Heat Stabilities of Ribosomal Subunits and Reassociated Ribosomes from *Bacillus stearothermophilus*

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Absorbance-temperature profiles reveal that both the 30S and 50S ribosomal subunits from *Bacillus stearothermophilus* are more thermostable than the comparable *Escherichia coli* particles. Thermophile ribosomes formed by the reassociation of subunits do not display functional heat stability.

Distinctive features of the protein-synthesizing machinery derived from thermophilic bacteria were recently summarized (3). The 70S ribosomes isolated from *Bacillus stearothermophilus* have been characterized as heat-stable ribonucleoprotein particles, both on the basis of physical determinations (4, 6, 9–11) and functional tests involving protein synthesis (1, 4). This study was undertaken to determine the relative heat stabilities of thermophile ribosomal subunits and reassociated ribosomes and to compare these results with parallel determinations on the equivalent particles from *Escherichia coli*.

*B. stearothermophilus* strain 2184 and *E. coli* strain B were grown at 65 and 37°C, respectively, and the S-30 fractions were prepared as previously outlined (4). The preincubated S-30 fraction from *B. stearothermophilus* was obtained as described earlier (5), with the exception that cytidine triphosphate and uridine triphosphate were eliminated from incubation mixture, and the preincubation was carried out at 55°C for 10 min. The *E. coli* preincubated S-30 fraction was prepared in the same manner, except that the preincubation was carried out at 35°C for 45 min. The preincubated S-30 fractions were then centrifuged at 122,000 × g for 90 min in the 50S rotor of the Spincro model L centrifuge. The supernatant fluids (S-122 fractions) were dialyzed overnight against standard buffer [0.01 M Tris(hydroxymethyl)aminomethane (Tris)-hydrochloride buffer (pH 7.8), 0.01 M magnesium acetate, 0.06 M KCl, and 0.006 M β-mercaptoethanol] and then stored in small samples at −70°C. The ribosomal pellets were suspended in standard buffer with the aid of a Teflon pestle. The ribosome suspensions were sedimented as described above, and the supernatant fluids were discarded.

Ribosomes were dissociated into subunits by suspending the pellets in 0.01 M Tris-hydrochloride buffer (pH 7.8) and 0.0001 M magnesium acetate, followed by dialysis against the same buffer overnight. After centrifugation at 10,000 × g for 10 min, 100 A260 units of the supernatant fluids were layered on 5 to 20% (w/v) linear sucrose density gradients made up in 0.01 M Tris-hydrochloride buffer (pH 7.8) and 0.0001 M magnesium acetate. After centrifugation at 19,000 rev/min for 16.5 hr at 5°C in a Spinco type SW 25.1 rotor, the gradients were fractionated, and the absorbancy of each fraction was measured at 260 nm. Tube contents corresponding to pure 30S and 50S subunit fractions were pooled separately and dialyzed overnight against standard buffer.

Absorbance-temperature profiles of ribosomal subunits and ribosomes from *B. stearothermophilus* and *E. coli* are presented in Fig. 1. From these data it can be seen that both the thermophile 30S (Tm = 77.0°C) and 50S (Tm = 80.2°C) subunits were more heat-stable than the comparable *E. coli* 30S (Tm = 66.0°C) and 50S (Tm = 73.5°C) subunits. While this manuscript was in preparation, similar results were reported by Altenburg and Saunders (2). In addition to these measurements of physical stability, studies with protein-synthesizing systems have demonstrated the functional stability of thermophile subunits (2, 7, 8). Tm values for 50S subunits from both the thermophile and *E. coli* were higher than Tm values for the corresponding 30S subunits. The greatest increment in transition temperatures between thermophile and mesophile ribosomal particles was found in the case of the 30S subunit (difference of 11°C). Tm values of 50S subunits from...
not designated, particles from 0.01; phosphoenol dissociated ribosomes in subsequent triphosphate, 0.25; guanosine adenosine a concentration of 0.045 and ing acetate, ribosomes; associated 12 methane-hydrochloride buffer unless otherwise specified): (pH multiple-sample absorbance temperature programmer units/ml. Measurements were stearothermophilus Bacillus polyuridylic nondissociated and reassociated Nondissociated a Time pre-incubated ribosomes

FIG. 1. Absorbance-temperature profiles of 30S (dotted line), 50S (broken line), and 70S (solid line) ribosomal particles from E. coli (A) and B. stearothermophilus (B). Particles were suspended in 0.01 M Tris-hydrochloride buffer (pH 7.8) containing 0.01 M magnesium acetate and 0.06 M KCl at initial concentrations of 0.5 to 0.6 A260 units/ml. Measurements were made on a Beckman DUR spectrophotometer attached to a Guild model 2000 multiple-sample absorbance recorder. The temperature was increased at the rate of 0.75 °C per min with the aid of a Neslab temperature programmer (model TP-2).

TABLE 1. Effect of preincubation on the ability of nondissociated and reassociated ribosomes from Bacillus stearothermophilus and Escherichia coli to support polyuridylic acid-directed phenylalanine incorporation

<table>
<thead>
<tr>
<th>Time preincubated at 60 °C (min)</th>
<th>Per cent of initial activitya</th>
<th>Nondissociated ribosomes</th>
<th>Reassociated ribosomes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E. coli</td>
<td>B. stearothermophilus</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>100</td>
<td>75</td>
<td>97</td>
</tr>
<tr>
<td>10</td>
<td>100</td>
<td>48</td>
<td>80</td>
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<tr>
<td>20</td>
<td>100</td>
<td>30</td>
<td>68</td>
</tr>
<tr>
<td>30</td>
<td>98</td>
<td>25</td>
<td>45</td>
</tr>
</tbody>
</table>

a Nondissociated and reassociated ribosomes suspended in 2 ml of standard buffer were preincubated at a concentration of 1.5 A260 units/ml. After the times designated, samples were removed and placed on ice for subsequent use in the protein synthesis assay.

The 0.4-ml incubation mixtures for protein synthesis contained the following components (in micromoles unless otherwise specified): tris(hydroxyethyl)amino-methane-hydrochloride buffer (pH 7.8), 4.0; magnesium acetate, 4.0; KCl, 24.0; β-mercaptoethanol, 1.4; adenosine triphosphate, 0.25; guanosine triphosphate, 0.01; phosphoenol pyruvate, 1.25; phosphoenolpyruvate kinase, 12 μg; a mixture of 14C-labeled amino acids excluding phenylalanine, 0.0125 of each; 14C-phenylalanine (45S mCi/mmole), 2.1 mmoles; 0.135 A260 units of nondissociated ribosomes or 0.09 A260 units of 50S subunits and 0.045 A260 units of 30S subunits in the case of reassociated ribosomes; 0.1 ml of the S-122 fraction from both organisms were slightly higher than those of the corresponding 70S ribosomes.

The thermostability of ribosomes formed by the reassociation of subunits was examined by preincubating these particles at 60 °C for various times and then assaying for their capacity to perform polyuridylic acid-directed protein synthesis at 37 °C. For purposes of comparison, nondissociated ribosomes were tested under identical conditions (Table 1). After 30 min of preincubation, 98% of the initial activity was retained by nondissociated ribosomes from B. stearothermophilus, whereas only 25% was retained by nondissociated ribosomes from E. coli. In contrast to these results, the kinetics of thermal inactivation for reassociated ribosomes from both organisms revealed similar liabilities. Thus, reassociated thermophile ribosomes retained 45% of the initial activity after preincubation for 30 min, and the corresponding value for E. coli reassociated ribosomes was 41%.

E. coli [0.2 mg of protein and 0.02 mg of ribonucleic acid (RNA)]; E. coli B transfer RNA, 50 μg; and polyuridylic acid (poly U), 100 μg. Samples were incubated at 37 °C for 30 min, after which they were processed as described previously (5). Initial activities (14C-phenylalanine incorporated in the presence of poly U minus that incorporated in the absence of poly U) for nondissociated ribosomes were 3,785 counts/min for B. stearothermophilus and 4,132 counts/min for E. coli. Initial activities for reassociated ribosomes were 2,821 counts/min for B. stearothermophilus and 2,648 counts/min for E. coli.
Since the *E. coli* S-122 fraction was used throughout this experiment regardless of the ribosome source, the possibility that the thermophile S-122 fraction contains a stabilizing factor for reassociated ribosomes was investigated (data not shown). When the S-122 fraction from *B. stearothermophilus* was substituted in the reaction mixtures, no effect on the lability of either *B. stearothermophilus* or *E. coli* reassociated ribosomes was noted. Although the mechanism remains unknown, the loss of functional heat stability in the case of reassociated thermophile ribosomes could result from a conformational change. Additional effects, such as the activation of a ribonuclease during the preincubation step, may also be involved.

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**LITERATURE CITED**


