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Replacement of Ribosomal Protein S1 by Interference Factor iα in Ribosomal Binding of Phage MS2 RNA

(gel electrophoresis/30S ribosomes/messenger RNA)

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ABSTRACT The MS2 RNA binding capacity of 30S ribosomal subunits, which is lost when protein S1 is removed, can be restored following incubation with interference factor iα and repelleting. Polyacrylamide-agarose gel electrophoresis shows that, under these conditions, a faster moving, non-RNA binding 30S species, which contains no S1, is converted to a slower moving RNA-binding one, having the same mobility as the 30S species that contains protein S1. Factor iα binds to single-stranded RNAs in a pattern that closely resembles the RNA binding pattern of initiation factor IF-3.

Binding of the messenger to the small ribosomal subunit is a primary event in translation (1). Binding and translation of poly(U) have been shown to require protein S1 (2, 3), the largest and most acidic of the 30S proteins (4, 5). S1 had been considered a fractional protein (6), i.e., a protein present in less than one copy per ribosome, but this is referable to loose binding of this protein to the 30S subunit. It has been shown that although the S1 to ribosome ratio is 0.1 to 0.3 in monosomes, it is about 1.0 in polysomes (3). Recently (7, 8) protein S1 has been found to be indistinguishable from interference factor iα, a protein isolated from the ribosomal wash (9, 10). This factor, which inhibits the overall translation of coliphage RNA, has also proved to be identical to the α subunit of Qβ replicase (11, 12), one of the three subunits of the enzyme contributed by the host (13, 14). The inhibitory effect of iα on translation may be due to the formation of an iα–mRNA complex, since inhibition is relieved by an excess of the RNA (15).

It was shown in this laboratory (16) that 30S subunits containing protein S1 form a weak, species-specific complex with MS2 RNA in the absence of initiation factors. *Escherichia coli* 30S subunits can be separated by polyacrylamide–agarose gel electrophoresis into two major subspecies (16, 17) of which only the more slowly moving one contains protein S1 and binds mRNA (16). In the present communication we show that factor iα restores the capacity of S1-deficient *E. coli* 30S subunits to bind MS2 RNA, simultaneously converting the faster moving into the slower moving subspecies. We also find that iα, like the initiation factor IF-3 (18), binds single-stranded RNA and synthetic polynucleotides.

MATERIALS AND METHODS

Ribosomes and Ribosomal Subunits. Unfractionated crude *E. coli* Q13 ribosomes were washed twice (15 hr and 4–5 hr) in a buffer (20 mM Tris·HCl, pH 7.8, 10 mM magnesium acetate, 0.5 mM dithiothreitol) containing 1.0 M NH_4Cl, by two different procedures. In the "concentrated procedure," the ribosomal pellet was suspended in the washing buffer at a concentration of 250–300 A_{260} units/ml; in the "diluted procedure," at 40–60 A_{260} units/ml. Ribosomal subunits were prepared from the washed ribosomes as previously described (18). 30S subunits depleted of S1 were obtained by dialysis (18 hr, 4°) against 1 mM Tris·HCl buffer, pH 7.8, as described by others (2, 3). Reconstitution with iα (35 μg of step-5 protein per 10–15 A_{260} units of 30S ribosomes) was carried out for 3 min at 40° in a buffer containing 10 mM Tris–acetate, pH 7.8, 10 mM magnesium acetate, 0.1 mM dithiothreitol, and 0.3 M KCl (3). The mixture was pelleted by high-speed centrifugation and the supernatant containing free iα was discarded.

Assays. Activity of iα was assayed through inhibition of translation of MS2 RNA (10, 19). The samples (0.125 ml) contained Tris·HCl buffer (pH 7.8), 60 mM; NH_4Cl, 70 mM; magnesium acetate, 12 mM; 2-mercaptoethanol, 16 mM; ATP, 1.3 mM; GTP, 0.3 mM; phosphocreatine (Sigma), 17 mM; creatine kinase (Worthington), 3 μg; *E. coli* tRNA (Schwarz/Mann), 50 μg; [^{14}C]lysine (New England Nuclear Corp.), 10 Ci/mol, 0.1 mM; the remaining unlabeled 19 amino acids (Sigma), 0.1 mM each; 1.0 M NH_4Cl-washed *E. coli* MRE 600 ribosomes, 5.0 A_{260} units; *E. coli* MRE 600 high-speed supernatant, 300 μg of protein; MS2 RNA, 40 μg; IF-1 (step 6, ref. 20), 0.95 μg; IF-2 (phosphocellulose step, ref. 21), 15 μg; and IF-3 (step 4, ref. 22), 0.7 μg. Incubation was for 15 min at 37°. A decrease of 1.0 pmol in [^{14}C]lysine incorporated into hot trichloroacetic acid-insoluble material was taken as one unit of activity.

For binding of MS2 RNA to 30S ribosomes (16), the samples (0.05 ml) contained 10 mM Tris–acetate, pH 7.4, 5 mM magnesium acetate, 50 mM NH_4Cl, 0.1 mM dithiothreitol, 0.8 A_{260} unit of 30S ribosomes, and 0.34 A_{260} unit of MS2 [^{3}H]RNA (6600 cpm). The samples were first incubated for 2 min at 37° without MS2 RNA, cooled in ice, and incubated for 5 min at 0° with the RNA; they were then diluted to 1.0 ml with the incubation buffer, filtered on Millipore membranes, washed twice with 1.0 ml of buffer, and the radioactivity retained was measured. Blanks without ribosomes were run with each experiment.

For the binding of iα to RNAs, the samples (0.05 ml) contained 6 μg of step 5 iα (or an equivalent amount of step 3 protein) and 1.0–1.6 μg of the RNA; incubation was for 10 min at 0° in either 20 mM sodium phosphate buffer, pH 7.0,

Abbreviation: IF, initiation factor.

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TABLE 1. *Binding of interference factor iα to labeled synthetic and natural polyribonucleotides*

	RNA retained on filter (% of input)	
	20 mM sodium phosphate buffer, pH 7.0	20 mM Tris·HCl pH 7.4, 10 mM Mg^{2+}
[^{3}H]Poly(U)	<1.0	44.3
[^{14}C]Poly(A)	67.6	13.2
Poly(A)·poly(U)	0.4, 0.0*	9.5, 9.0*
[^{14}C]tRNA	6.3	1.6
16S ribosomal [^{14}C]RNA	88.0	36.8
23S ribosomal [^{14}C]RNA	93.5	54.5

Binding was measured by the Millipore filter assay as described in *Materials and Methods*.

* The figures indicate % of [^{14}C]poly(A) and [^{3}H]poly(U), respectively, retained on filters.

or in 20 mM Tris-acetate buffer, pH 7.4, containing 10 mM magnesium acetate. Samples were diluted to 1.0 ml with the corresponding buffer and filtered through Millipore membranes; the filters were washed three times with buffer (2.0 ml each) and the retained radioactivity was measured. For assays with labeled poly(U), and for those with labeled poly(A) carried out in Tris-Mg buffer, filters were pretreated with 0.5 M KOH (23) to reduce blanks without iα. The following RNAs were used: [^{3}H]poly(U) (14,400 cpm/μg), [^{14}C]poly(A) (3930 cpm/μg), *E. coli* W [^{14}C]tRNA (5370 cpm/μg), all purchased from the Miles Laboratories; 16S (5050 cpm/μg) and 23S (4650 cpm/μg) ribosomal [^{3}H]RNAs were prepared from *E. coli* Q13 cells grown with 0.5 μCi/ml of [^{3}H]uracil (24).

Purification of iα. iα was purified from *E. coli* MRE 600 by a procedure previously outlined (10). It involves ammonium sulfate fractionation (0.35 to 0.55 saturation) of the 1.0 M NH_4Cl ribosomal wash (step 1), followed by chromatography on DEAE-cellulose (step 2), phosphocellulose (step 3), hydroxylapatite (step 4), and agarose A-1.5 m (step 5). Purification was about 125-fold. Step 5 iα was homogeneous as judged by polyacrylamide-sodium dodecylsulfate gel electrophoresis. The standard activity assayed for was inhibition of MS2 RNA translation. Binding of the factor to MS2 RNA (see ref. 15) copurified with the inhibitory activity.

Polyacrylamide-Agarose Gel Electrophoresis of 30S Subunits. The gel (2.25% polyacrylamide, 0.5% agarose) was prepared according to published procedures (refs. 25 and 26, see also ref. 16). Column dimensions were 0.6 × 9 cm. The samples (0.05 ml) contained 0.3-0.5 A_{260} unit of 30S ribosomes, 10 mM Tris-acetate (pH 7.4), 5 mM magnesium acetate, 50 mM ammonium acetate, and 0.1 mM dithiothreitol; they were made 4% in sucrose before application to the gel. A buffer containing 25 mM Tris-acetate, pH 7.2, 5 mM magnesium acetate, and 50 mM ammonium acetate was used in both chambers and changed every 1.5 hr. The temperature was 5-9°. After a preliminary run (45 min at 4 V/cm) without samples, these were applied and electrophoresis was performed for 20 min at 4 V/cm and then for 5 hr at 9 V/cm. For location

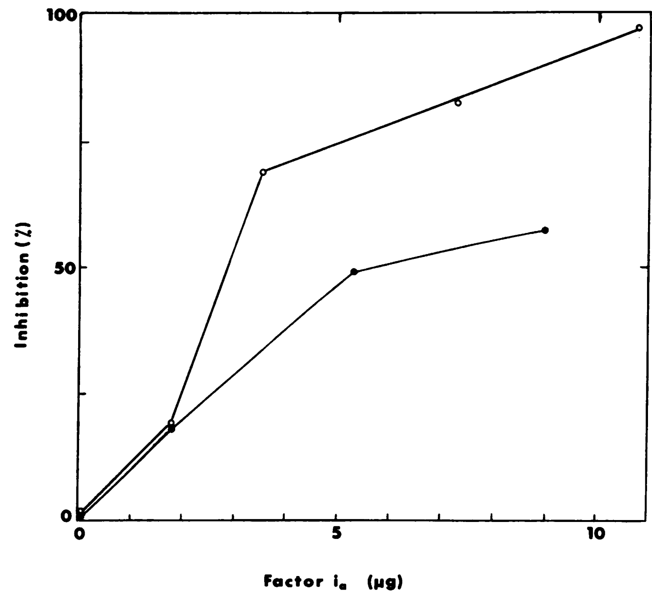

FIG. 1. Inhibition of MS2 RNA translation by interference factor iα. O, 0.36 A_{260} unit; ●, 1.0 A_{260} unit of MS2 RNA/sample.

of the bands, the gels were either scanned at 260 nm (Gilford model 2400) or stained with methylene blue (25) or both.

Polyacrylamide-Dodecyl Sulfate Gel Electrophoresis of iα and of Ribosomal Proteins. The gel (10% polyacrylamide, 0.5% bis acrylamide, 0.1% sodium dodecyl sulfate) was prepared as described (27). Column dimensions are indicated in legends. Samples containing 10-35 μg of protein or 0.7-1.0 A_{260} unit of 30S ribosomal subunits were suspended in 10 mM sodium phosphate, pH 7.0, 1.0% sodium dodecyl sulfate, 1.0% mercaptoethanol, and 10% glycerol (final volume 0.05 ml), incubated for 10 min at 65°, and electrophoresed at room temperature in 25 mM sodium phosphate, pH 7.2, containing 0.1% sodium dodecyl sulfate for about 2 hr at 8 mA per gel until the tracking dye (bromophenol blue) reached the bottom of the column. Gels were stained with Coomassie Brilliant Blue and scanned at 550 nm (Gilford 2400). The areas under the traces of the S1, or iα, bands were measured and standardized according to Vermeer *et al.* (28).

RESULTS

As seen from Fig. 1, iα inhibits the translation of MS2 RNA and, in agreement with results by others (15), inhibition is substantially relieved when the concentration of MS2 RNA is increased.

Table 1 presents data on the binding of iα at 0° to several polynucleotides in sodium phosphate buffer and in Tris·HCl buffer containing Mg^{2+}. In phosphate buffer there is good binding to the single-stranded, stacked poly(A) helix (29) and virtually no binding to poly(U) which, under these conditions, exhibits an almost unstacked structure (30). In Mg^{2+}-containing Tris·HCl buffer, however, the binding of poly(A) drops markedly and that of poly(U) becomes rather high. The increase in poly(U) binding apparently reflects an increased affinity of iα for poly(U) when the polymer assumes an ordered structure, as is the case in the presence of Mg^{2+} (31, 32). There is no binding of iα to the double-stranded poly(A)·poly(U) complex in sodium phosphate buffer but some binding occurs in Tris-Mg^{2+} buffer; it may reflect partial

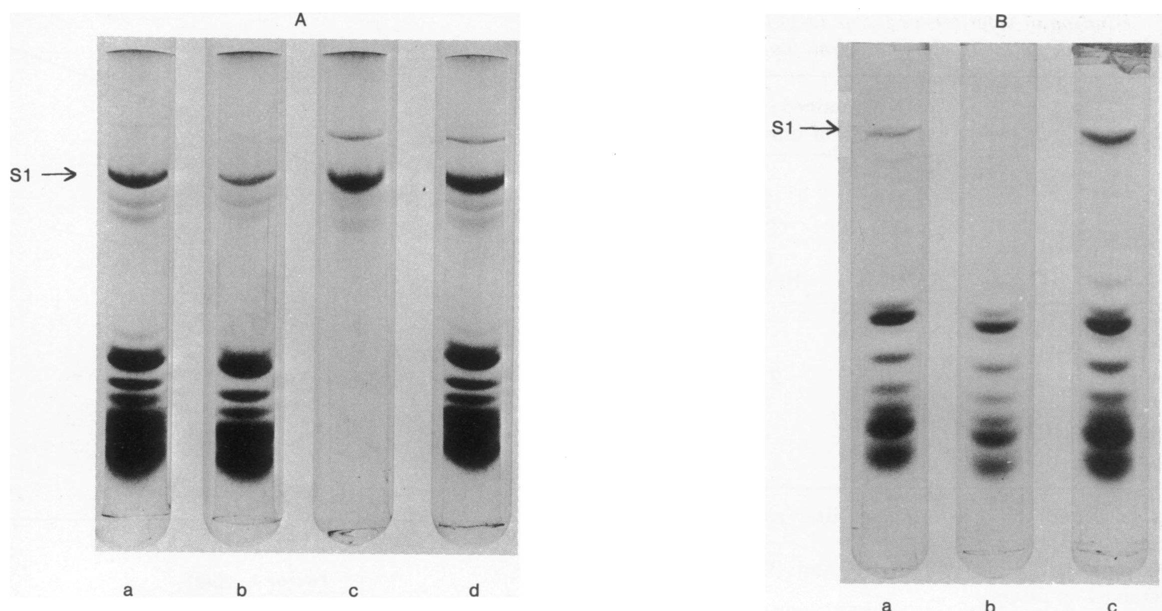

FIG. 2. Polyacrylamide–dodecyl sulfate gel electrophoresis of ribosomal proteins from 30S ribosomal subunits containing various amounts of protein S1, and subunits reconstituted with iα. (A) a, washed 30S, concentrated procedure; b, washed 30S, diluted procedure; c, factor iα, 5 μg; d, coelectrophoresis of the samples in b and c. (B) a, washed 30S, diluted procedure; b, washed 30S (diluted procedure), depleted of S1; c, as in b but reconstituted with iα. The column dimensions were: A, 0.6 × 8.0 cm; B, 0.6 × 9.5 cm. The iα used in A, gels c and d, had been stored for 4 months. Prior to storage it had shown only one band on dodecyl sulfate gel electrophoresis. The slower-moving upper-most band may have resulted from aggregation on aging. However, there was no significant decrease in the specific activity of the preparation.

rearrangement of the double- to a triple-stranded structure with concomitant appearance of single strands which bind the protein. Transfer RNA with its compact structure and short single-stranded regions binds very poorly in phosphate buffer and still less in Tris–Mg^{2+}. Both ribosomal RNAs show very good binding, particularly in phosphate buffer in which they assume a more extended conformation. The data of Table 1 indicate that the binding of iα is not base-specific but rather depends on structural features of the polynucleotides. The RNA binding properties of iα are strikingly similar to those of IF-3 (18).

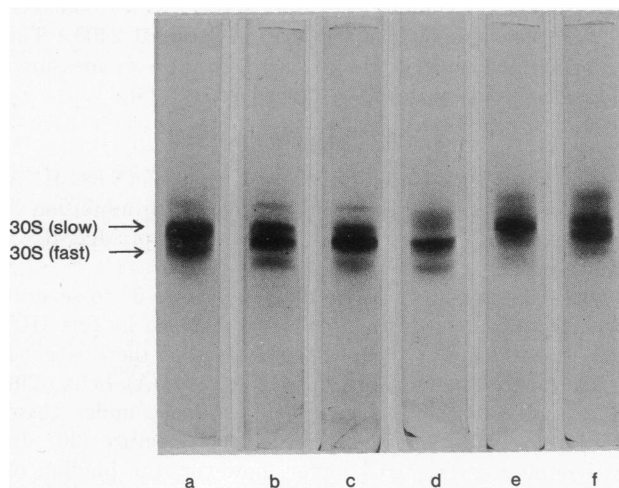

FIG. 3. Polyacrylamide–agarose gel electrophoresis of 30S subunits containing various amounts of protein S1 and subunits reconstituted with iα. a, unwashed; b, washed by the concentrated procedure; c, washed by the diluted procedure; d, washed, depleted of S1; e, as in b but reconstituted with iα; f, as in d but reconstituted with iα. See Table 2 for relative S1 (iα) content and MS2 RNA binding of the preparations shown.

Reconstitution of 30S Subunits with iα. As seen in Fig. 2A, polyacrylamide-dodecyl sulfate electrophoresis of proteins from 30S subunits prepared under concentrated and diluted conditions showed virtually identical patterns except for the lower content of S1 in the latter preparation (gels a and b). Protein S1, the largest and most acidic of the *E. coli* 30S proteins, is easily identified by one dimensional gel electrophoresis (3, 28). It may be seen that iα (gel c), when mixed with 30S ribosomal proteins, comigrated with the S1 band (gel d). Fig. 2B shows the results of a typical reconstitution experiment. Protein S1 was removed from the 30S subunits by dialysis (3) against diluted Tris·HCl buffer (compare gels a and b), the S1-depleted 30S subunits were incubated with iα, and the reconstituted particles were isolated from the mixture. The new protein band which appears in the reconstituted particle (gel c) corresponds to S1. This experiment shows that iα is incorporated into the 30S subunit.

MS2 RNA Binding Activity and Electrophoretic Mobility of 30S Subunits Reconstituted with iα. E. coli 30S subunits are separated into two major subspecies by electrophoresis on polyacrylamide–agarose gels and only the more slowly moving component contains protein S1 (16, 17). The S1-containing species, but not the other, binds MS2 RNA in the absence of initiation factors (16). As seen in Fig. 3, the relative amount of the slow moving species decreases on washing the ribosomes under concentrated or diluted conditions and there is good correlation with the S1 content of these preparations (compare Fig. 2A). In the S1-depleted 30S (gel d), the more slowly moving band is virually absent. Gels e and f show 30S subunits reconstituted with iα; incorporation of iα converts the

TABLE 2. *Binding of MS2 [³H]RNA to 30S subunits containing various amounts of protein S1 or inteference factor iα (designations a to f correspond to those of Fig. 3)*

30S Ribosomes	MS2 [³H]RNA retained (cpm)*	Relative content of protein S1 or iα†	Relative content of active 30S species‡
a Unwashed	3160 (48.2)	—	0.7
b Washed (concentrated procedure)	1509 (22.9)	0.46	—
c Washed (diluted procedure)	580 (8.8)	0.26	0.2
d Washed, depleted of S1	381 (5.8)	0.12	<0.1
e As in b, reconstituted with iα	3930 (60.5)	1.0	>0.9
f As in d, reconstituted with iα	2680 (40.5)	0.72	0.5

Figures in parentheses indicate % of input cpm retained on filters.

* Standard assay with 0.34 A_{260} unit of MS2 [³H]RNA (6600 cpm). Blanks without ribosomes (50–70 cpm) were subtracted.

† Polyacrylamide–dodecyl sulfate gels of ribosomal proteins from the various 30S preparations were scanned at 550 nm and the areas under the S1 (iα) peaks were measured. The S1 content of preparation e was taken as 1.0 (Fig. 3, gel e).

‡ Polyacrylamide–agarose gels (Fig. 3) were scanned at 260 nm and the areas under the corresponding peaks were measured. The combined area of the two major bands was taken as 1.0.

faster moving species to the slower one. Conversion is nearly complete for the 30S obtained by concentrated washing (compare gels b and e) but only partial with S1-depleted 30S subunits (compare gels d and f).

Table 2 shows the MS2 RNA binding capacity of the 30S subunit preparations of Fig. 3. Binding decreases when the S1 content is reduced by washing or by dialysis against dilute Tris buffer. Reconstitution with iα of 30S preparations containing little or no S1 restores their MS2 RNA binding activity. There is good correlation in all cases between (*1*) MS2 RNA binding, (*2*) relative content of S1 (iα), and (*3*) relative amount of the slow moving species.

DISCUSSION

The present studies demonstrate that interference factor iα can act, depending on the experimental conditions, either as an inhibitor of translation of MS2 RNA or as a protein which promotes binding of MS2 RNA to 30S ribosomal subunits. Both activities are probably related to the affinity of this protein for single-stranded RNA, on the one hand, and for the 30S subunit on the other. The precise temperature and ionic conditions favoring these interactions are not known, and whether or not other proteins affect iα interactions is also a matter for speculation. When it becomes part of the 30S subunit, iα behaves like protein S1 in conferring to this subunit both the electrophoretic properties of S1-containing 30S ribosomes and restoring MS2 RNA binding capacity. This observation is in line with previous findings that mRNA binding to 30S subunits is protein S1-dependent (16) and that interference factor iα is protein S1 (7, 8). Our work indicates that iα, like IF-3 (18), has high affinity for some structurally unique features of single-stranded RNAs. This may be related to the involvement of these two proteins in the interaction of mRNA with the 30S subunit.

One may look upon S1 (iα) as an easily dissociable ribosomal protein which, upon infection of *E. coli* with phage Qβ, may become part of Qβ replicase. Further studies on the molar ratio of S1 to ribosomes in the cell and its affinity for 30S subunits and mRNA will be required to evaluate its postulated role (9) as a translational repressor.

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