THE RELATIONSHIP BETWEEN THE 3′-END OF 16 S RNA AND THE BINDING OF INITIATION FACTOR IF-3 TO THE 30 S SUBUNIT OF *E. COLI*

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1. Introduction

The absence of proteins S1, S11, S12, S19 and S21 does not seem to influence the physical integrity of IF-3's binding site on the 30 S subunit [1]. However, proteins removed from 30 S subunits by CsCl centrifugation may [2] or may not [3] contribute to the integrity of this binding site. It seems clear, however, that the 16 S RNA does play a role in IF-3 binding [1]. Furthermore, IF-3 and the 3′-end of the 16 S RNA appear to be functionally [4] as well as topographically related [5]. These results, as well as speculations about the cause of the IF-3's dissociation activity [6], make plausible the idea that some of the nucleotides adjacent to the 3′-end of 16 S RNA may be in the immediate neighborhood of IF-3, or that they may be an important part of the IF-3 binding site on the 30 S subunit.

We describe here the results of experiments done to identify whether an intact 3′-end of 16 S RNA is required for IF-3 binding activity.

Mixtures of IF-3 and various 30 S subunit preparations were analyzed by sucrose gradient centrifugation at either 53 000 rev./min or 24 000 rev./min. We find that the stability of the IF-3 interaction with the 30 S subunit is strongly dependent, at high rotor speed, on the presence of the last 49 nucleotides of the 3′-end of 16 S RNA. This dependence is much less obvious at low speed. Furthermore, we find that 30 S cores lacking S1, S2, S3, S9, S10, S14, S20 and S21 are unaffected, whatever the rotor speed, in their IF-3 binding ability. This last observation is in contrast to identical experiments done previously on S1 [7], another protein which may be functionally [4] as well as topographically related to the 3′-end of 16 S RNA [8,9].

2. Materials and methods

NH_4Cl cores, 3 M, were prepared as in [10]. Control 30 S subunits and 30 S subunits lacking the 3′-end of 16 S RNA were prepared by the 30 mM Tris method [10]. Normal 30 S subunits were prepared by the 0.85 M NH_4Cl method [4].

IF-3, prepared as in [11], was 95% pure on SDS gels. It was made radioactive by reductive methylation [12], the resulting specific activity was 21 150 cpm/μg.

3. Results

Subunits, 30 S, lacking both the 3′-end of 16 S RNA and S1 (30 S (-E3)) were resuspended in reconstitution buffer [12] and annealed by incubation at

40°C to restore native configuration [10]. The products were mixed with IF-3 and analyzed by sucrose gradient centrifugation at 53 000 rev./min in the Spinco SW60 rotor. The amount of IF-3 bound was estimated from the ratio ^{14}C to A_{260} across the 30 S peak in the gradients.

We observe very little IF-3 binding to the 30 S (-E3) particles (fig.1a). On the other hand, control 30 S (i.e., uncleaved 30 S subunits which went through the procedure used to remove the colicin E3 fragment) bind IF-3 with high affinity (fig.1a) and in a manner undistinguishable from that of normal 30 S subunits (fig.1b).

The absence of IF-3 binding to the 30 S (-E3) particles could be explained in 4 ways. IF-3 binding could have been prevented by:

(i) Colicin E3 cleavage alone.
(ii) Absence of S1 on the 30 S (-E3) particles.
(iii) Absence of the colicin E3 fragment on the 30 S (-E3) particles.
(iv) Pressure-dependent dissociation which would manifest itself only upon IF-3-30 S (E3) interaction and not upon IF-3 interaction with the other 30 S preparations.

Explanations (i) and (ii) can be excluded because IF-3 binds with high affinity to cleaved 30 S subunits (fig.1b) and because binding is not improved when 30 S (-E3) particles are preincubated with a 6-fold molar excess of S1 (fig.1b).

To verify whether pressure-dependent dissociation might be responsible for the apparent lack of IF-3 affinity for 30 S (-E3), the experiments were repeated at lower rotor speed (fig.1c,1d). A striking observation emerges: speed reduction from 53 000–24 000 rev./min results in a 10–40-fold increase in the apparent affinity of IF-3 for 30 S (-E3), whether the particles are preincubated or not with S1 (fig.1c). On the other hand, the affinity of IF-3 for control 30 S, cleaved 30 S and normal subunits is nearly unchanged (compare fig.1a,b with fig.1c,d).

The demonstrable pressure dependence of the IF-3-30 S (-E3) complex indicates that the molar volume of such an interaction [18,19] is positive and much larger than when IF-3 binds to either control 30 S, cleaved 30 S, or normal 30 S subunits. This excess molar volume of interaction can be obtained in either of 2 ways. IF-3 binding to 30 S (-E3), and to 30 S (-E3) only, could:

(1) Create an expansion of the complex.
(2) Result in the trapping of excess solvent, e.g., in a groove normally occupied by the missing RNA.

We cannot rule out hypothesis (1) but we consider (2) a simpler explanation for the pressure effect we observe.

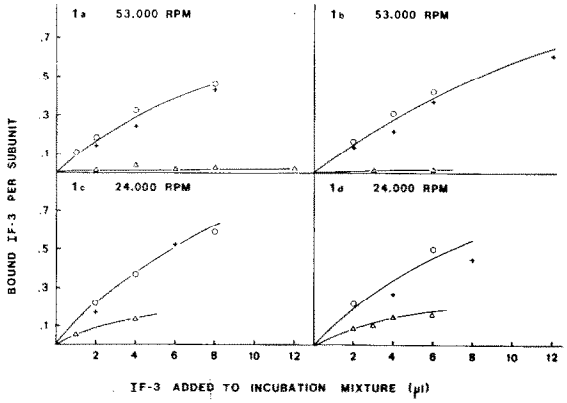

Fig.1. Stoichiometry of IF-3 binding to various types of MRE600 30 S subunits. 54 pmol 30 S subunits were incubated for 15 min at 37°C [14,15] with ^{14}C-labelled IF-3 (23.7 pmol/μl) in 0.18 ml containing 14 mM Tris, 6.6 mM magnesium acetate, 66 mM KCl, 40 mM NH_4Cl, 0.2 mM EDTA, 6 mM 2-mercaptoethanol (pH 7.5). Samples were layered onto 5–20% sucrose gradients containing 18 mM Tris, 6.5 mM magnesium acetate, 90 mM NH_4Cl, 0.4 mM EDTA (pH 7.5), centrifuged (SW 60 rotor, 4°C) and analyzed for absorbance and radioactivity content. The specific radioactivity of IF-3 was calculated assuming mol. wt 20 500 [16]. 30 S subunit concentrations were determined from A_{260} using the extinction coefficient obtained in [17].

Fig.1a. Centrifugation at 53 000 rev./min for 2.5 h. (○) Stoichiometry on control 30 S; uncleaved 30 S subunits which went through the procedure used to remove the colicin E3 fragment. (+) Stoichiometry on NH_4Cl cores, lacking S1, S2, S3, S9, S10, S14, S20, S21 [10]. (△) Stoichiometry on 30 S subunits lacking the 3'-end of 16 S RNA and S1 (30 S (-E3)) [10].

Fig.1b. Centrifugation as in fig.1a. (○) Stoichiometry on normal 30 S subunits [4]. (+) Stoichiometry on colicin E3 cleaved 30 S subunit. (△) Stoichiometry on 30 S subunits lacking only the 3'-end of 16 S RNA. 30 S (-E3) were incubated with 6-fold excess S1 (0°C, 10 min) before addition of IF-3.

Fig.1c. Centrifugation at 24 000 rev./min for 12 h. Otherwise as in fig.1a.

Fig.1d. Centrifugation as in fig.1c. Otherwise as in fig.1b.

Since the colicin E3 RNA fragment does not appear to be the main ribosomal component responsible for IF-3 binding we looked for protein involvements. Cores, 30 S, prepared by 3 M NH_4Cl washing and lacking S1, S2, S3, S9, S10, S14, S20 and S21 [10], bind IF-3 with normal affinity (fig.1a,1c). These split proteins should not therefore be involved in IF-3 binding unless an additional IF-3 binding site gets uncovered by removal of these proteins.

4. Discussion

Procedures for removing the colicin E3 fragment are not gentle [10]. In order to use such particles for IF-3 binding studies, we must assume that they suffer from no injury other than their lack of a normal 3′-end. The validity of this assumption was examined in [10]. It was shown that the sedimentation and electrophoretic properties of these stripped 30 S particles are normal. Furthermore stripping conditions removed no proteins, except S1, and S1 binding properties of 30 S (-E3) particles are identical to those of reactivated, normal subunits. We conclude that 30 S (-E3) subunits, prepared as described, are physically intact (except for their lack of a proper 3′-terminus) and that the IF-3 binding data obtained with these particles are likely to be relevant to an understanding of the relationship between the 3′-end of the 16 S RNA and the IF-3 binding site on normal subunits.

Our results demonstrate both similarities and contrasts between the requirements for S1 and IF-3 binding to the 30 S subunit.

1. S1 does not require the colicin E3 fragment in order to bind to its high affinity binding site on the 30 S subunit [10]. We have shown here that IF-3 does not seem to require that fragment either, though we cannot exclude the possibility that as much as 10% of the free energy of binding IF-3 to the 30 S subunit is contributed by the 3′-end of 16 S RNA. On the other hand we observed that IF-3 binding to 30 S particles lacking the 3′-end of 16 S RNA results in an increased positive value for the molar volume of interaction, as if IF-3 binding to 30 S (-E3) was trapping extra solvent in a putative 'groove' left by the removal of the 3′-end of 16 S RNA.
2. S1 requires S9 in order to bind with high affinity to the 30 S subunit [7]. IF-3 seems to require neither S2, S3, S9, S10, S14, S20, nor S1, S11, S12, S19, S21 [1].

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