EVIDENCE FOR A CONFORMATIONAL CHANGE IN THE 30 S *E. COLI* RIBOSOMAL SUBUNIT UPON FORMATION OF 70 S PARTICLES

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1. Introduction

The association of the 50 S and 30 S ribosomal subunits has been shown to be accompanied by a conformational change in one or both particles [1,2]. This result is interesting in light of the probable functional importance of the 30 S:50 S interface in protein synthesis. The tRNA molecule is thought to span the interface [3], the 30 S-50 S association stimulates both EF-Tu and EF-G dependent GTP hydrolysis [4,5] and translocation is thought to involve the movement of mRNA along the 30 S subunit and subsequent realignment of the 30 S decoding region with the 50 S aminoacyl tRNA binding site [6].

We have employed enzymatic iodination as a surface probe [7–9] to monitor the differences between the isolated 30 S subunit in solution and the 30 S moiety of the 70 S ribosome. This paper reports that the 30 S proteins in the 70 S ribosome are 18% more accessible to enzymatic iodination than in the free 30 S particle. The largest increases in iodine incorporation were observed for S9 and S18. These two 30 S proteins were previously reported to be near the subunit interface or involved in interface related functions (e.g., tRNA binding) [5,10,11].

2. Materials and methods

2.1. *Preparation of 70 S ribosomes and subunits*

70 S ribosomes and 30 S subunits were prepared from midlogarithmic phase A19 cells [12]. The following buffers were used: Buffer A (10 mM Tris [pH 7.6] –30 mM NH_4Cl–10 m $Mg(OAc)_2$), Buffer B (Buffer A plus 6 mM β-mercaptoethanol), Buffer C (10 mM Tris [pH 7.4] – 50 mM NH_4Cl–0.5 mM $Mg(OAc)_2$–6 mM β-mercaptoethanol). Analytical gradients were run on 5–20% sucrose gradients for 1.75 hr at 45 000 rpm, in a SW 50.1 rotor at 4°C.

2.2. *Enzymatic iodination of 70 S ribosomes and 30 S subunits*

Lactoperoxidase (Sigma Chemical Co.) was purified as previously described [13] to remove nuclease contaminants. 30 S or 70 S ribosomes in Buffer A were iodinated for 30 min as previously described [9]. 30 S and 70 S iodination reactions were always run in paralle. The $Na^{125}I$ was usually 20 mCi/mM. H_2O_2 was added in five aliquots over a 5-min period.

2.3. *Electrophoresis and data analysis*

30 S ribosomes were stripped of protein by the acetic acid procedure in the presence of 0.2 M $Mg(OAc)_2$ [14]. Two dimensional gel electrophoresis was performed as described by Kaltschmidt and Wittman [15]. Protein spots were cut out with a scalpel, dried at 75°C, dissolved in 0.9 ml of 30% H_2O_2 for 7 hr at 75°C, and counted in ~ 14 ml of a dioxane based scintillation fluid [9].

The counts obtained from two dimensional gels were treated mathematically as previously described [9]. This treatment enables one to directly compare proteins labeled with different specific activity ^{125}I or protein from gels with different amounts of protein.

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2.4. *Protein synthesis*

The activity of labeled 70 S or 30 S ribosomes was assayed using a poly U dependent phenylalanine incorporation system [16]. The assay was performed under conditions of limiting ribosomes. 100 μg of 70 S ribosomes, or 35 μg of 30 S subunits plus 200 μg of 50 S subunits were assayed. Iodinated ribosomes to be assayed were labeled with low specific activity ^{125}I (~ 1 mCi/mM) and their intrinsic trichloroacetic acid precipitable counts were subtracted from the counts obtained in the protein synthesis assay. The intrinsic counts never amounted to more than 5% total counts.

3. Results

3.1. *The extent of iodination*

Table 1 summarizes the results from four independent labeling experiments. In each experiment the 30 S and 70 S iodination reactions were run in parallel. The ribosomes were always iodinated to less than saturation. 30 S particles are characterized by the ratio of iodines per 30 S particle. The iodines/subunit ratio for 30 S subunits labeled as isolated particles is designated I_{30} and the ratio for 30 S ribosomes obtained from iodinated 70 S particles is designated I_{70}. Although the I_{30} or I_{70} ratio was somewhat variable between experiments I_{30}/I_{70} was fairly reproducible with an average of 0.82 ± .05 for the four experiments.

The I_{30}/I_{70} ratio allows us to compare incorporation of iodine by the 30 S particle in the free and associated state. The data in table 1 shows that the proteins of the 30 S moiety of the 70 S ribosomes are more exposed to the enzyme lactoperoxidase than the proteins in the free 30 S subunit.

Table 1
Iodine incorporation by free 30 S subunits and 30 S particles derived from iodinated 70 S ribosomes*

	I_{30}	I_{70}	I_{30}/I_{70}
Experiment 1	16.0	18.3	0.875
Experiment 2	19.4	23.5	0.830
Experiment 3	14.0	18.5	0.724
Experiment 4	17.9	21.8	0.821
Average			0.82 ± .05

* I_{30} is the ratio of iodines per 30 S particle labeled as an isolated subunit. I_{70} is the ratio of iodines per 30 S particle derived from radio-labeled 70 S ribosomes.

Sucrose gradient sedimentation was employed to determine whether extensive iodination produces any distortion of the ribosomal structure. The 30 S particles labeled in the free state had an I_{30} ratio of 17.9 and the 30 S derived from labeled 70 S ribosomes had an I_{70} ratio of 19.5. As can be seen from fig. 1, heavily iodinated 30 S particles have almost the same sedimentation velocity as unlabeled 30 S subunits. This indicates that no gross conformational distortion has occurred as a result of iodination.

3.2. *The effect of iodination of protein synthesis activity*

Chemical modification of the ribosomes always involves the risk that the topography of the particle

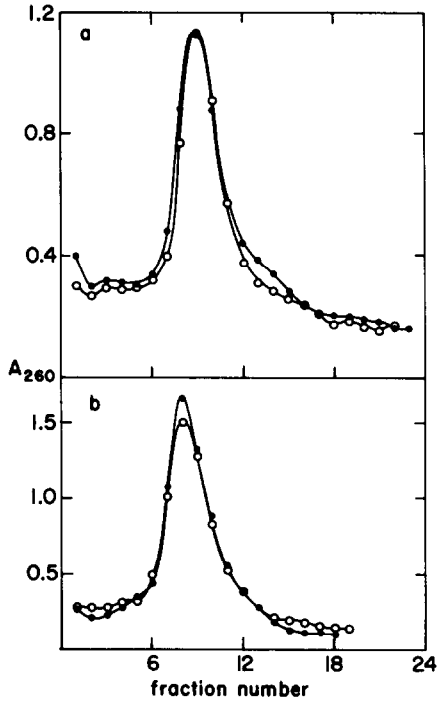

Fig. 1. Sucrose gradient profiles of iodinated and untreated 30 S subunits. 5 to 20% sucrose, 45 000 rpm, SW 50.1 rotor, 1.75 hr, 17 drop fractions. a) (o–o–o) control 30 S, (•–•–•) 30 S iodinated as free subunit I_{30} = 17.9; b) (o–o–o) control 30 S, (•–•–•) 30 S iodinated as 70 S ribosomes, I_{70} = 19.5. Sedimentation was from left to right.

may be altered as a result of covalcnt reaction. The most stringent criterion of native ribosomes is functional activity. 30 S subunits labeled as free subunits as well as iodinated 70 S ribosomes, which should reflect the minimum activity of the 30 S moiety, were assayed for competence in a poly (U) directed phenylalanine incorporation assay.

Fig. 2 shows the protein synthesis ability of subunits and ribosomes as a function of the number of iodines incorporated. As can be seen, the ribosomes are almost linearly deactivated by iodination. Heavily labeled ribosomes, however, retain significant protein synthesis ability. Ribosomes used in the report retain more than 50% activity, 30 S subunits derived from iodinated 70 S ribosomes are at least as active as the 70 S ribosomes from which they were derived.

3.3. *Identification of iodinated 30 S proteins*

Two dimensional gels were run on the protein extracted from iodinated 30 S subunits and from 30 S particles derived from 70 S ribosomes (30 via 70 ribosomes). 1 to 2 mg of ribosomal protein was electrophoresed on each gel. The radioactive protein spots were removed and counted as in Materials and methods. The data shown in fig. 3 represents three independent experiments and nine two-dimensional gels for each 30 S subunit or 30 S via 70 S subunit. The data was treated as described in reference [9].

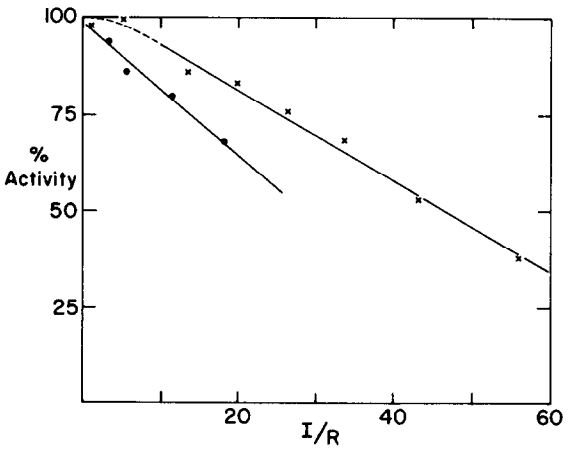

Fig. 2. Protein synthesis ability of 30 S subunits and 70 S ribosomes. Iodinated 70 S ribosomes (X–X–X) were compared to untreated 70 S ribosomes. Iodinated 30 S particles (•–•–•) were compared to unlabeled 30 S particles.

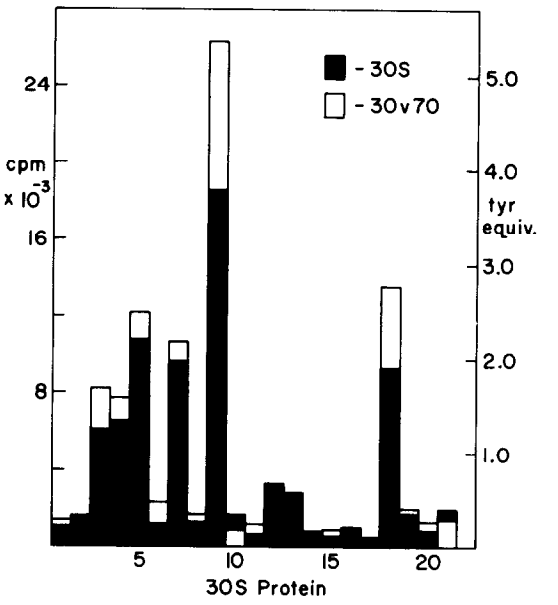

Fig. 3. Iodine incorporation into 30 S proteins. (■) iodine incorporation by 30 S particles labeled as isolated subunits, (□) iodine incorporation by 30 S particles derived from labeled 70 S ribosomes. The left hand ordinate is cpm observed; the right hand ordinate is the calculated tyrosine equivalents labeled per protein.

As can be seen from fig. 3 the 30 S proteins vary greatly with respect to their degree of iodination. Most proteins incorporate iodine to the same extent irrespective of whether they were derived from labeled free 30 S particles or 30 S subunits derived from labeled 70 S ribosomes. Several proteins, notably S3, S6, S9 and S18, are labeled to a significantly greater extent in the 70 S ribosome. Two proteins, S9 and S18, account for 60% of the increased iodination of the 30 via 70 particle over that of the 30 S particles. One protein, S10, is more protected in the 70 S ribosome than in the 30 S particle.

4. Discussion

Increased reactivity towards lactoperoxidase catalyzed iodination is most likely the result of increased exposure of tyrosine residues. The results presented above show that several tyrosines of at least two 30 S ribosomal proteins, S9 and S18, are

more exposed in the 70 S particles than the free 30 S subunit. Similar smaller effects are seen with a third protein, S3. The only simple interpretation of such a change is that a conformational alteration in the 30 S particle accompanies 70 S formation. The iodination results suggest that proteins S9 and S18 either undergo local conformational changes or that structural reorganization of other nearby proteins unmasks tyrosines on S9 and S18.

Earlier results using fluorescein isothiocyanate showed that another 30 S protein, S21, is also more reactive and therefore presumably more exposed in the 70 S particle than in the free 30 S [17]. Other results from this laboratory have demonstrated that a fluorescent dye attached to 30 S proteins S20 and one or both of S16 and S17 is more accessible to quenching by solvent iodide ions in the 70 S ribosome than in the isolated 30 S subunit (Huang and Cantor, unpublished results). This again can only be due to conformational changes. Thus the structural change in the 30 S which accompanies 70 S formation can be shown to involve no fewer than five proteins. Since each shows enhanced exposure, it strongly suggests that at least part of the 30 S opens or expands when the 70 S complex is formed.

It is interesting to note that data exists implicating virtually all of these five proteins as being either near the 30 S–50 S interface in the 70 S particles or involved in functions which seem to involve both subunits and therefore may also reflect events at the interface [5,10,18]. The simplest expectation for an interface protein would be reduced reactivity in a 70 S complex due to masking by the 50 S subunit. This is the opposite of what has actually been observed. Thus the conformational changes attendant in 70 S formation may either entail a fairly substantial reorganization of the arrangement of ribosomal proteins or else may involve rather complex conformation changes within quite a number of individual ribosomal proteins.

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