STOICHIOMETRY OF INITIATION FACTOR IF-3 IN EXPONENTIALLY GROWING *ESCHERICHIA COLI* MRE 600

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1. Introduction

The initiation factors IF-1, IF-2 and IF-3 mediate initiation of polypeptide synthesis in *E. coli* [1–3]. All three factors are present on native 30 S ribosomes [4, 5] as was shown by the ability of these particles to initiate protein synthesis. Although these proteins can be removed from the 30 S ribosome by washing in 1 M NH_4Cl and are not normally classified as ribosomal proteins, it would be of interest to demonstrate their presence by a simple technique and to determine their stoichiometry. SDS gel electrophoresis suggested itself as a suitable tool, since the published molecular weights of the initiation factors [5, 6] differ from the molecular weights of the 30 S ribosomal proteins, at least in *E. coli* B and in *E. coli* MRE 600 [7–9]. IF-1 has a molecular weight of 9000 [5, 6] and thus should migrate well ahead of the smallest known ribosomal proteins (molecular weights approx. 10 900, [8]) in SDS gel electrophoresis. The molecular weight of IF-3 is 25 000 as determined by glycerol gradient centrifugation and 21 000 on SDS polyacrylamide gels [5]. At least in *E. coli* B and in *E. coli* MRE 600 there is no 30 S ribosomal protein with a molecular weight between 26 700 (S4) and 19 600 (S5) [8, 9]. Therefore in SDS gel electrophoresis of 30 S proteins IF-3 should show up between these two proteins. In *E. coli* K12 (Q13) the 30 S ribosome contains one protein with a molecular weight that is different from the corresponding protein in strains B and MRE 600: S7K (K-protein) [9, 10] has a reported molecular weight of 22 700 as compared to 19 200 in B and MRE 600 [9]. For this reason the presence of IF-3 on 30 S ribosomes of *E. coli* K12 might be obscured by S7K in SDS gel electrophoresis. The situation with regard to IF-2 is much less clear. The data for the molecular weight of this protein vary from 80 000 [11] to 55 000 [5] and furthermore preparations of IF-2 are reported to contain more than one protein species [5]. As will be shown below the major component of our IF-2 preparation co-migrates with the 30 S ribosomal protein S1 in gel electrophoresis.

This paper describes an attempt to identify the initiation factors in native 30 S particles by SDS gel electrophoresis and to determine the stoichiometry of IF-3.

2. Materials and methods

2.1. *Growth of bacteria and isolation of subunits*

E. coli Q13 cultures were grown as described previously [12]. *E. coli* MRE 600 was cultured on minimal salts medium with glucose [13]. Both strains were harvested in mid-log phase. The cells were harvested and lysed as described previously [12], except that the DNAase treatment was omitted. The cell-free lysates were centrifuged on 15–30% sucrose gradients in 0.01 M Tris, pH 7.6; 0.01 M Mg acetate; 0.06 M NH_4Cl (standard buffer). The native 30 S subunits were collected by centrifugation at 50 000 rpm in the Spinco 50 Titanium rotor and resuspended in a small volume of standard buffer. Derived subunits were prepared by dissociating the polyribosomes which were sedimented to the bottom of the swing-out tube in standard buffer containing 0.0002 M Mg acetate and preparative gradient centrifugation. In accordance with Dubnoff et al. [5] we found initiation factors

Table 1
Molecular weights and stoichiometry of some 30 S ribosomal proteins of *E. coli* MRE 600 determined by SDS gel electrophoresis.

Protein	Molecular weight Our values	Data from Dzionara et al. [8]	Protein	Stoichiometry Our values	Data from Voynow and Kurland [21] [b]	Data from Weber [22]
S1	65 000	65 000	S1	0.22	0.29	0.10
S2, S3	28 800	28 300 (S2) 28 200 (S3)	S2 + S3	0.98	1.24	1.15
S4	27 000	26 700	S4	0.73	0.87	1.20
S7K	23 500	22 700	S7K	0.82	–	–
S7, S5	19 700	19 600 (S5) 19 200 (S7)	S7 + S5	1.89	1.53	2.20
IF-3	23 800	21 000[a]	IF-3	0.52[c]	–	–

[a] This value is taken from Dubnoff et al. [5].
[b] Data from recovery measurements, except for S5 which was only determined by a dilution method.
[c] Number of copies per native 30 S ribosome.

only at the position of native 30 S particles in the gradients of lysates (unpublished).

2.2. *Polyacrylamide gel electrophoresis of ribosomal proteins*

Gel electrophoresis was essentially according to Weber and Osborn [14], using the sample preparation of Bickle and Traut [15] for ribosomal proteins. Coomassie Brilliant Blue (0.1% solution in 7% acetic acid, 25% methanol) was used as the protein stain. The quantitative staining of proteins by this dye was checked by running separate samples of increasing amounts of several ribosomal proteins and initiation factors and determining the surface areas of the peaks obtained after scanning of the gels at 600 nm in a Gilford spectrophotometer. Staining of the bands was linear with the amount of protein applied to the gels (compare [15]). We therefore consider the surface areas of the peaks on the Gilford-chart to be proportional to the mass of protein present in the bands.

As molecular weight markers on the gels were used: pyruvate kinase (57 000), glutamic dehydrogenase (53 000), Carbonic anhydrase (29 000), chymotrypsinogen (25 700), and myoglobin (17 200). The molecular weights of some of the 30 S ribosomal proteins determined with these markers are shown in table 1.

Results and discussion

3.1. *Detection of IF-3 on native 30 S particles by SDS gel electrophoresis*

Most of the 30 S ribosomal proteins have a molecular weight from 11 000 to 17 000 [8] and do not split up into separate bands in this type of electrophoresis [15]. Six proteins, viz, S1, S2, S3, S4, S5 and S7 are found in five more or less well separated bands, at least in *E. coli* K12 (Q13) (fig. 1A). S2 and S3 co-migrate in accordance with their almost identical size [8]. The protein S7K is found to have a molecular weight of 23 500 (table 1) and could mask the presence of IF-3 protein (mol. wt 23 800, table 1) if it were present on 30 S ribosomes of this strain. In *E. coli* MRE 600 S7 has shifted to the position of protein S5 (fig. 1B) and if a substantial amount of IF-3 were present in this preparation a peak would be recorded between S4 and S5 S7. Indeed, if purified IF-1 (fig. 1E), IF-2 (fig. 1F) and IF-3 (fig. 1G) are added to derived 30 S subunits of *E. coli* MRE 600, a peak is found at the position of S7K (fig. 1C). It is also readily seen from this panel that IF-1 migrates faster than any of the ribosomal proteins and that IF-2 co-migrates with S1.

The gel pattern of native 30 S ribosomes from *E. coli* MRE 600 (fig. 1D) is dominated by a number of heavily stained protein bands of unidentified nature. These high molecular weight proteins, which we have

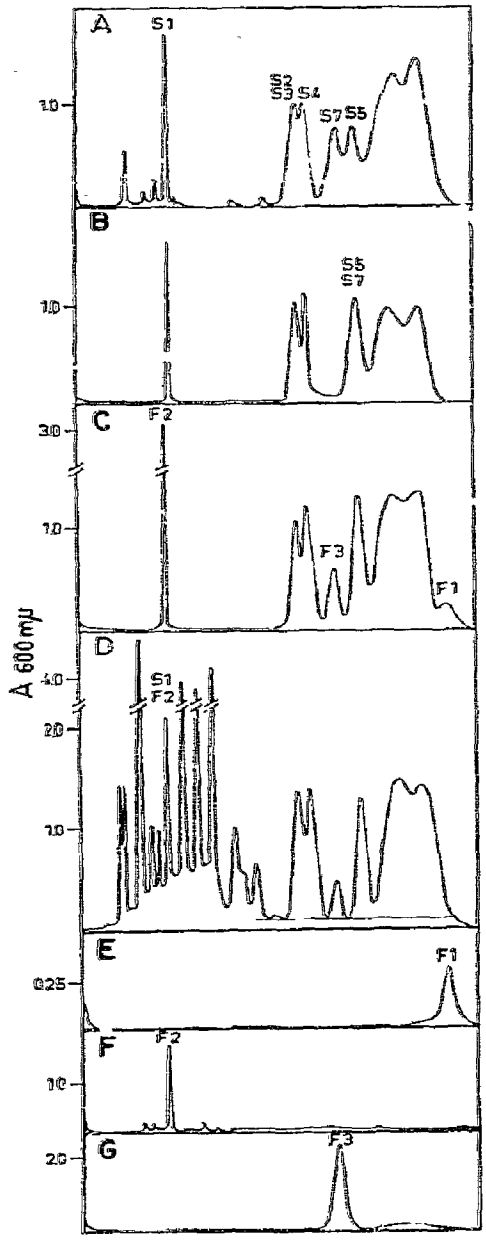

Fig. 1. Gel scan tracings of 30 S ribosomal proteins and initiation factors. SDS gel electrophoresis of ribosomal proteins (from approx. 1 A_{260} unit of 30 S ribosomes) was carried out as described in Materials and methods. A) Derived 30 S subunits of *E. coli* Q13. B) Derived 30 S subunits of *E. coli* MRE 600. C) Derived 30 S subunits of *E. coli* MRE 600 in the presence of purified IF-1, IF-2 and IF-3. D) Native 30 S subunits of *E. coli* MRE 600. E) Purified IF-1. F) Purified IF-2. G) Purified IF-3.

also shown previously to be present in pH 4.5 urea gels [16], are probably partly contaminants and otherwise may contain the interference factor *i* and *j* discovered by Revel et al. [17]. The position of the proteins IF-2 and S1 is indicated in panel D. IF-3 is readily identified in the gels between the bands of S4 and S5 S7. It is very reproducibly present in roughly the same quantities on all native 30 S particles we have analyzed so far. Apart from the fact that purified IF-3 co-migrates with this band, the protein is also very easily removed by washing with 1 M NH_4Cl (data not shown). Furthermore when native 30 S particles are incubated with 50 S ribosomal subunits only 30–40% association to 70 S is found. The 30 S particles which do not associate contain the putative IF-3 in increased relative amount (data not shown), in agreement with the dissociating [18] or anti-associating [19] activity of this factor.

The usefulness of this technique for following the fate of IF-3 during initiation [20] is demonstrated by Vermeer and Bosch in a forthcoming paper [23].

3.2. *The stoichiometry of IF-3 on native 30 S ribosomes*

The number of copies of a ribosomal protein can be calculated when the mass fraction of that protein and its molecular weight are known. Voynow and Kurland [21] and later on Weber [22] determined the mass fraction of all 30 S ribosomal proteins by a radioactive recovery method and then used these data to compute the stoichiometry of the proteins. As a variant to their methods we determined the mass fractions of S1, S2 + S3, S4 and S5 + S7 from Coomassie Brilliant Blue stained gels of derived 30 S subunits. After having established that this dye stains protein bands quantitatively (Materials and methods, compare [15]) we determined the surface areas of the peaks on the recorder-chart with a planimeter. Dividing the obtained values by the total surface area, measured by the same method, gives the mass fractions of the proteins present in the peaks. Then we applied the same calculations as Voynow and Kurland [21], using the molecular weights determined from our own data (table 1) and taking 260 000 daltons for the total molecular weight of protein present in purified 30 S ribosomes [7]. Table 1 shows that our results fit in fairly well with the literature data and warrant a rough determination of the stoichiometry of initiation factor IF-3.

Unfortunately the above described method could not be applied to proteins of native 30 S particles since they are always contaminated with large amounts of non-ribosomal proteins (fig. 1D). However, it did not seem too unrealistic to consider the combined proteins S5 and S7 as an internal standard, since they are both listed as unit proteins [22]. The same holds true for protein S4. From the ratio of the surface area of a protein peak of unknown stoichiometry (e.g. IF-3) to the surface area of the internal standard protein(s) (e.g. S5 + S7), the stoichiometry (e.g. $N_{IF\text{-}3}$) can be calculated by the formula:

$$N_{IF\text{-}3} = \frac{O_{IF\text{-}3}}{O_{S5+S7}} \times \frac{MW_{S5,S7}}{MW_{IF\text{-}3}} \times N_{S5+S7}$$

where O denotes surface.

From three experiments with separate batches of native 30 S ribosomes we determined $O_{IF\text{-}3}/O_{S5+S7}$ ratios of 0.31, 0.33 and 0.35, respectively, yielding an average value for $N_{IF\text{-}3}$ of 0.52. The stoichiometry of IF-3 determined in up to ten experiments, also employing S4 or S2 + S3 + S4 as internal standards, varied from 0.4 to 0.6. We conclude that the initiation factor IF-3, residing on native 30 S particles has to be classified as a fractional protein on this subclass of ribosomes.

Since in logarithmic phase *E. coli*, native 30 S particles represent approx. 10% of all the 30 S ribosomes in the cell (P.H. van Knippenberg, unpublished results), the stoichiometry of IF-3 approaches 0.05 when the total population of 30 S ribosomes is considered. This means that roughly one out of every twenty 30 S ribosomes in the cell is equipped with a factor that enables it to start initiation of protein synthesis.

Because of its low molecualr weight, factor IF-1 would be undetectable by our method if it had a stoichiometry of less than 0.3. Conversely this means that this factor is present in less than 0.3 copies per native 30 S subunit since we could never detect the slightest shadow of a protein band at this position in the gel.

The situation with regard to IF-2 has to await another method to separate this protein from S1. In urea gels at pH 4.5 we have also been unsuccessful to separate the main component of our IF-2 preparation from purified S1 protein. At pH 8.7 we found two major bands in the IF-2 preparation, one of which again co-electrophoresed with S1.

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