Antagonistic Action between Spermidine and Putrescine on Association and Dissociation of Purified, Run-off Ribosomes from *Escherichia coli*

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The effects of polyamines on the equilibrium between prokaryotic ribosomal subunits and 70 S ribosomes have been studied as a function of concentration of Mg$$^{2+}$$ from 2.5 to 7.5 mM. Run-off ribosomes were obtained from *Escherichia coli* and were washed with buffered 1 M NH$_4$Cl. Spermidine at 1 mM favors association of subunits at all concentrations of Mg$$^{2+}$$. Putrescine, at concentrations above 5 mM, favors net dissociation at concentrations of Mg$$^{2+}$$ below 4.5 mM. Streptomycin behaves like spermidine, while putrescine behaves like initiation factor 1 and initiation factor 3. The effect of putrescine on dissociation is time-dependent and appears to have a half-life of about 3.5 min at 30°C. When added after the effects of spermidine or streptomycin on association have occurred, putrescine still causes dissociation. The data suggest that putrescine may reduce net formation of vacant 70 S ribosomes. Another possibility is that spermidine and putrescine may act antagonistically to maintain a stable equilibrium between ribosomal subunits and vacant 70 S ribosomes. It may be significant that the putrescine effect is observed at the concentration of Mg$$^{2+}$$ found to be optimum for initiation.

Mg$$^{2+}$$ and spermidine (1, 2) have been shown to shift the equilibrium between 70 S ribosomes and 50 S + 30 S subunits toward 70 S couples, whereas monovalent cations (1-3) and initiation factors, IF-1 and IF-3, have a dissociation effect on 70 S particles (4, 5). Evidence is presented in this report indicating that putrescine, like IF-1 and IF-3, shifts the equilibrium between monosomes and subunits toward dissociation. The putrescine effect is observed below 4 mM Mg$$^{2+}$$. In this respect it is interesting to note that the rate of initiation of protein synthesis in vitro is optimum at 2 to 4 mM Mg$$^{2+}$$(6).

**MATERIALS AND METHODS**

The ribosome suspension, a mixture of 70 S vacant couples and native subunits (see Fig. 1, upper left section) was obtained from polysomes isolated from exponentially growing *Escherichia coli* B cells by the lysosome-Brij 58 procedure (7). The polysome suspension was incubated for 1 h at 37°C to allow run-off of ribosomes and hydrolysis of endogenous mRNA. The run-off ribosomes were di-alyzed against Tris-buffered 0.01 M MgCl$_2$ at pH 7.5 and washed by centrifugation with 1 M NH$_4$Cl in Tris-buffered 0.01 M Mg at pH 0.1.

The washed ribosomes were suspended in buffer (10 mM Tris/HCl, pH 7.5, 5 mM MgCl$_2$, 50 mM KCl and 6 mM mercaptoethanol) and stored at -80°C.

The incubation mixture consisted of 0.01 M Tris/hydrochloride (pH 7.5), 0.03 M KCl, 2 mM dithiothreitol, and Mg$$^{2+}$$ at 2.5 to 7.5 mM. The incubation mixture of 0.2 ml contained 2 optical density units of ribosomes (800 µg/ml). After incubation at 30°C, the ribosome suspension was centrifuged in an SW 50.1 rotor at 43,000 rpm for 75 min through a 10 to 40% sucrose gradient containing 0.01 M Tris/HCl (pH 7.5), 10 mM MgCl$_2$ and 50 mM KCl. The peaks were resolved by top flow through a Gilford model 2480 density gradient scanner, and the areas under the peaks were measured with a Dietzgen planimeter. An increase in the percentage of the area under the 70 S peak is taken as a measure of the shift toward net association of subunits, while a decrease is taken as a measure of the shift toward net dissociation of 70 S vacant couples.

**RESULTS AND DISCUSSION**

Fig. 1 illustrates the opposing net effects of putrescine and spermidine on the equilibrium between 70 S vacant couples and free subunits incubated at 2.5 mM Mg$$^{2+}$$.

Table I illustrates the effect of concentration of Mg$$^{2+}$$ on the ability of putrescine to promote net dissociation of vacant 70 S couples. At 7.5 mM Mg$$^{2+}$$, putrescine has no net effect on the equilibrium between subunits and 70 S vacant couples. At 4.5 mM Mg$$^{2+}$$, a slight but significant effect is observed and the shift toward dissociation is pronounced at 2.5 mM Mg$$^{2+}$$. Putrescine and spermidine result in a shift toward dissociation (lower right).

Fig. 2 summarizes the effects of concentration of putrescine at three different concentrations of Mg$$^{2+}$$ on net dissociation of 70 S couples. The dissociative effect of putrescine becomes more pronounced with increasing concentration of putrescine and decreasing concentration of Mg$$^{2+}$$. Putrescine and spermidine have a dissociative effect on 70 S ribosomes and subunits after 30 min incubation is shown in the upper left portion of Fig. 1. The distribution of ribosomes and subunits was the same as that found at zero time incubation. As seen from the upper right portion of Fig. 1, the equilibrium between monosomes and subunits shifts toward dissociation after a 30 min incubation with 15 mM putrescine and toward association after incubation with 1 mM spermidine (lower left). Incubation in the presence of both putrescine and spermidine results in a shift toward dissociation (lower right).
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FIG. 1. Effects of putrescine and spermidine (spd) on the equilibrium between 70 S monosomes and subunits. See "Materials and Methods" for details of the procedure. The distribution of ribosomes and subunits was obtained by centrifugation through a 10 to 40% sucrose density gradient after a 30-min incubation in buffer containing 2.5 mM Mg$^{2+}$.

TABLE I

**Effect of putrescine on net dissociation of 70 S ribosomes as function of concentration of Mg$^{2+}$**

The procedure is described under "Materials and Methods" and the legend under Fig. 1. The shift toward association or dissociation of 70 S couples is measured as an increase or decrease of the area under the 70 S peak expressed as a percentage of the total area under 30 S, 50 S, and 70 S peaks.

<table>
<thead>
<tr>
<th>Mg$^{2+}$</th>
<th>% 70 S</th>
<th>% decrease of 70 S</th>
</tr>
</thead>
<tbody>
<tr>
<td>mM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.5</td>
<td>71.0</td>
<td>51.0</td>
</tr>
<tr>
<td>4.25</td>
<td>71.0</td>
<td>64.6</td>
</tr>
<tr>
<td>7.5</td>
<td>72.0</td>
<td>72.0</td>
</tr>
</tbody>
</table>

Ribosomes incubated at 2.5 mM Mg$^{2+}$ at 30° for 30 mm were divided into two portions, one of which was treated with 4% formaldehyde to freeze the ribosome equilibrium (8). The ribosome suspensions were then centrifuged through sucrose gradients containing either 2.5 mM Mg$^{2+}$ or 10 mM Mg$^{2+}$ as indicated in Table II.

The ribosome suspensions centrifuged through a gradient containing 10 mM Mg$^{2+}$ was found to contain 71% monosomes. Treatment with formaldehyde did not affect this distribution. When centrifuged through a gradient containing 2.5 mM Mg$^{2+}$, the percentage of 70 S monosomes was slightly reduced (to 67%). When treated with formaldehyde before centrifugation, the small decrease in the percentage of 70 S monosomes was abolished. Since formaldehyde has been shown to "freeze" ribosome equilibria (8), we have concluded that centrifugation through a gradient containing 10 mM Mg$^{2+}$ did not alter the ribosome equilibria achieved during incubation.

In Fig. 1, we show that the effect of putrescine on net dissociation of 70 S couples is both time- and temperature-dependent. At 2.5 mM Mg$^{2+}$, there is no net change in the equilibrium between subunits and 70 S couples after a 30-min incubation at 30° or below, while at 37°, a slight shift toward dissociation appears after 5 to 10 min. In the presence of putrescine at 15 mM, there is a slow but steady shift toward dissociation even at 0°. The rate and extent of dissociation increases sharply at 30°, with further but significant increases at 37°. At 30°, it takes about 3.5 min to obtain 50% of the total shift in the equilibrium produced by putrescine at 15 mM.

Since putrescine and spermidine appeared to have opposite effects on the equilibrium between monosomes and subunits (see Fig. 1), we next determined the effect of putrescine on the associative effect of spermidine. As seen from Table III, addition of spermidine at 1 mM increases the percentage of ribosomes present in 70 S couples from 70 to 85% at all three concentrations of Mg$^{2+}$. At 2.5 mM Mg$^{2+}$, addition of putrescine at 15 mM reverses the spermidine effect. As concentration of Mg$^{2+}$ increases, the dissociative effect of putrescine decreases.

Streptomycin has been shown to shift the equilibrium between subunits and 70 S couples toward association (9). As
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seen from Table IV, the effect of streptomycin at 0.125 mM on the equilibrium at 2.5 mM Mg\textsuperscript{2+} is similar to the effect of 1 mM spermidine. As in the case with spermidine, addition of putrescine at 15 mM shifts the equilibrium toward dissociation.

It is commonly believed that neutralization of negative phosphate charges by Mg\textsuperscript{2+} and by spermidine are required to promote association of ribosomal subunits, although there is evidence that the neutralized charges are probably not identical. Thus Weiss et al. (10) have shown that to maintain ribosomal integrity and function, 20% of the cationic binding sites (Class I) must be charged with Mg\textsuperscript{2+}. Spermidine cannot substitute for Mg\textsuperscript{2+} at these sites. Class II sites can be charged with either Mg\textsuperscript{2+} or spermidine, while the remaining sites (Class III) can be charged with Mg\textsuperscript{2+}, spermidine, or monovalent cations. The present data suggests that putrescine at concentrations of Mg\textsuperscript{2+} below 4 to 7 mM may affect the role played by Class II and Class III sites in promoting association of subunits.

The protein-synthesizing activity of a cell-free system decreases with excess stability and excess instability of 70 S ribosomes, as evidenced by effects of Mg\textsuperscript{2+} and spermidine on stability and on protein-synthesizing activity of ribosomes. At concentrations of Mg\textsuperscript{2+} above 8 mM and below 2 mM, protein synthesis by Escherichia coli polysome extracts is markedly inhibited (10, 11). Addition of spermidine, which shifts the equilibrium of ribosomal subunits toward association, also increases the inhibitory effects of excess Mg\textsuperscript{2+} (11, 12).

Noll and Noll (5) have suggested that run-off ribosomes may be preserved as undissociated couples in "native" 50 S subunits seem to be susceptible to loss of ability to reform either vacant couples or initiation complexes. It would therefore appear to be advantageous to cells to maintain a high proportion of the subunits not actively engaged in protein synthesis in the form of vacant 70 S couples. Spirin (13), on the other hand, has emphasized the importance of the lability of this equilibrium between subunits and vacant couples in order to provide a readily available source of free subunits for the initiation sequences. The present work suggests that the ratio of putrescine to spermidine in E. coli may be important in maintaining the lability of this equilibrium.

Although it is difficult to relate the concentrations of polyamines in our in vitro system to the concentration of polyamines in intact cells, it can be calculated that the total internal concentration of putrescine and spermidine in E. coli may be as high as 30 to 40 mM and 2 to 4 mM, respectively. Dubin (14) has reported that E. coli contains 15 to 20 pmol of putrescine and 1 to 2 pmol of spermidine per g wet weight. Since we have found that about 50% of the water in a cellular pellet collected by centrifugation is extracellular, the true internal concentration of polyamines may be proportionately higher.

REFERENCES


Table III

<table>
<thead>
<tr>
<th>Mg\textsuperscript{2+} (mM)</th>
<th>% 70 S - Putrescine</th>
<th>% 70 S + Putrescine</th>
<th>% decrease of 70 S</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5</td>
<td>85</td>
<td>65</td>
<td>23</td>
</tr>
<tr>
<td>4.25</td>
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<td>72</td>
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<td>7.5</td>
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<td>6</td>
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Table IV

<table>
<thead>
<tr>
<th>Condition</th>
<th>% 70 S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minus streptomycin</td>
<td>71</td>
</tr>
<tr>
<td>Plus streptomycin (0.125 mM)</td>
<td>86</td>
</tr>
<tr>
<td>Plus streptomycin (0.125 mM) and putrescine (15 mM)</td>
<td>64</td>
</tr>
</tbody>
</table>
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