STUDIES ON POLYPEPTIDE CHAIN INITIATION FACTORS F_1 AND F_2

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1. Introduction

AUG-directed binding of fMet-$tRNA_F$ to *E. coli* ribosomes needs initiation factors F_1 and F_2. However, while considerable binding may occur in the presence of F_2 alone, e.g., at 0°, F_1 by itself is completely inactive [1]. Although the precise mechanism by which F_1 increases binding is not clear, the present study indicates that this factor may function by increasing the affinity of AUG for the ribosome.

In further work on F_2 it was found that chromatography of a crude fraction (prepared from 1 M NH_4Cl wash of *E. coli* Q_{13} ribosomes) on phosphocellulose columns resulted in the elution of F_2 as two major peaks of activity (F_2a and F_2b). At 37°, F_2a is much less effective than F_2b in promoting the formation of an initiation complex, directed by AUG, GUG or MS2 RNA.

2. Materials and methods

Phosphocellulose (P 11, capacity 7.4 meq/gm), was a product of Whatman. AUG was purchased from Miles Laboratories. Ribosomal binding of fMet(^{14}C)~tRNA and fMet-puromycin synthesis was measured as described previously [1]. Highly purified F_2, F_1 and F_3 were prepared as described previously [2–4]. Unless mentioned otherwise, F_2a and F_2b were routinely assayed at 0° without F_1 [1]. F_2a and F_2b were prepared as follows. Crude ribosomes from *E. coli* Q_{13} were washed with 1 M NH_4Cl and the wash was precipitated with ammonium sulfate. The precipitate obtained between 30 and 50% saturation was dissolved in a minimal volume of buffer containing 50% glycerol, 20 mM Tris-HCl, pH 7.8, 0.2 mM Mg^{2+}, 20 mM NH_4Cl, 1 mM dithiothreitol (DTT) and stored at −20°. Prior to chromatography, this fraction was dialyzed against 20 mM Tris-HCl, pH 7.4, 1 mM DTT (Buffer A) for 3 hr. Any precipitate formed during dialysis was discarded. 3.3 ml of the dialyzed solution, containing about 92 mg protein, was applied to a phosphocellulose column (0.8 × 25 cm) previously equilibrated with Buffer A. A part (15–20%) of the applied F_2 activity (F_2a) was eluted with Buffer A along with the bulk of the protein. The column was then eluted with 250 mM NH_4Cl, 50 mM Tris-HCl, pH 7.4, 1 mM DTT (Buffer B). About 30% of

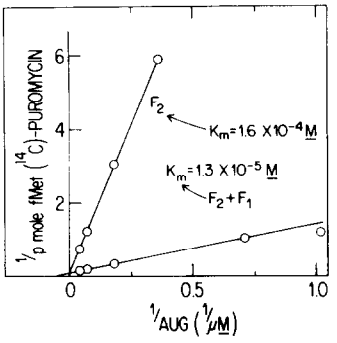

Fig. 1. Determination of K_m for AUG. Assay mixtures contained (in a final volume of 0.05 ml), NH_4Cl, 130 mM; Tris-HCl buffer, pH 7.2, 50 mM; magnesium acetate, 4 mM; DTT, 5 mM; GTP, 0.2 mM; fMet~tRNA labelled with ^{14}C methionine, 100 pmole (24,000 cpm); ribosomes, 2.2 A_{260} units; puromycin, 1 mM; F_2, 1.1 μg and when present, F_1, 0.5 μg. Reaction was started by the addition of ribosomes. Incubation was for 15 min at 25°. Blanks without AUG subtracted from all values.

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Table 1
Activity of F_2a and F_2b with trinucleotide codons in the presence of F_1.

		Net fMet(^{14}C)~tRNA bound (pmoles)				Activity remaining at 37° (%)	
		F_2a		F_2b		F_2a	F_2b
Expt.		25°	37°	25°	37°		
1	AUG	3.48	0.53	3.66	2.26	15	62
2	GUG	1.15	0.02	1.78	0.59	1	33

Assay mixtures contained (in a final volume of 0.05 ml): Tris-HCl buffer, pH 7.3, 50 mM; NH_4Cl, 100 mM; magnesium acetate, 6 mM; DTT, 5 mM; GTP, 0.2 mM; fMet(^{14}C)~tRNA, 20 pmole (360 cpm/pmole); ribosomes, 2.75 A_{260} units; either AUG (expt. 1) or GUG (expt. 2), 0.05 A_{260} units; F_2a, 40 μg (expt. 1), 25 μg (expt. 2); F_2b, 3.9 μg (expt. 1), 2.2 μg (expt. 2) and F_1, 1.5 μg. Ribosomes were added last. Incubation was for 15 min at the indicated temperatures. Blanks without F_2 at both temperatures, 0.42 (expt. 1) and 0.32 (expt. 2) pmole, subtracted.

Table 2
Activity of F_2a and F_2b with MS2 RNA in the presence of F_1 and F_3.

	Net fMet(^{14}C)~tRNA bound (pmole)		
	25° (AUG)	37° (MS2 RNA)	Activity remaining at 37° (%)
F_2a	5.0	0.63	13
F_2b	4.15	2.27	55

Conditions similar to those of table 1 with F_3, 1 μg; either F_2a, 18 μg or F_2b, 2 μg and either AUG, 0.05 or MS2 RNA, 0.96 A_{260} units. Incubation was for 15 min as indicated. Blanks without F_2 (1.41 pmole at 25° and 0.65 pmole at 37°) subtracted. MS2 RNA cannot be used at 25° because of its high degree of ordered structure at this temperature.

the applied F_2 activity was eluted as a peak with Buffer B (F_2b). A minor peak of F_2 activity eluting ahead of F_2b has also been observed. It may represent a mixture of F_2a and F_2b. F_2 is very sensitive and is easily inactivated; this may account for the low total recovery of F_2 activity. When necessary, F_2a was concentrated by dialysis overnight against a solution (adjusted to pH 7.5) containing 50 mM Tris-HCl, 20 mM NH_4Cl, 2 mM Mg^{2+}, 10 mM 2-mercaptoethanol and ammonium sulfate at 0.7 saturation (Buffer C). The precipitated protein was collected by centrifugation, dissolved in a minimal volume of Buffer B, dialyzed against the same buffer for 3 hr, diluted with an equal volume of glycerol and stored at −20°. F_2b was concentrated by ultrafiltration, made 50% with respect to glycerol and stored at −20°. For some experiments (table 2), F_2a and F_2b were chromatographed successively on DEAE-cellulose and Sephadex G-200.

3. Results and discussion

3.1. *Effect of F_1 on the ribosomal affinity of AUG*

The rate of the fMet-puromycin reaction as a function of AUG concentration was studied with F_2 alone and F_2 plus F_1. The results plotted according to Lineweaver and Burk [5] are shown in fig. 1. Analysis of such a plot reveals that F_1 lowers the K_m for AUG by approximately a factor of 10 without significantly affecting the V_{max}. In this respect, F_1 resembles the S factor which is involved in the release of ribosome-bound fMet-tRNA as formylmethionine [6]. It was shown that, although S factor lowered the K_m for release codons in the presence of either R_1 or R_2, this factor by itself was ineffective in causing release.

3.2. *Properties of F_2a and F_2b*

Both F_2a and F_2b need sulfhydryl groups for activity as judged by their sensitivity to *p*-hydroxymercuribenzoate [7]. Table 1 summarizes results of experiments in which the binding of fMet-tRNA to ribosome was studied at 25° and 37° using F_1 with either F_2a or F_2b. Both F_2a and F_2b were active at 25° in promoting binding directed by AUG or GUG. However, at 37°, F_2a was much less active than F_2b with either codon. Similar results were obtained when the ribosomal binding of fMet-tRNA was studied at 37° using the natural messenger, MS2 RNA (table 2). In this experiment, F_3 was also included in assay samples since little MS2 RNA-directed binding of fMet-tRNA occurs in the absence of this factor. The results suggest that F_2a differs from F_2b in that it has relatively little activity at 37° in promoting for-

mation of an initiation complex directed by AUG, GUG or MS2 RNA.

Since the fraction of total F_2 present as F_2a is likely to be functionally inactive at 37°, conversion of F_2b to F_2a may provide a means for controlling the rate of protein synthesis. The possibility that F_2b is converted to the apparently more acidic F_2a by phosphorylation or adenylylation and the question whether the relative proportion of F_2a increases under conditions where the rate of protein synthesis slows down, e.g., by amino-acid deprivation of an auxotrophic strain, is currently under investigation.

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