SPECIFIC INHIBITORS OF MS2 AND LATE T4 RNA TRANSLATION IN E. COLI

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SUMMARY

1.0 M NH_4Cl washes of E. coli ribosomes contain two protein factors affecting activities of the polypeptide chain initiation factor 3 (IF3). One (iα) inhibits functions of IF3α, the IF3 species which recognizes MS2, E. coli, and early T4 RNA; the other (iβ) inhibits functions of IF3β, the species recognizing late T4 RNA. Initiation complex formation and translation of the mRNA are inhibited to the same extent but the ribosome dissociation activity of IF3 is not affected.

Revel and collaborators (1) recently isolated from 1.0 M NH_4Cl washes of E. coli ribosomes a protein factor that inhibits the IF3-dependent translation of MS2, but not late T4 RNA. They refer to this protein as the i (interference) factor. This factor was further identified (2) as the α subunit of Q_β replicase, one of the three subunits contributed by the host (3, 4). Weissmann and collaborators (5) isolated α-less Q_β replicase and found that it is inactive with Q_β RNA plus strands but active with Q_β RNA minus strands or poly C as template, and that full Q_β replicase activity is restored by Revel's i factor as well as by the purified α subunit.

We have described the isolation of two messenger-discriminating species of E. coli IF3 (6, 7). One of these species, IF3α, is selective for such messengers as MS2, E. coli, and early T4 RNA whereas the other, IF3β, is specific for late T4 RNA. We now report that 1.0 M NH_4Cl washes of E. coli MRE600 ribosomes contain two i factors which will hereinafter be referred to as iα and iβ. Factor iα, which inhibits the IF3α-promoted translation of MS2

RNA, is Revel's i factor. Factor iβ inhibits the IF3β-promoted translation of late T4 RNA. Curiously the i factors do not affect the ribosome dissociation factor (DF) activity of IF3α or IF3β.

Isolation of i factors

Factor iα activity was assayed through inhibition of translation of MS2 RNA, in the presence of IF1, IF2, and IF3α. Factor iβ activity was assayed in an identical manner except for the substitution of late T4 RNA for MS2 RNA and of IF3β for IF3α. The IF3 assays have been described (6, 7). It is evident from Table 1 that low $(NH_4)_2SO_4$ cuts of the ribosomal wash have both iα and iβ activity, and that the two activities decrease in the higher $(NH_4)_2SO_4$ fractions. The precipitate obtained between 0.35 and 0.45 $(NH_4)_2SO_4$ saturation was used for further purification. Stepwise elution from DEAE cellulose with NH_4Cl-containing buffers yielded a fraction enriched in iβ activity at 0.125 M and one enriched in iα activity at 0.25 M NH_4Cl. Each was separately chromatographed on phosphocellulose in a buffer containing 0.05 M NH_4Cl. The bulk of iα activity (from DEAE cellulose 0.25 NH_4Cl eluate) came through with the wash. On stepwise elution, factor iβ activity (from DEAE cellulose 0.125 M NH_4Cl eluate) was eluted at 0.5 M NH_4Cl. Factor iβ was not purified beyond this step but factor iα was purified further by hydroxylapatite chromatography; the activity eluted at 0.15 M phosphate. The specific activity of the iβ preparation was about one-fifth that of the iα fraction. Purification is being continued and will be reported in detail elsewhere.

Inhibition of translation and of ribosomal binding of $fMet\text{-}tRNA_f$

The effect of unresolved (0.35-0.45 $(NH_4)_2SO_4$ saturation fraction) and of purified iα and iβ on the IF3α-dependent translation of MS2 RNA and the IF3β-dependent translation of late T4 (T4L) RNA is shown in Fig. 1. Under our

Table 1. Inhibition of IF3-dependent translation of mRNA by $(NH_4)_2SO_4$ fractions of ribosomal wash

Fraction		Translation of MS2 RNA with IF3α		Translation of late T4 RNA with IF3β	
$(NH_4)_2SO_4$ saturation	Protein (μg)	cpm	Inhibition (%)	cpm	Inhibition (%)
0	0	4994		5588	
0-0.35	600	529	89	1236	78
0.35-0.45	606	0	100	0	100
0.45-0.50	610	0	100	1496	73
0.50-0.55	665	1658	65	3446	38
0.55-0.80	554	4736	4	7063*	

*The bulk of the IF3 activity is present in the 0.55-0.70 $(NH_4)_2SO_4$ saturation fraction (6, 7). This may account for the stimulation (1475 cpm) observed here.

Translation was measured as the amount of ^{14}C-lysine incorporated into hot trichloroacetic acid-insoluble material under the conditions of the standard IF3 assay (6, 7). The samples contained, in a final volume of 0.125 ml, Tris-HCl buffer (pH 7.8), 60 mM, ATP (Sigma), 1.3 mM; GTP (P-L Biochemicals), 0.3 mM; phosphocreatine (Sigma), 17 mM; creatine kinase (Worthington), 3 μg; magnesium acetate, 12 mM; dithiothreitol, 8 mM; E. coli W tRNA (Schwarz/Mann), 50 μg; ^{14}C-lysine (New England Nuclear, specific radioactivity 10 μci/μmol), 0.1 mM; the remaining unlabeled 19 amino acids (Sigma), 0.1 mM each; 1.0 M NH_4Cl-washed E. coli MRE600 ribosomes, 6 A_{260} units; E. coli MRE600 high-speed supernatant, 300 μg of protein; NH_4Cl, 70 mM; homogeneous IF1 (10), 1 μg; purified IF2, 2.4 μg; messenger RNA, 40 μg; IF3α or IF3β (7), 0.6 μg. The assay components were added in the order given. After incubation for 20 min at 37°, the amount of ^{14}C-lysine incorporated was determined as in earlier work (6).

assay conditions, the crude $(NH_4)_2SO_4$ fraction had equal amounts of each activity. At 12.5 μg/sample the inhibition of MS2 RNA translation by purified iα was about 55% whereas the translation of T4L RNA was inhibited roughly 10%. As for the purified iβ, 40 μg inhibited T4L RNA translation about 40% and MS2 RNA translation 3%. We also tested the activity of the homogene-

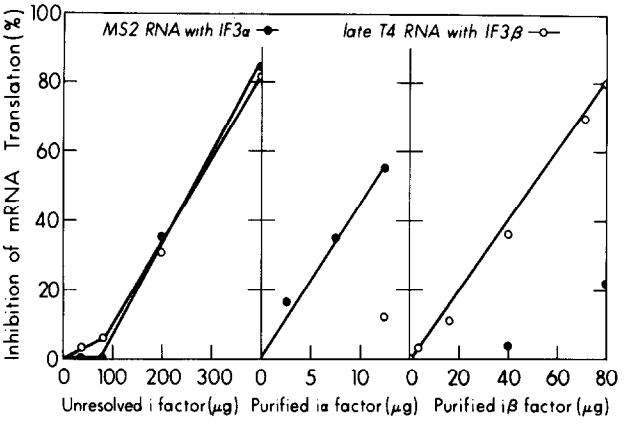

Fig. 1. Effect of unresolved and of purified iα and iβ factors on the IF3-dependent translation of mRNA. Assay conditions as in Table 1 with various amounts of i factors.

ous preparation of the α subunit of Q_β replicase (5) provided by Dr. C. Weissmann. At 12.5 μg it inhibited MS2 RNA translation by approximately 55%, whereas translation of T4L RNA was inhibited only about 2%. Virtually the same results were obtained, as shown in Table 2, for the MS2-directed, IF3α-dependent and the T4L-directed, IF3β-dependent ribosomal binding of fMet-$tRNA_f$. As already mentioned, the ribosome dissociation activity of IF3 is not impaired by the i factors.

Regarding the mode of action of the i factors one could imagine that each complexes with its cognate IF3 and prevents its binding to the 30S ribosomal subunit (8). This, however, should also inhibit DF activity (9) unless the IF3-i complex is itself capable of binding. If so one would have to assume that the i factor, while not affecting 30S binding site(s) of IF3, would block or otherwise alter initiation binding site(s) of the IF3 molecule. Alternatively, the i factors might not complex with free but with 30S-bound IF3. One other possibility that would explain the lack of inhibition of DF activity is that i factors interact with the messenger rather than with IF3.

Table 2. Inhibition by i factors of mRNA- and IF3-dependent ribosomal binding of fMet-tRNA$_f$

mRNA	IF3	i Factor (μg)	fMet-tRNA$_f$ bound* (pmol)	Inhibition (%)
MS2	α	None	2.44	0
		Q_βα subunit+ (11.2)	1.35	44.5
		α (12.5)	1.07	56
		β (64)	2.35	4
Late T4	β	None	5.89	0
		Q_βα subunit+ (11.2)	5.69	4
		α (12.5)	5.55	5.5
		β (64)	2.15	63

*Net values, in the presence of IF3, values in the absence of IF3 having been subtracted. They were as follows: MS2 RNA, no i factor, 0.3 pmol; late T4 RNA, no i factor, 0.56 pmol.

+Phage Q_β RNA replicase α subunit (5).

Assays were conducted as previously described (6, 7). Samples contained, in a final volume of 0.05 ml, Tris-HCl buffer (pH 7.2), 100 mM; NH_4Cl, 54 mM; magnesium acetate, 5.5 mM; GTP, 0.2 mM; mRNA, 40 μg; f[^{14}C]Met-tRNA, 20 pmol (8000 cpm); 1.0 M NH_4Cl-washed E. coli Q13 ribosomes, 3 A_{260} units; homogeneous IF1, 1 μg; purified IF2, 3.0 μg; and IF3α or IF3β, 0.5 μg. Incubation was for 10 min at 37°. All messenger RNA'swere incubated at 37° in 0.1 mM EDTA for 3 min before use.

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