COMPARISON OF RIBOSOMAL PROTEIN S1 AND THE A-PROTEIN FROM *ESCHERICHIA COLI*

Lack of structural or functional homology

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Received 15 January 1979

1. Introduction

The 30 S subunits of *Escherichia coli* ribosome contain (even after washing with 1 M NH_4Cl) two high molecular weight proteins of similar size (mol. wt $68–76 \times 10^3$) and amino acid composition of which the larger one corresponds to protein S1 while the other is denoted A-protein [1]. The content of A-protein in the ribosomes is dependent on the growth cycle of the bacterial culture; the content of protein S1, on the other hand, is relatively constant ([1], A.R.S., unpublished).

A protein very similar to A-protein in properties has been isolated from *E. coli* [2]. It was designated as S1A, presumably to stress its possible homology to protein S1. For example, from another bacterial species *(Neisseria gonorrhoeae)* an 'S1-type' protein was noted to react with antibodies against both S1 and S1A from *E. coli* MRE600 indicating such a homology.

Protein-A is obtained in pure form during the purification of protein S1 from post-exponential *E. coli* ribosomes [3]. By isolating large amounts of A-protein in connection with our studies on protein S1 [3,4], it became possible to compare structure/function properties of proteins S1 and A in detail. The results presented below show an absence of observable structural/functional homology between these two proteins isolated from *E. coli* ribosomes.

2. Materials and methods

Proteins S1 and A were isolated from *E. coli* (MRE600 and A19) ribosomes as in [1,3]. Translation of phage f_2 RNA in vitro was carried out as in [5]; further specific details are given in the legend to fig.3. Antibodies against the purified proteins were raised in rabbits and analyzed by double immunodiffusion [6]. The antisera were subjected to $(NH_4)_2SO_4$ fractionation and Sephadex G-150 chromatography to obtain IgG antibodies. The N-terminal sequences were determined using a modified Beckman sequenator [7]. Protein determination and dodecylsulfate–gel electrophoresis were done as in [1].

3. Results and discussion

3.1. *Terminal sequence*

Figure 1 shows the N-terminal sequences of proteins S1 and A up to residue 23–24. No sequence homology is apparent in this region of the two proteins. The N-terminal sequence of S1 up to residue 5 and of S1A up to residue 12 have been published (original references in [2]) and these data are in

	1				5				10				
S1	Met-	Thr-	Glu-	Ser-	Phe-	Ala-	Gln-	Leu-	Phe-	Glu-	Glu -	Asp-	Leu-
A	Ala-	Ala-	Lys-	Asp-	Val-	Lys-	Phe-	Gly-	Asn-	Asp-	Ala-	(Arg)-	Val-

	15					20				
S1	Lys-	(Glu)-	Leu/Thr	(Glu)-	Asn-	(Arg)-	Pro -	Gly -	Asp-	Gly-
A	Lys -	Met -	Leu-	(Glu)-	Gly -	Val -	Asn-	(Arg)-	(Leu)-	Ala-	Tyr -

Fig.1. N-terminal sequence of proteins S1 and A from *E. coli*, as derived from sequenator degradations.

agreement with the results in fig.1. This supports the conclusion that 'A' and 'S1A' refer to one and the same protein in the case of *E. coli*.

The sequenator results of protein S1 gave some ambiguities beyond residue 10 which are caused partly by sequence specific problems (e.g., the Glu–Glu–Asp sequence tends to increase overlapping) and partly by increase of the background (probably due to labile peptide bond(s) in S1). Therefore, the N-terminal sequence of S1 as given in fig.1 has to be confirmed further by sequence analysis of the N-terminal peptides of this protein. This work is in progress.

Incubation of either S1 or A-protein with carboxypeptidase A (7 μg enzyme/mg protein, 37°C, 2 h) did not release any free amino acids. Incubation with carboxypeptidase B under similar conditions released lysine and arginine in the case of both S1 and A-protein. Thus the two proteins terminate with –Arg, Lys.

3.2. *Immunological tests*

Double immunodiffusion patterns of proteins S1 and A against their antisera are shown in fig.2. The two proteins do not show any apparent immunological homology. The absence of immunological cross-reactivity also has been noted [2] between S1 and S1A proteins isolated from *E. coli* MRE600.

3.3. *Protein–chemical properties*

Proteins S1 and A migrate to clearly different positions (see [1,8] for electrophoretic patterns) in both a two-dimensional gel electrophoresis resolving acidic proteins [9] as well as in the standard system [10].

Proteins S1 and A behave differently in several protein–chemical tests. For example, protein S1 gives a large fragment in high yield when cleaved by CNBr treatment ([11], A.R.S., Giorginis, unpublished). A-protein by the same treatment yielded only a mixture of small fragments. A-protein which had been dialyzed without SH-group protection showed 2 extra bands of lower mobility by dodecylsulfate–gel electrophoresis, while S1 gave only a single band under similar conditions (data not shown).

3.4. *Functional tests*

Antibodies to protein S1 strongly inhibit protein synthesis when added to in vitro systems translating either synthetic or natural mRNA [12,13]. Figure 3

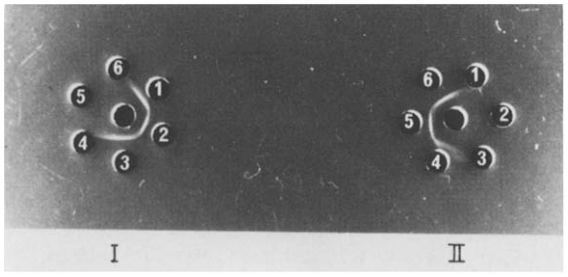

Fig.2. Double immunodiffusion test for proteins S1 and A and the absence of cross-reaction. (I) Reaction of purified S1 and A with anti-S1 serum. (II) Reaction with anti A-serum. Wells numbered 1–3 contained 1, 2 and 4 μg S1; wells 4–6 contained 1, 2 and 4 μg A. Center well contained 10 μl anti-S1 serum in I and 16 μl anti-A serum in II.

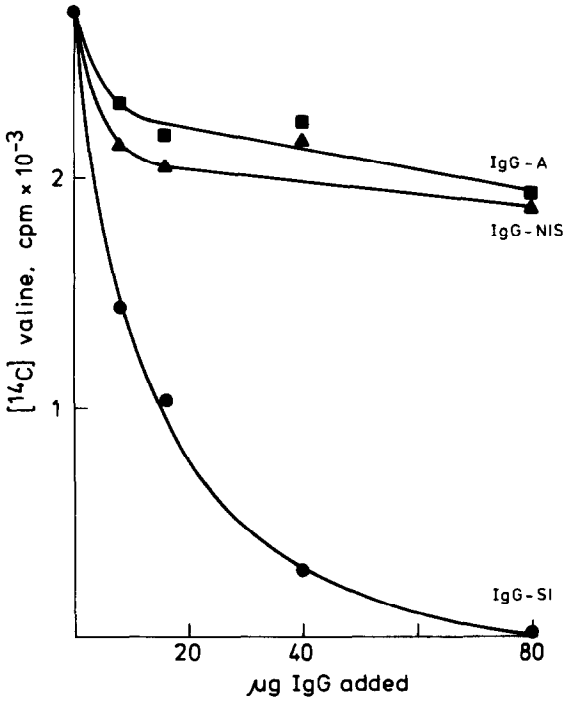

Fig.3. Effect of anti-A and anti-S1 IgG on the translation of f_2 RNA by *E. coli* ribosomes. The incubation mixture contained 50 μg f_2 RNA, an extract of *E. coli* (from early logarithmic cells, 60 μg ribosomes) and other required components including [^{14}C]valine as in [5]. The mixture was incubated at 37°C for 30 min and hot trichloroacetic acid-insoluble peptides were determined [5]. NIS, serum before immunization.

Table 1
Effect of proteins A and S1 on the translation of phage f_2 RNA

Addition		[^{14}C]Valine incorporated	
protein	μg	cpm	%
None	–	9357	100
A	20	9571	102
A	40	9198	98
A	100	9902	105
S1	100	768	8

The conditions of protein synthesis are the same as in the legend to fig.3

shows the effect of adding purified A-protein antibodies to a system translating phage f_2 RNA. There was no clear specific inhibition of this system by A-protein antibodies. Antibodies against S1 are strongly inhibitory, as expected.

Protein S1 by itself in large excess is known to inhibit protein synthesis [14]. Table 1 shows the effect of adding large amounts of A-protein to an f_2 RNA translating system. There was no effect by A-protein on the extent of amino acid incorporation. Moreover, analysis of the labeled polypeptides by dodecylsulfate–gel electrophoresis (followed by slicing and radioactivity determination) did not reveal any effect of A-protein on the pattern of the labeled products.

Protein S1 binds to poly(U) very strongly and this binding can be assayed by Millipore filtration [15]. We have found that A-protein binds only very weakly to poly(U) under the same assay conditions (fig.4). A-protein has also been found, unlike protein S1 [16], incapable of stimulating the coat protein synthesis in an in vitro system from *Bacillus stearothermophilus* using phage f_2 RNA (S. Isono, personal commun.).

3.5. *Conclusion*

The above results lead to the conclusion that S1 and A are two distinct proteins (i.e., encoded in distinct genes) and bear no structural homology to each other. There is also no similarity in their known functions. Protein S1 is a required component for ribosome function in vitro (e.g. [17]) and presumably in vivo. So far no in vitro function has been noted for

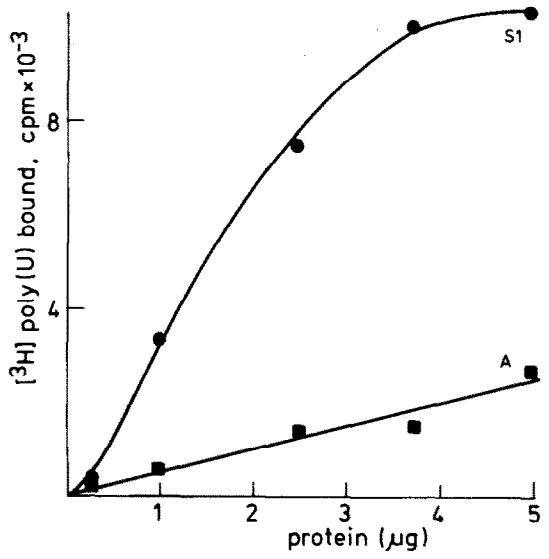

Fig.4. Binding of [^{3}H]poly(U) by proteins S1 and A. The proteins were incubated with 0.05 μCi [5-^{3}H]poly(U) (Miles, 1.9 μCi/μg) at 0°C and assayed by Millipore filtration [15].

A-protein. However, A-protein accumulates in *E. coli* in a characteristic manner towards the end of exponential growth; under certain growth conditions A-protein is present in *E. coli* in amounts comparable to that of S1 [1,18]. This finding and the strong association of A-protein with the 30 S ribosomal subunit [1] would suggest a possible role in the regulation of protein biosynthesis. But whatever the role of A-protein of *E. coli*, the present results argue against the usefulness of correlating it directly to protein S1 by the designation 'S1A'.

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