CHARACTERIZATION OF AN S1-LIKE PROTEIN IN *MYCOBACTERIUM SMEGMATIS* RIBOSOMES

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1. Introduction

An important role of *Escherichia coli* ribosomal protein S1 is known to be the correct binding of mRNA to the ribosome. In addition, protein S1 shows cistron discrimination. Thus, in the cell-free system derived from *Bacillus thermophilus*, f2 RNA coat protein is inefficiently translated, but the addition of *E. coli* S1 remarkably stimulates the incorporation of valine into coat protein [1,2].

During a study of ribosomes from *Mycobacterium smegmatis*, we were faced with the difficulty of translating f2 RNA in this particular cell-free system. In this connection, S1 protein from this organism was characterized and studied. The results show that the translational barrier of *M. smegmatis* ribosomes was due not only to the incompatibility of S1 protein but also the whole structure of the ribosomes.

2. Materials and methods

2.1. *Buffers*

TMNSH, 10 mM Tris–HCl (pH 7.8), 60 mM NH_4Cl, 10 mM Mg acetate, 6 mM 2-mercaptoethanol. T*x*M*y*N*z*, *x* mM Tris–HCl (pH 7.8), *y* mM Mg acetate, *z* mM NH_4Cl.

2.2. *Strains and culture*

Mycobacterium smegmatis strain Rabinowitchi (R)-15 (*argA-6*, *met-5*) and *E. coli* HAK10 (*rna*, *rnb*, *trp*, *met*) were used. *Escherichia coli* HAK10 was generously given by Dr M. Kuwano through Dr Imamoto. The culture medium contained following ingredients, per liter of distilled water: broth 10 g; polypeptone, 10 g; NaCl, 2 g; glycerol, 40 ml. The final pH was adjusted to 7.0 with 10% NaOH. The cells were grown by shaking at 37°C to mid-logarithmic phase, harvested and washed with T20M50N60SH buffer 3 times and then with TMNSH once.

2.3. *Ribosomes and ribosomal subunits*

The cells were suspended in 2-fold volumes of TMNSH buffer and disrupted by sonic oscillation (Tomy model UR-150P [Japan]) for 2 min, 3 times at 4–15°C. After removing cell debris by centrifugation at 20 000 × *g* for 30 min (2 times), ribosomes and supernatant fluid were separated by centrifugation at 104, 5000 × *g* for 2 h. The supernatant fraction was dialyzed against 600-fold volume of TMNSH for 24 h, and stored frozen at −80°C until use. To obtain ribosomal subunits, ribosomes were dialyzed against TM2N60SH for 24 h, samples loaded on 10–30% sucrose gradients in the same buffer and the centrifugation was done at 21 000 rev./min for 17 h at 4°C in a Beckman SW 27 rotor. The gradients were analyzed using a ISCO density gradient fractionator. Fractions containing 50 S or 30 S subunits were pooled and concentrated by ultrafiltration. The purity of each subunit preparation was re-examined in the same gradient by using Beckman SW 41 rotor. Centrifugation was performed at 18 000 rev./min for 21 h; no cross-contamination of subunits was found.

2.4. *Preparation of protein S1 and S1-deficient 70 S ribosomes*

For isolation of protein S1 the procedure in [2] was used. 70 S ribosomes were suspended in TMN1000SH buffer, left in ice for 4 h, and loaded on poly(U)–Sepharose 4B column (Pharmacia Fine Chemicals) which had been equilibrated with 10 mM Tris–HCl buffer (pH 7.5) containing 1 M NaCl followed with

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TMN1000SH buffer. The column was washed with the same buffer. The S1-deficient 70 S ribosomes, which are not retained, were pooled and immediately dialyzed against TMNSH for 24 h, and stored frozen at −80°C. The ribosomes from *M. smegmatis* were unstable in TMN1000SH buffer. Protein S1 was eluted with TMN1000SH containing 6 M urea, concentrated by ultrafiltration with the membrane UH 100/25 (Schleicher and Schull GmbH), dialyzed against TMNSH for 24 h, and stored frozen at −80°C until use.

2.5. *Preparation of initiation factors*

Crude initiation factors were prepared as in [3,4]. Protein S1 from crude initiation factors were removed by adsorption on poly(U)–Sepharose 4B. The factors thus obtained were dialyzed against TMNSH, and stored frozen at −80°C.

2.6. *Preparation of f2 RNA*

f2 phage was generously given by Dr K. Isono. The procedure for preparing RNA followed generally the same procedure as for ribosomal RNA [5].

2.7. *Assay for polypeptide synthesis*

Experimental conditions for poly(U)-dependent polyphenylalanine synthesis were as in [6], with a few modifications. The standard reaction mixture (0.1 ml) contained the following: 95 mM Tris–HCl (pH 7.8); 5 mM 2-mercaptoethanol; 7 mM phosphoenolpyruvate monopotassium salt (Sigma); 0.9 mM ATP; 0.028 mM GTP; 0.1 mM [^{14}C]phenylalanine (spec. act. 100 μCi/5.5 μmol, Radiochemical Centre, Amersham); 48 mM NH_4Cl; 1.5 μg phosphoenolpyruvate kinase (Sigma); 10 μl supernatant fluid of *E. coli* HAK10 (A_{280} = 14); 0.5 μg poly(U) (Miles); 50 μg *E. coli* transfer RNA (Calbiochem.); 11 mM Mg-acetate for *M. smegmatis* system or 14 mM Mg-acetate for *E. coli* system; 3 A_{260} units of S1-deficient 70 S ribosomes. After incubation at 37°C for 45 min, the reaction was stopped by adding 1 ml 10% trichloroacetic acid, and the mixture was heated at 90°C for 15 min. The resulting acid-insoluble material was collected on glass-fiber papers (Whatman GF83) and radioactivity counted in a liquid scintillation spectrometer.

The standard reaction mixture (0.1 ml) for translation of f2 RNA contained the following: 95 mM Tris–HCl (pH 7.8); 5 mM 2-mercaptoethanol; 7 mM phosphoenolpyruvate monopotassium salt (Sigma); 0.9 mM ATP; 0.028 mM GTP; 48 mM NH_4Cl; 1.5 μg pyruvate kinase; 2.5 μg (±)-L-tetrahydrofolic acid (Sigma), 10 μl supernatant fluid of *E. coli* HAK10 (A_{280} = 14); 1 A_{260} unit of f2 RNA; 20 μl initiation factors, 3 A_{260} units of S1-deficient 70 S ribosomes or 1 A_{260} unit of 30 S subunits and 2 A_{260} units of 50 S subunits; 1 μM [^{14}C]valine (spec. act. 178 μCi/μmol); 0.186 mM each of 19 other amino acids. Further experimental procedures were as described for poly(U) system.

3. Results and discussion

3.1. *Electrophoretic and immunological analysis of S1 proteins*

To check molecular similarity of proteins S1 of *E. coli* and *M. smegmatis*, samples were first analysed in slab-gel electrophoresis. As can be seen in fig.1, S1 derived from *M. smegmatis* moved slightly faster than *E. coli* S1. This indicated it to be smaller than *E. coli* S1

To see molecular similarity more precisely, the

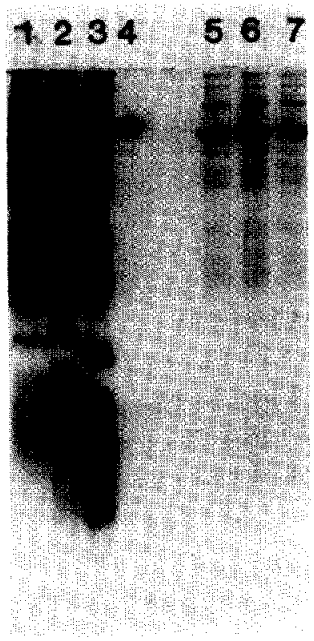

Fig.1. Removal by affinity chromatography on poly(U)–Sepharose of S1 like protein from *M. smegmatis* ribosomes. SDS gel electrophoresis patterns of: (1–3) *M. smegmatis* ribosomes after passage through poly(U)–Sepharose; (5–7) 6 M urea effluent of bound proteins from poly(U)–Sepharose; (4) *E. coli* ribosomal protein S1.

double immunodiffusion patterns of the isolated S1 proteins against anti-*E. coli* S1 serum were examined. However, no cross-reactions were observed between *M. smegmatis* S1 and anti-*E. coli* S1, whereas *E. coli* S1 reacted with anti-*E. coli* S1 as expected. The results indicated the poor molecular similarity of S1 proteins derived from these unrelated species. At least, the antigenic determinants of *E. coli* S1 proteins are not common with *M. smegmatis* S1.

3.2. *Functional properties of S1*

One of the characteristic property of S1 is known to be stimulation of poly(U)-directed polyphenylalanine synthesis. This property was tested using S1 deficient *M. smegmatis* 70 S ribosomes. As shown in fig.2A, S1 from *M. smegmatis* was efficient for full activity of homologous ribosomes, whereas S1 derived from heterologous *E. coli* was virtually inactive. Therefore, we could conclude *M. smegmatis* S1 protein was functionally similar to *E. coli* S1 with respect to stimulating poly(U)-directed polyphenylalanine synthesis. The next question is whether *M. smegmatis* S1 protein can replace *E. coli* S1 in an *E. coli* system. At a variety of concentrations of *M. smegmatis* S1, polyphenylalanine synthesis on S1-deficient *E. coli* 70 S ribosomes was examined. As can be seen in fig.2B, only slight stimulation by added S1 was observed. The addition of *E. coli* S1 protein gave enhancement of polyphenylalanine synthesis on *E. coli* ribosomes as expected. In conclusion, we can say *M. smegmatis* S1 protein retains functional domains which are concerned with poly(U)-directed polyphenylalanine synthesis. However, these particular domains of *M. smegmatis* protein are structurally different from *E. coli* protein, as suggested by immunoelectrophoresis experiments.

Another characteristic property of *E. coli* S1 is that it plays an important role in the initiation of translation of natural mRNA. Thus, it stimulates the synthesis of f2 RNA coat protein in the cell-free system of *Bacillus thermophilus*, which can translate very few coat proteins without added *E. coli* S1 [6].

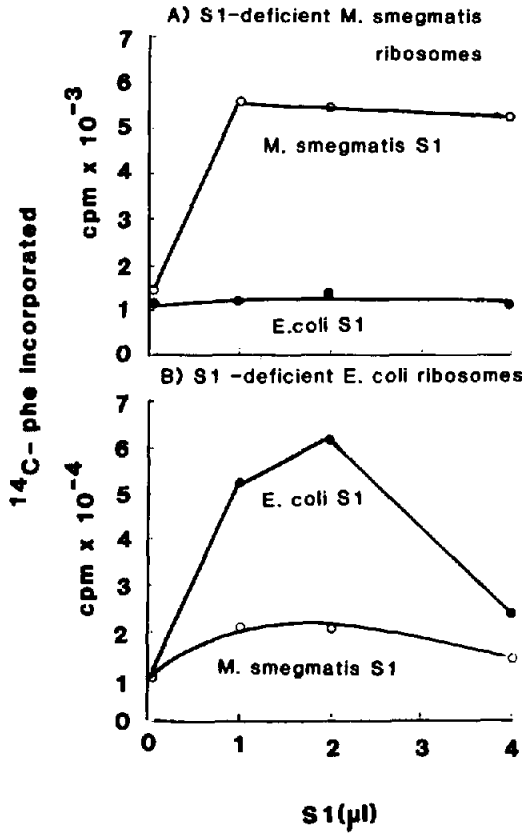

Fig.2. Effect of proteins S1 derived from *M. smegmatis* (○——○) and *E. coli* (●——●) in the translation of poly(U) using: (A) S1-deficient *M. smegmatis*; (B) *E. coli* ribosomes; S1 proteins were adjusted to 35 A_{280} units/ml.

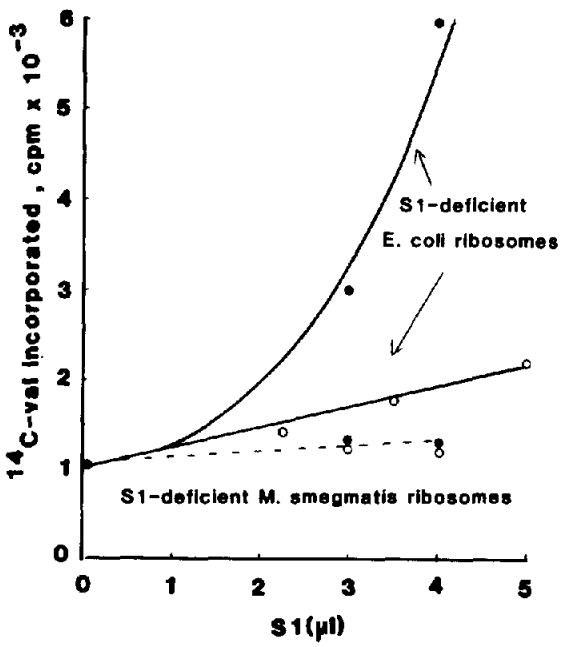

Fig.3. Effect of protein S1 derived from *M. smegmatis* (○——○, ○– – –○) and *E. coli* (●——●, ●– – –●) in the translation of f2 RNA using S1-deficient *M. smegmatis* (○– – –○, ●– – –●) or *E. coli* (○——○, ●——●) ribosomes; 20 µl initiation factors (6 A_{280} units/ml) was used; S1 proteins were adjusted to 35 A_{280} units/ml.

Therefore the translation of f2 RNA was tested in the cell-free system of *M. smegmatis*. S1 proteins and S1-deficient 70 S ribosomes were prepared from *M. smegmatis* and *E. coli*. They were mixed in all combinations and the capability of translating f2 RNA was tested. The results are presented in fig.3. The control system which contained S1-deficient *E. coli* 70 S ribosomes and increasing amounts of homologous S1 proteins showed sufficient amounts of translation. *M. smegmatis* S1 substituted for that of *E. coli* with less efficiency. *M. smegmatis* ribosomes, however, could not translate f2 RNA even in the presence of *E. coli* S1. It may be concluded that not only S1 but also ribosomes of *M. smegmatis* are inactive for the translation of f2 RNA. The fact that *E. coli* S1 could not support the translation of f2 RNA on *M. smegmatis* ribosomes leads us to speculate that *E. coli* S1 might not be able to fit in to *M. smegmatis* 30 S subunits. To further test this possibility, ribosomal subunits were prepared both from *M. smegmatis* and *E. coli* and exchanged in all combinations. The translation of f2 RNA was examined in each system. The results are presented in table 1. In these systems, isolated 30 S subunits contained sufficient amounts of S1 proteins, since the addition of isolated S1 did not stimulate the incorporation any more. Data show that the homologous *E. coli* system translated f2 RNA, but the homologous system of *M. smegmatis* did not, as expected from fig.2. Interestingly, heterologous ribosomes containing *E. coli* 30 S subunits or 50 S subunits showed translation to a significant amount and *E. coli* S1 protein stimulated the translation on heterologous ribosomes containing *M. smegmatis* 30 S subunits.

Table 1
Translation of phage f2 RNA[a]

Source of subunits[b]		Phage RNA	S1 from[c] *E. coli*	[^{14}C]Val incorp.
50 S	30 S			
E. coli	*E. coli*	–		741
		+		5096
M. smegmatis	*E. coli*	–		379
		+		3099
E. coli	*M. smegmatis*	–		866
		+		1711
		+	+	2669
M. smegmatis	*M. smegmatis*	–		217
		+		204
		+	+	428

[a] Experimental procedures are described in the text
[b] Absence of contamination of counterpart subunits was checked
[c] Concentrations of S1 proteins were 35 A_{280} units/ml

In conclusion, *M. smegmatis* ribosomes contain a high-M_r protein which can be isolated, like *E. coli* S1, by affinity chromatography on poly(U)–Sepharose. The *M. smegmatis* protein supports poly(U)-directed polyphenylalanine synthesis, but only in the homologous ribosomes. The functional domain for the initiation of f2 RNA in ribosomes seems to have been much changed through evolution.

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References

[1] Isono, S. and Isono, K. (1975) Eur. J. Biochem. 56, 15–22.
[2] Subramanian, A. R., Rienhardt, P., Kimura, M. and Suryanarayana, T. (1981) Eur. J. Biochem. 119, 245–249.
[3] Minks, M. A., Suryanarayana, T. and Subramanian, A. R. (1978) Eur. J. Biochem. 82, 271–277.
[4] Subramanian, A. R., Haase, C. and Giesen, M. (1976) Eur. J. Biochem. 67, 591–601.
[5] Dolme, F. and Nierhaus, K. H. (1976) J. Mol. Biol. 107, 585–599.
[6] Yamada, T., Masuda, K., Mizuguchi, Y. and Suga, K. (1976) Antimicrob. Agents Chemother. 9, 817–823.