
17. S1 is a fractional protein on the ribosome [41].
18. S1 is a unit protein in polyribosomes [42].
19. S1 is removed by washing ribosomes in high salt solutions [43].
20. The amino-terminal sequence of S1 begins with Ala–Ala–Lys [30].
21. S1 can be purified by CMC column chromatography from salt-washed ribosomes [23].
22. S1 can be separated into two species, one which binds to a column denatured DNA and one which does not (Grunberg-Manago personal communication).
23. S1 binds 23 S rRNA [44].

Manago, personal communication). Controlled physiological studies are underway to assess whether these variations are growth related or some subtle function-related chemical modification.

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