# MOLECULAR MORPHOLOGY OF RIBOSOMES: EFFECT OF CHAIN INITIATION FACTOR 3 ON 30 S SUBUNIT CONFORMATION

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## 1. Introduction

Chain initiation factor 3 (IF-3) binds to a single site on the *Escherichia coli* 30 S ribosomal subunit [1–4]. Recent studies have indicated that this binding site is in close proximity to a number of ribosomal proteins [4–8]. Various lines of evidence suggest that the association of the factor with 30 S subunits causes a conformational change of the particles [9,10]. Such a change may be responsible for the activity of IF-3 in promoting the binding of message to ribosomes [11–14], and in preventing ribosomal subunit association [15–18]. However, the specific components of the 30 S subunit which are involved in the conformational change induced by IF-3 are not known.

For the work presented here, lactoperoxidase-catalyzed iodination was used to examine the influence of IF-3 upon the conformation of the 30 S ribosomal subunits. This probe has previously allowed us to demonstrate structural alterations of ribosomes following changes in ionic strength, temperature and selective removal of proteins L7 and L12 from the 50 S subunit [19–22]. Our studies reveal that the enzymatic iodination of 5 ribosomal proteins is altered following the 30S–IF-3 interaction.

## 2. Materials and methods

### 2.1. *Materials*

Growth conditions of *E. coli* MRE600, labelling of r-proteins with [$^3$H]tyrosine, ribosome preparations and extraction of r-proteins for gel electrophoresis were as previously described [19,21,23]. Lactoperoxidase obtained from Sigma Chemical Co. was further purified according to the method of Morrison and Hultquist [24].

### 2.2. *Ribosome–IF-3 complexes*

IF-3 was isolated from the 55–75% $(NH_4)_2SO_4$ fraction of the 1.0 M $NH_4Cl$ ribosomal wash and purified as previously described [25,26]. The purity of the protein was estimated to be greater than 95% as judged by SDS–polyacrylamide gel electrophoresis [27]. Complexes of 30 S and IF-3 were obtained by incubating 25 $A_{260}$ units of 30 S ribosomes with 142 $\mu$g of purified IF-3 in the presence of 60 mM $NH_4Cl$, 10 mM magnesium acetate and 50 mM Tris–HCl, pH 7.2 for 20 min at 37°C. Under these conditions stoichiometric amounts of IF-3 were bound to the 30 S subunits.

2.3. *Enzymatic iodination of 30 S–IF-3 complexes*

Lactoperoxidase-catalyzed iodination of the 30 S–IF-3 complexes was performed at 30°C as described previously [19,21,22]. 30 S subunits in the absence of IF-3 were also iodinated under the same conditions and served as a control in all experiments. Following iodination all ribosome preparations were mixed with an equal amount of unlabelled 30 S particles, pelleted and resuspended in buffer prior to protein extraction and gel electrophoresis. The factor remained bound to the 30 S subunits following iodination. This was evident from the appearance of IF-3 protein next to S7 on the two-dimensional gel slabs.

2.4. *Two-dimensional gel electrophoresis*

Two-dimensional gel electrophoresis of the 30 S r-proteins after iodination was according to the method of Kalschmidt and Wittmann [28] with the following modifications. Approximately 150 μg of 30 S r-proteins were separated in the first-dimension in gel tubes of 5 mm inside diameter and 10 cm in length. After 6.5 h at 3.5 mA/tube, the first-dimension gels were polymerized to slabs, 10 cm × 10 cm, and electrophoresed for 16 h at 13 mA/slab. Staining and destaining of gel slabs and the preparation of individual protein spots for liquid scintillation counting were as previously described [21,23].

## 3. Results

The following procedure was employed to examine the influence of IF-3 upon the susceptibility of r-proteins of the 30 S subunit to enzymatic iodination. 30 S ribosomal subunits labelled with [$^3$H]tyrosine

Table 1
Isotope content (normalized ratios of $^{131}$I/$^3$H) of 30 S ribosomal proteins from exponentially growing cells incubated for three generations with [$^3$H]tyrosine and iodinated with Na$^{131}$I in the presence of lactoperoxidase

| | Normalized ratios of $^{131}$I/$^3$H for 30 S r-proteins | |
|---|---|---|
| Protein | 30 S Subunits | 30 S + IF-3 |
| S2 | 0.95 | 1.32 (+) |
| 3 | 1.08 | 1.10 |
| 4 | 0.39 | 0.38 |
| 5 | 1.16 | 1.66 (+) |
| 6 | 0.11 | 0.11 |
| 7 | 2.89 | 3.07 |
| 8 | 0.31 | 0.51 (+) |
| 9 | 1.28 | 1.23 |
| 10 | 1.88 | 1.86 |
| 11 | 1.72 | 1.39 (-) |
| 12 | 1.38 | 1.37 |
| 13 | 0.71 | 0.69 |
| 14 | 0.69 | 0.74 |
| 15 | 0.21 | 0.19 |
| 16 | 0.26 | 0.25 |
| 17 | 1.15 | 0.86 (-) |
| 18 | 0.78 | 0.72 |
| 19 | 0.95 | 0.91 |
| 20 | 0.25 | 0.25 |
| 21 | 1.51 | 1.64 |

The mean ($^{131}$I/$^3$H) ratio was calculated and the normalized ratios determined by dividing the appropriate mean ($^{131}$I/$^3$H) ratio into the individual ($^{131}$I/$^3$H) ratios.

during the exponential growth phase of *E. coli* MRE600 were isolated and enzymatically iodinated in the presence and absence of IF-3. Following electrophoretic separation of the r-proteins, the normalized ratio of $^{131}I/^{3}H$ was determined for each r-protein. Iodination patterns of r-proteins from 30 S subunits in presence and absence of IF-3 appear in table 1.

With the exception of 5 ribisomal proteins, the pattern of iodination remains essentially unchanged following incubation of 30 S subunits with IF-3. Three proteins, S2, S5 and S8 were found to be consistently more susceptible to enzymatic iodination under these conditions. The increase in normalized ratios for S2, S5 and S8 ranged from 30–40% of the control values. Two proteins, S11 and S17 were less susceptible to enzymatic iodination and had normalized ratios which were 20–25% lower than the control values.

## 4. Discussion

The stoichiometric binding of IF-3 to 30 S subunits has been shown to occur independently of temperature and $Mg^{2+}$ concentration [1–4]. How the attachment of the factor influences the structure of the ribosomal particle has not been precisely defined. Hydrodynamic methods and low-angle X-ray diffraction suggested a tightening of the 30 S particle upon binding of IF-3 [29], whereas circular dichroism studies indicated a conformational modification of some 30 S ribosomal proteins [10]. The specific components involved in this conformational change have not been established.

The data presented in this communication indicate that binding of IF-3 to 30 S subunits produces an altered susceptibility of r-proteins S2, S5, S8, S11 and S17 to enzymatic iodination. These alterations suggest that when IF-3 binds to the small subunit a conformational change occurs. The observation that only a small number of the proteins is affected indicates that IF-3 modifies the structure in a restricted area of the 30 S subunit. Also, cross-linking studies have revealed that r-proteins S2, S5 and S8 are near neighbors and therefore, in close proximity on the 30 S ribosome [30,31].

The relative position of r-proteins S2, S5, S8, S11 and S17 is apparently altered during the binding of IF-3 to the 30 S subunit, as indicated by the iodination patterns in table 1. This suggests several possibilities with respect to the function of IF-3. S2 and S5 have been implicated in the binding of fMet–tRNA to the 30 S subunit [32,33], while antibodies to S8 and S11 have been shown to interfere with EF-Tu-dependent polyU-directed Phe-tRNA binding [33]. Ginzburg and Zamir have indicated that S17 is involved in polyU-directed Phe-tRNA binding [34]. Other studies have also indicated that fMet–tRNA and aminoacyl-tRNA binding sites may overlap [33,34]. IF-3, on the other hand, has been shown to stimulate fMet-tRNA binding and to induce the destabilization of 30 S, aminoacyl-tRNA complexes [35,36].

An attractive interpretation of the data presented here is that the binding of IF-3 to the 30 S ribosome promotes a localized conformational change in the small subunit. This change, revolving around the relative position of r-proteins S2, S5, S8, S11 and S17, results in a conformation favorable to the binding of fMet–tRNA. This should increase the rate of translation of messenger RNA.

In addition to binding to 30 S subunits, recent evidence from our laboratory suggests that IF-3 also interacts with 50 S and 70 S particles. The labeled factor was crosslinked with dimethyl-suberimidate to 30 S, 50 S and 70 S particles. From the stoichiometry of crosslinking, we consider the IF-3 binding site on the 70 S ribosome to be composed of contributions from each ribosomal subunit [37]. Ribosomal proteins S9, S12, L2, L5 and L17 were found to be located in close proximity to IF-3 in purified IF-3 subunit complexes [4,5]. In support of an interaction between IF-3 and 30 S and 50 S subunits, Van Duin et al. [38] have shown that the IF-3 binding site may be composed of rRNA from both subunits. We have also found that the translation of synthetic and natural messenger RNA, at 12–14 mM $Mg^{2+}$, is strictly dependent on the presence of IF-3 and ribosomal protein S1 [25,39], and we have recently demonstrated direct binding of IF-3 to 70 S ribosomes by analyzing [$^{14}C$]IF-3–70 S mixtures on polyacrylamide gel electrophoresis [40]. Whether IF-3 also attaches to 50 S subunits and/or 70 S monosomes inducing a conformational change as measured by lactoperoxidase-catalyzed iodination has not been established and is currently under investigation.

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