RELEASE OF IF2 FROM NATIVE RIBOSOMES BY DILUTION

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1. Introduction

In E. coli, IF2 and other polypeptide chain initiation factors have been shown to be associated with the native 30S ribosomal subunits [1, 2]. These factors are removed by washing the ribosomes with a buffer containing relatively high (0.5–1.0 M) concentrations of NH$_4$Cl. However, in certain eukaryotic systems, e.g., Artemia salina embryos [3], rat liver [4], wheat germ [5], initiation factors, functionally similar to IF2, are found in the post-ribosomal supernatant even though the subcellular fractions are prepared in a buffer containing a relatively low concentration of monovalent cation. In the reticulocyte system under these conditions, there is evidence that the initiation factors are ribosome-associated [6] as well as free in the cytoplasm [7].

The present study shows that much of the IF2 is released when native ribosomes are simply diluted in a buffer containing relatively low (0.05 M) concentration of NH$_4$Cl. These results are consistent with the idea [8] that, as in the case of IF3 [9], the interaction of IF2 with the 30S subunit may involve the equilibrium: IF2 + 30S $\leftrightarrow$ [IF2–30S].

2. Materials and methods

The pH of all buffers was measured at 25°C. Native ribosomes were prepared as follows: Fresh E. coli Q13 cells were washed once with buffer A containing 10 mM magnesium acetate, 20 mM Tris–HCl, pH 7.8, 50 mM NH$_4$Cl and 0.5 mM dithiothreitol (DTT). The cells were broken by grinding in a mortar with alumina and then suspended in buffer A (2 g of alumina and 1.5 ml of buffer A were used per gram of cells). Cell debris and alumina were removed by centrifugation at 30 000 g for 30 min. The supernatant solution was incubated at 37°C for 30 min, cooled in ice, incubated for 5–10 min at 0°C with 3 µg of DNAase per ml and centrifuged again at 30 000 g for 30 min. The supernatant (S-30 extract) was centrifuged for 2.5 hr at 60 000 rpm in the Spinco No. 65 ultracentrifuge rotor. The ribosomal pellet was suspended in a minimal volume of buffer A and centrifuged for 30 min at 10 000 rpm in the SS-34 rotor of the Servall centrifuge. The supernatant was used as a source of native ribosomes.

For table 1, experiment 1, three different dilutions of native ribosomes were made. Aliquots of native ribosomes containing a total of 4680, 1944 and 1020 $A_{260}$ units were diluted with buffer A to give a final ribosome concentration of 390, 81 and 17 $A_{260}$ units per ml, respectively. The diluted ribosome samples were then repelleted by centrifugation at 60 000 rpm for 3 hr in the Spinco No. 65 rotor and resuspended in a volume of buffer A approximately equal to the original aliquot (low-salt ribosomes). The supernatant solutions were dialyzed overnight against 80% saturated (NH$_4$)$_2$SO$_4$ solution, pH 7.3, containing 0.2 mM dithiothreitol. After centrifugation, the precipitates were taken up in a minimal volume of buffer A without Mg$^{2+}$, and dialyzed against this buffer for 3–4 hr with several changes of buffer (supernatant). For table 1, experiment 2, native ribosomes were prepared from another batch of Q13 cells. Aliquots containing a total of 4637 and 1003 $A_{260}$ units were diluted to 393 and 17 $A_{260}$ units.
Table 1
Effect of dilution on the release of IF2 from native ribosomes

<table>
<thead>
<tr>
<th>Expt no.</th>
<th>Concentration of native ribosomes* (A260 units/ml)</th>
<th>(a) Supernatant</th>
<th>(b) Low-salt ribosomes</th>
<th>(c) High-salt ribosomal wash</th>
<th>Total units (a) + (b) or (c)</th>
<th>Percent of total units in supernatant</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>390</td>
<td>798 (0.04)</td>
<td>13 290</td>
<td>-</td>
<td>14 088</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>81</td>
<td>968 (0.07)</td>
<td>-</td>
<td>3541</td>
<td>4509</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>832 (0.12)</td>
<td>-</td>
<td>-</td>
<td>1271</td>
<td>65</td>
</tr>
<tr>
<td>2</td>
<td>393</td>
<td>388 (0.02)</td>
<td>-</td>
<td>9261 (0.90)</td>
<td>9649</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>796 (0.10)</td>
<td>-</td>
<td>383 (0.13)</td>
<td>1179</td>
<td>68</td>
</tr>
</tbody>
</table>

* See 'Materials and methods' for details regarding dilution.

** Net values (blanks without IF2 subtracted); 1 unit = 1 pmole of f[14C] Met-tRNA bound to ribosomes.

# Results

It may be seen from table 1 that (a) the specific activity of IF2 in the supernatant fractions increases with increasing dilution of native ribosomes (column 4). (b) The fraction of total IF2 activity recovered in the supernatant fraction also shows a progressive increase as a result of increasing dilution of ribosomes (last column). This is true regardless of whether the total IF2 activity is expressed as the sum of IF2 activities present in the supernatant and low-salt washed ribosomes.
Table 2
Effect of dilution on release of IF2 from native ribosomes

<table>
<thead>
<tr>
<th>Expt. no.</th>
<th>Concentration of native ribosomes ($A_{260}$ units/ml)</th>
<th>Total amount of native ribosomes ($A_{260}$ units)</th>
<th>Content of IF2</th>
<th>Supernatant Total units Sp.Act. b/a</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(a)</td>
<td></td>
<td>(b)</td>
</tr>
<tr>
<td>1</td>
<td>310</td>
<td>931</td>
<td>115</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>31</td>
<td>186</td>
<td>61</td>
<td>0.1</td>
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<tr>
<td>2</td>
<td>387</td>
<td>1162</td>
<td>40</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>29</td>
<td>174</td>
<td>14</td>
<td>0.03</td>
</tr>
</tbody>
</table>

IF2 activity in the supernatant fractions was assayed essentially as described in table 1.

Ribosomes (expt. 1, columns 3, 5, 8) or as the sum of that present in the supernatant and the high-salt ribosomal wash (expt. 2, columns 3, 6, 8). (c) In contrast, the specific activity of IF2 recovered in the high-salt ribosomal wash is considerably lowered by prior dilution of native ribosomes (expt. 2, column 7), since as mentioned above, more IF2 is released from the ribosomes into the supernatant by lowering the concentration of native ribosomes. It should be pointed out that the recovery of total IF2 activity appears to be less, the higher the dilution of native ribosomes. In expt. 1, for example, the yield of total IF2 activity (per 100 $A_{260}$ units of native ribosomes) is about 300, 230 and 130, respectively for the three dilutions used. The reasons for these lower yields at higher dilutions probably include an increased adsorption of IF2 (and IF3) to glass surfaces [12, 13] and an increased loss of IF1 during dialysis [14].

Analytical sucrose gradient centrifugation revealed that small but detectable amounts of ribosomal sub-units were still present as contaminants in these high-speed supernatant fractions. In addition, the level of contaminating subunits was more, the higher the dilution of native ribosomes. This is presumably due to the fact that dilution of 50S–30S couples (which constitute the bulk of native ribosomes) leads to an increased formation of free 50S and 30S subunits by shifting the equilibrium: 50S–30S $\rightleftharpoons$ 50S+30S, to the right [15]. However, since 50S–30S couples contain no IF2 or other initiation factors [1,2,16], the effect of dilution on the equilibrium just described should not contribute to the amount of IF2 which is recovered in the high-speed supernatant fractions. This is further substantiated by the results summarized in table 2. The supernatants used in these experiments were prepared by a different procedure (see Materials and methods) and did not contain detectable amounts of ribosomal subunits. It may be seen that the IF2 activity (per $A_{260}$ unit of native ribosomes layered on sucrose gradients) recovered in the supernatant fractions again increases with dilution of native ribosomes (column 6, table 2). There is a parallel rise in the specific activity of IF2 also (column 5, table 2). The total recovery of IF2 activity from the more highly diluted sample of native ribosomes was, however, very low in these experiments. This is presumably due to the fact that the experiments of table 2 involved prolonged periods of centrifugation (14 hr) as well as increased dilutions (3 ml of native ribosomes layered on 50–55 ml sucrose gradients). It appears likely, therefore, that much of the IF2 which was released into the supernatant by dilution of native ribosomes, was not detected in the experiments of table 2.

5. Discussion

It has recently been proposed [8] that the first step in the IF2-dependent, AUG-directed binding of fMet-tRNA to factor-depleted ribosomes may involve the equilibrium: IF2 + 30S $\rightleftharpoons$ [IF2–30S]. If so, a higher proportion of the total IF2 should exist free when the concentration of the [IF2–30S] complex is lowered. The results of the present study...
are consistent with this view, since dilution of native ribosomes, which contain bound IF2 (as well as bound IF1 and IF3), in a buffer with relatively low concentration of NH₄Cl, leads to release of IF2 activity in the supernatant. It is likely that the observed distribution of polypeptide chain initiation factors (prokaryotic and eukaryotic) between ribosomes and postribosomal supernatant may reflect the dissociation constants of the respective factor(s)—ribosome complexes as well as the dilution of such complexes during isolation. It would also appear that the dissociation constants of IF2—ribosome complexes are increased in the presence of buffer containing a relatively high concentration of NH₄Cl, since only traces of IF2 activity are present in high-salt washed ribosomes. Finally, it should be mentioned that Fakunding and Hershey [17], using radioactively labelled IF2, have directly demonstrated that this factor binds to the 30S subunit and that this binding is stabilized by IF1 and IF3. Similar results have been obtained in other laboratories [18,19].

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References