Mechanism of Resistance to Antibiotic Synergy in Enterococci

ROBERT A. ZIMMERMANN, JR., ROBERT C. MOELLERING, JR., AND ARNOLD N. WEINBERG

Department of Bacteriology, Harvard Medical School and Department of Medicine (Infectious Disease Unit), Massachusetts General Hospital, Boston, Massachusetts 02114

Received for publication 3 December 1970

Enterococci exhibit two types of resistance to streptomycin. Moderately high-level resistance is observed in most naturally occurring strains and can be overcome by simultaneous exposure to penicillin. In addition, very high-level resistance is found in those strains against which penicillin plus streptomycin fail to produce synergism in vitro. To study the mechanism of streptomycin resistance in enterococci, ribosomes from a wild-type strain and from a highly streptomycin-resistant mutant were isolated, characterized, and studied in an in vitro amino acid incorporation system. The ribosomes from the organism with moderately high-level streptomycin resistance were sensitive to streptomycin in vitro, suggesting that this type of resistance is caused by failure of streptomycin to reach the ribosomes. Very high-level resistance (and lack of penicillin-streptomycin synergism), on the other hand, appears to be due to ribosomally mediated streptomycin resistance.

Most strains of enterococci are resistant to streptomycin by conventional antibiotic sensitivity testing (22). The addition of penicillin overcomes this resistance (i.e., produces synergism with streptomycin) in many but not all strains of enterococci (14). Our previous investigations (15) showed that penicillin increases the uptake of labeled streptomycin both in cells which exhibit synergism and in mutants with very high-level streptomycin resistance, against which synergism no longer occurs. Hence, failure of synergism does not appear to be related to ineffectiveness of penicillin against these organisms. Other recent studies from our laboratory (R. C. Moellering, Jr., et al., Abstr. 10th Intersci. Conf. Antimicrob. Ag. Chemother., Chicago, 1970, p. 164) show a good correlation between very high-level streptomycin resistance [minimal inhibitory concentration (MIC) of streptomycin >2 mg/ml] and failure of synergism of penicillin and streptomycin. These findings suggest that enterococci have two types of streptomycin resistance: (i) moderately high-level resistance which occurs in most natural isolates and appears to be a permeability phenomenon, since it is reversed by agents which inhibit cell wall synthesis (15), and (ii) very high-level resistance which appears to be mediated by a different mechanism and is not overcome by agents which inhibit formation of bacterial cell walls. Strains of enterococci with very high-level streptomycin resistance occur frequently in clinical isolates (R. C. Moellering, Jr., et al., Abstr. 10th Intersci. Conf. Antimicrob. Ag. Chemother., Chicago, 1970, p. 164).

The present study was initiated to attempt to determine the mechanism of very high-level streptomycin resistance, and hence of failure of synergism, in enterococci. Extensive studies of the mechanism of action of streptomycin have been performed with Escherichia coli (13). In particular, streptomycin has been found to inhibit polypeptide synthesis both in vivo (6) and in vitro (7, 8). Response to the drug in vitro is mediated by the ribosomes; the antibiotic exerts its inhibitory effect only when the ribosomes are derived from a strain sensitive to the drug but not when they are isolated from streptomycin-resistant cells (9). Streptomycin also evokes extensive misreading or mistranslation of synthetic polynucleotide templates by ribosomes from sensitive cells, whereas ribosomes from streptomycin-resistant mutants are insensitive to this influence (4, 5). The streptomycin-sensitive site in E. coli ribosomes has been localized to the 30S ribosomal subunit (2, 3). Similar experiments have not been performed previously with enterococci, although studies of in vitro amino acid incorporation by enterococcal ribosomes have been described (12, 16).

MATERIALS AND METHODS

Bacteria: routine bacteriological studies. The orga-
nism used for this study was an enterococcus (Streptococcus faecalis var. liquefaciens) isolated from the blood of a patient with bacterial endocarditis. The MIC of streptomycin for this organism was 400 μg/ml, and the minimal bactericidal concentration (MBC) was 800 μg/ml. Penicillin plus streptomycin exhibited synergism against this organism (13). A highly streptomycin-resistant mutant of this strain (MIC, MBC >5,000 μg/ml) was also used. This mutant occurred spontaneously when the parent strain was incubated with a streptomycin concentration slightly greater than its MIC. Penicillin and streptomycin together produced no synergistic effect on the high-level resistant strain. For purposes of this study, the wild-type parental strain will be designated streptomycin sensitive (Str⁺), and the highly resistant mutant, streptomycin resistant (Str⁻).

The bacterial preparation for ribosomes. For preparation of ribosomes, bacteria were grown in dextrose phosphate broth (Albini, catalogue No. A-113), harvested in mid-logarithmic phase, washed in normal saline, and frozen at −70°C until further use. Approximately 10 g of cells (wet weight) were suspended in 15 ml of modified Ni-
renberg buffer (MNB; reference 17) containing 0.01 M magnesium acetate, 0.06 M ammonium acetate, 0.006 M β-mercaptoethanol in 0.01 M tris(hydroxymethyl)aminomethane (Tris)-acetate, pH 7.4. Small samples were disrupted by sonic treatment for 2 min/ml at 0°C. The viscous suspension was centrifuged for 10 min at 12,000 × g to remove unbroken cells and most of the cell debris. After treatment with deoxyribonuclease (EC 3.1.4.5; Worthington Biochemical Corp., 10 μg/ml) for 5 min at 25°C, the remaining debris was removed from the suspension by centrifugation for 30 min at 30,000 × g.

The supernatant was centrifuged at 150,000 × g for 90 min to sediment the ribosomes, which were then resuspended in MNB and centrifuged for 5 min at 12,000 × g to remove insoluble matter. Ribosomes were resedimented from this supernatant by a second cycle of centrifugation at 150,000 × g for 90 min. The pellets were once again resuspended in MNB and centrifuged for 5 min at 12,000 × g, and the supernatant was frozen in small samples at −70°C after adjustment of the ribosome concentration to 10 mg/ml. The concentration of a ribosomal suspension registering 16 absorbance units at 260 nm was taken as 1 mg/ml.

The ribosomes used in this study were grown in large excess of buffer containing 0.0003 M magnesium acetate, 0.03 M ammonium acetate, 0.006 M β-mercapto-
ethanol in 0.01 M Tris-acetate, pH 7.8 [essentially the TMA II buffer of Traub and Nomura (23)]. The sub-units were fractionated on a linear 5 to 20% sucrose gradient in the same buffer by centrifugation for 10 hr at 25,000 rev/min in a Spinco SW41 rotor. Gradients were analyzed with a Gilford absorbance recorder fitted with a flow cuvette. The separate subunit fractions were diluted three- to fourfold with MNB and concentrated by sedimentation at 150,000 × g for 8 hr. Pellets were resuspended in MNB, after which the suspensions were cleared by a 5-min centrifugation at 120,000 × g and stored at −70°C.

To check the purity of the subunit fractions and to investigate the dissociation of washed ribosomes at low Mg²⁺ concentrations, small quantities of material (e.g., 2 to 4 absorbance units, 260 nm) were centrifuged on 5 to 20% sucrose gradients in an appropriate buffer for 2 to 3 hr at 40,000 rev/min in a Spinco SW41 rotor. The distribution of ribosomal particles on the gradients was analyzed with the aid of an Isco model D gradient fractionator and model UA-2 ultraviolet analyzer.

Amino acid incorporation directed by synthetic polynu-onucleotides. The capacity of ribosomes from enterococcus to support amino acid incorporation in vitro (17) was measured in 0.10-ml reaction mixtures containing the following components: 0.052 M Tris-acetate (pH 7.4), 0.017 M magnesium acetate (except as noted), 0.073 M ammonium acetate, 0.009 M β-mercaptoethanol, 0.0001 M guanosine triphosphate, 0.001 M adenosine triphosphate, 0.005 M phosphoenolpyruvate, 0.3 unit of pyruvate kinase (EC 2.7.1.40, Calbiochem), 40 μg of stripped E. coli transfer ribonucleic acid (General Biochemicals), from 300 to 400 μg of an S-150 protein fraction from E. coli strain A19 rns 1° prepared as described below, 12.5 μg of polyuridylic acid (poly U; Miles Laboratories) as indicated, and 19 nonradioactively labeled amino acids at a concentration of 0.0004 μg each. The concentrations of 14C-labeled amino acids were: phenylalanine, 22.9 μM (10 μCi/μmole); proline, 12.2 μM (20 μCi/μmole); isoleucine, 6.4 μM (50 μCi/μmole); serine, 6.9 μM (50 μCi/μmole); and histidine, 6.2 μM (50 μCi/μmole). When needed, 2.0 μg of streptomycin or 1.0 μg of paromomycin was added to the reaction mixture. In general, each assay tube contained 200 μg of washed ribosomes; however, when subunits were tested, 150 μg of 30S or 150 μg of 50S particles (or both) in MNB was used.

Samples containing poly U were incubated for 15 min, and those containing polyctidylic acid (poly C) were incubated for 30 min at 30°C. Incorporation was halted by treatment with hot trichloroacetic acid, and the resulting precipitate was collected on membrane filters (Millipore Corp.) which were then glued to plan-
chets. Radioactivity was measured in a low-background gas-flow counter with an efficiency of about 20%. Data reported represent net poly U- or poly C-dependent amino acid incorporation, corrected for background incorporation assayed separately in the absence of polyribonucleotide template.

The S-150 protein fraction was prepared as follows. Packed E. coli cells, strain A19, were ground with twice their weight of alumina and extracted with three volumes of MNB. After low-speed centrifugation (12,000 × g) for 10 min, treatment with 5 μg of deoxyribonu-
clease per ml for 10 min at 37°C, and centrifugation at 30,000 × g for 30 min, the resulting extract was centrifuged for 2 hr at 150,000 g. The upper two-thirds of the supernatant was removed from the tube and recentri-
fuged for 2 hr at 150,000 × g. The upper two-thirds of the supernatant was once again removed, dialyzed overnight against MNB, and frozen in small portions at −70°C. The dialyzed extract constitutes the S-150 frac-
tion employed in the incorporation experiments.

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RESULTS

Antibiotic sensitivity of whole cells. Growth curves of the wild-type parent strain (Str\(^{**}\)) and the mutant with very high-level streptomycin resistance (Str\(^{***}\)) are shown in Fig. 1. Streptomycin was lethal to the Str\(^{**}\) strain at a concentration of 25 \(\mu g/ml\) in the presence of 10 units of penicillin per ml but not in the absence of the second drug. Synergism was not observed with the Str\(^{**}\) mutant. Figure 2 shows the frequency distribution of the MIC and MBC values for streptomycin against 27 strains of enterococci isolated from blood cultures. The MIC for streptomycin was 62 \(\mu g/ml\) or greater for all strains, and hence they were "resistant" to streptomycin by the usual criteria. [Peak serum levels obtainable in patients are 25 to 30 \(\mu g/ml\) (24).] In addition, a significant number (40.7\%) had an MIC of \(>2,000 \mu g/ml\)—i.e., exhibited very high-level resistance to streptomycin.

Characterization of enterococcus ribosomes. At an Mg\(^{2+}\) ion concentration of 0.01 M, washed enterococcus ribosomes sedimented at approximately 70S, 50S, and 30S in a sucrose density gradient. When the Mg\(^{2+}\) concentration was reduced to 0.0003 M by dialysis, all of the 70S ribosomes were dissociated into subunits. Restoration of divalent cations to the suspension promoted substantial re-formation of 70S particles.

Dialysis of the ribosomes into 0.01 M Tris without Mg\(^{2+}\) prior to sucrose gradient analysis in the same buffer resulted in the formation of a prominent shoulder at approximately 43S on the more slowly sedimenting side of the 50S subunit and produced a slight broadening of the 30S peak. Sequestration of divalent cations by dialysis against ethylenediaminetetraacetate produced particles of approximately 23S and 28S, partially overlapping in the gradient pattern.

To find the Mg\(^{2+}\) ion concentration optimum for cell-free amino acid incorporation, ribosomes from the Str\(^{**}\) enterococcus strain were incubated in vitro with a poly U template. The incorporation of \(^{14}\)C-phenylalanine was measured as a function of Mg\(^{2+}\) concentration from 2 mM, the lowest level achievable with the buffers employed, to 37 mM in 5-mM steps. The optimum Mg\(^{2+}\) concentration for poly U-directed phenylalanine incorporation was found to be 17 mM.

Incorporation of phenylalanine into hot trichloroacetic acid-precipitable material under the direction of poly U was also studied as a function of ribosome concentration while Mg\(^{2+}\) concentration was held constant at 17 mM. The rate of incorporation was proportional to the ribosome concentration from 0 to 150 \(\mu g/0.10\) ml and thereafter declined sharply as more ribosomes were added to the system. Accordingly, subsequent incubations were carried out at a ribosome concentration of 200 \(\mu g/0.10\) ml to insure optimal utilization of exogenous polyribonucleotide template.

Sensitivity and resistance of enterococcus ribo-
somes to streptomycin. The effect of streptomycin on amino acid incorporation in vitro was investigated by using washed 70S ribosomes prepared from both Str<sup>sen</sup> and Str<sup>res</sup> cells. The S-150 protein fraction for these experiments was derived from <i>E. coli</i> (strain A19, <i>rns</i> I<sup>−</sup>) to avoid any possible effect of homologous Str<sup>sen</sup> and Str<sup>res</sup> supernatants on the incorporation system.

Poly U-directed incorporation of phenylalanine by ribosomes from Str<sup>sen</sup> cells was inhibited approximately 50% by streptomycin (Table 1, line 1). In corresponding assays with ribosomes from the Str<sup>res</sup> mutant, the incorporation of phenylalanine was unaffected by the presence of streptomycin. Paromomycin caused a 15 to 25% inhibition of phenylalanine incorporation with ribosomes from both Str<sup>sen</sup> and Str<sup>res</sup> strains.

Ribosomes from the two strains were also tested for misreading in a poly U-directed system. The incorporation of isoleucine and serine, amino acids not normally coded by poly U, was measured in the presence of streptomycin and paromomycin. The results are presented in the second and third lines of Table 1. Ribosomes from both strains incorporated low levels of isoleucine and serine in the absence of antibiotics. In the presence of streptomycin, however, the incorporation of isoleucine by Str<sup>sen</sup> ribosomes was stimulated roughly 13-fold and that of serine, 4-fold. Streptomycin had no such effect on Str<sup>res</sup> ribosomes, and the same low levels of misreading were observed both in the presence and in the absence of this drug. Paromomycin-induced misreading was nearly equal in the two strains.

Measurements of poly C-dependent amino acid incorporation are shown in Table 1, lines 4 to 6. The "correct" incorporation of proline and the misincorporation of histidine and serine by Str<sup>sen</sup> ribosomes were all stimulated in the presence of both streptomycin and paromomycin. Ribosomes from the Str<sup>res</sup> mutant were indifferent to streptomycin, however, whereas their response to paromomycin was unchanged by the mutation.

Incorporation of phenylalanine, isoleucine, and proline by Str<sup>sen</sup> ribosomes and of phenylalanine by Str<sup>res</sup> ribosomes as a function of Mg<sup>2+</sup> concentration is illustrated in Fig. 3. Parallel experiments were performed both in the presence and in the absence of streptomycin. Streptomycin inhibited the incorporation of phenylalanine by sensitive ribosomes at all Mg<sup>2+</sup> concentrations (Fig. 3A). Inhibition varied from 50 to 70% near the Mg<sup>2+</sup> optimum of 17 mM to 30 to 40% at higher concentrations. Even though per cent inhibition declined at Mg<sup>2+</sup> concentrations above the optimum, streptomycin clearly manifested its ability to interfere with phenylalanine incorporation throughout the entire Mg<sup>2+</sup> range. As was the case for sensitive ribosomes, Str<sup>res</sup> ribosomes displayed a sharp maximum at 17 mM Mg<sup>2+</sup>, and their indifference to inhibition was evident throughout the range of Mg<sup>2+</sup> concentrations tested (Fig. 3B).

The optimum Mg<sup>2+</sup> concentration for poly U-directed isoleucine incorporation was 12 mM (Fig. 3C), although, in the presence of streptomycin, the maximum misreading occurred at decidedly higher concentration, i.e., 17 mM. Furthermore, the extent of misreading evoked by the drug increased with Mg<sup>2+</sup> concentration from two- to threefold at 12 mM to approximately eightfold at 17 mM and above. Nevertheless, streptomycin was capable of exerting its stimulatory effect on
The optimum fell at 17 mM for incorporation of this amino acid, both in the absence and presence of streptomycin. Ribosomes from Str<sup>sen</sup> and Str<sup>res</sup> strains of enterococcus were dissociated into 30S and 50S subunits by dialysis against buffer containing Mg<sup>2+</sup> ions at a concentration of 0.0003 M, and the subunits were fractionated by sucrose density gradient centrifugation. The separated subunits were assayed independently for their ability to promote the incorporation of phenylalanine under the direction of poly U. Subunits were then reassOCIated to form the four possible combinations of 70S ribosomes which were tested for their response to streptomycin in vitro.

Isolated 30S and 50S subunits from either Str<sup>sen</sup> or Str<sup>res</sup> cells showed scant ability to support poly U-directed incorporation of phenylalanine (Table 2, lines 1–4). The reassociated ribosomes were all active in promoting phenylalanine incorporation, however, and it is quite apparent that the combinations containing 30S subunits from Str<sup>sen</sup> cells were inhibited by streptomycin, whereas those containing 30S subunits from Str<sup>res</sup> were not (Table 2, lines 5–8). The origin of the 50S subunit appeared to be immaterial in determining sensitivity to the antibiotic.

When the poly U-directed incorporation of isoleucine was tested with the four sets of reassociated ribosomes, the high levels of streptomycin stimulation found with untreated 70S ribosomes were not detected (Table 2, lines 9–12). Nevertheless, the recombined particles which contained 30S subunits from the Str<sup>sen</sup> strain were stimulated to misread by a factor of slightly less than two, whereas the combinations containing 30S subunits from Str<sup>res</sup> were insensitive to streptomycin.

**DISCUSSION**

In our previous studies of antibiotic synergism against enterococci, we noted that these organisms exhibit at least two different types of resistance to streptomycin. The first is a moderately high-level resistance (MIC 62 to 500 µg/ml) which is present in most naturally occurring strains. Moderate resistance can be overcome by agents which inhibit cell wall synthesis leading to synergism in combination with aminoglycoside antibiotics (15). The second is a very high-level

**TABLE 2. Specificity of ribosomal subunits in streptomycin-induced inhibition and misreading in vitro with a poly U template**

<table>
<thead>
<tr>
<th>Amino acid tested</th>
<th>Source of ribosomal subunit</th>
<th>Addition</th>
<th>Per cent inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30S</td>
<td>50S</td>
<td>0</td>
</tr>
<tr>
<td>Phe</td>
<td>sen</td>
<td>35</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>— sen</td>
<td>86</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>res</td>
<td>0</td>
<td>—</td>
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<tr>
<td></td>
<td>— res</td>
<td>110</td>
<td>—</td>
</tr>
<tr>
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<td>sen sen</td>
<td>740</td>
<td>346</td>
</tr>
<tr>
<td></td>
<td>sen res</td>
<td>839</td>
<td>617</td>
</tr>
<tr>
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<td>res sen</td>
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<td>640</td>
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<tr>
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<td>res res</td>
<td>881</td>
<td>867</td>
</tr>
<tr>
<td>Ile</td>
<td>sen sen</td>
<td>23</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>sen res</td>
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</tr>
<tr>
<td></td>
<td>res res</td>
<td>7</td>
<td>8</td>
</tr>
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</table>

* Figures represent counts per minute; —, not tested. Mg<sup>2+</sup> was 17 mM. Abbreviations: Phe, phenylalanine; Ile, isoleucine; sen, subunit derived from Str<sup>sen</sup> strain; res, subunit derived from Str<sup>res</sup> strain.
resistance (MIC > 2,000 μg/ml), and strains which exhibit this property are not sensitive to the synergistic effects of penicillin plus streptomycin (R. C. Moellering, Jr., et al., Abstr. 10th Intersc. Conf. Antimicrob. Ag. Chemother., Chicago, 1970, p. 164).

The moderately high-level resistance to streptomycin in naturally occurring strains of enterococci appears to be due to a relative impermeability of the organisms to streptomycin. It is overcome when the permeability barriers are circumvented. Our previous experiments which show increased uptake of labeled streptomycin in the presence of agents which inhibit cell wall synthesis (15) are consistent with such a hypothesis, but this argument would be strengthened by showing that the ribosomes of these moderately resistant organisms are sensitive to streptomycin.

Very high-level resistance to streptomycin occurs frequently among clinical strains of enterococci (noted in 11 out of 27 isolates from blood cultures studied here). Strains with this type of resistance are no longer susceptible to synergism by penicillin (or other agents which affect cell wall synthesis) in combination with streptomycin (R. C. Moellering, Jr., et al., Abstr. 10th Intersc. Conf. Antimicrob. Ag. Chemother., Chicago, 1970, p. 164), even though such agents still cause an increased uptake of labeled streptomycin into these cells (15). The present studies were undertaken to define the mechanism of this high-level resistance to streptomycin and to determine whether such resistance is ribosomally mediated.

Ribosomes and ribosomal subunits from enterococci proved to be physically similar to those from E. coli. At a high magnesium ion concentration (0.01 M), much of the ribosomal material was present in the 70S form. At lower concentrations of magnesium (e.g., 0.0003 M), the 70S particles reversibly dissociated into 30S and 50S subunits, as do E. coli ribosomes (21). Further depletion of Mg²⁺ ions, either by dialysis into Mg²⁺-free buffer or by using ethylenediaminetetraacetate to chelate the divalent ions, resulted in the formation of subunit particles with decreased sedimentation rates. Similar decreases in sedimentation rates have been noted in Mg²⁺-depleted ribosomal subunits from E. coli and have been attributed to an expansion or unfolding of the normally compact ribosomal structure (10, 11). It has been suggested that this structural alteration occurs because of mutual electrostatic repulsion among negatively charged groups within the ribosome that are normally screened by bound Mg²⁺ ions (20).

Even though the wild-type parental strain used for these studies exhibited moderately high-level resistance to streptomycin, its ribosomes were sensitive to the effects of this drug. Streptomycin caused a decrease in the incorporation of labeled phenylalanine into trichloroacetic acid-precipitable peptides when added to a cell-free system containing wild-type enterococcal ribosomes and a poly U template. The antibiotic also promoted substantial misreading by these ribosomes; that is, the incorporation of isoleucine and serine in the presence of poly U and of histidine and serine in the presence of poly C, although the synthetic templates do not normally code for these amino acids. Ribosomes from the mutant strain with very high-level streptomycin resistance, however, were insensitive to inhibition of poly U-directed phenylalanine incorporation by the drug as well as to streptomycin-induced misreading.

The foregoing observations demonstrate that sensitivity and resistance to streptomycin in the enterococcus is ribosomally mediated, as previously found in E. coli (9, 13). The effects of streptomycin on sensitive and resistant ribosomes were noted throughout the entire range of Mg²⁺ concentrations in which significant amino acid incorporation occurred. This strongly suggests that sensitivity or resistance to streptomycin in vitro reflects a property of the enterococcus ribosomes employed and that the experimental results cannot be attributed to the use of any one particular Mg²⁺ concentration in the cell-free system. The influence of supernatant factors on streptomycin or on enterococcus ribosomes was minimized by the use of a nonhomologous protein fraction from E. coli for incorporation studies. Furthermore, the mutation to streptomycin resistance is highly specific to that drug, since it does not alter the response of the ribosomes to paromomycin, a related aminoglycoside antibiotic. The mutation to streptomycin resistance in E. coli is also specific, altering the in vitro sensitivity of ribosomes to streptomycin but not to a variety of other aminoglycosides (5).

By dissociating the ribosomes from sensitive and resistant bacteria into 30S and 50S subunits and then reassociating the subunits in various combinations, it was found that the streptomycin-sensitive site is located in the 30S subunit. Mutation to streptomycin resistance changes this subunit in such a way that it is no longer susceptible to the production of inhibition and misreading by the antibiotic. Streptomycin sensitivity and resistance in E. coli is also mediated by the 30S ribosomal subunit (2, 3), and it has recently been demonstrated that this characteristic is determined by a single structural protein of that particle (18). In light of the extensive similarities between the ribosomes of enterococci and E. coli and the like manner in which ribosomes from the Str** and Str*** derivatives respond to aminoglycoside antibiotics in amino acid incorporation assays, it is likely that the streptomycin charac-
teristic in enterococci is also determined by a protein component of the 30S ribosomal subunit.

Although the studies reported here suggest that the ribosomes of enterococci are similar in many respects to those of *E. coli*, the physiological response of the two bacterial species to streptomycin is not the same. Thus, most naturally occurring strains of enterococci are resistant to streptomycin as judged by conventional testing procedures. Nonetheless, ribosomes from the wild-type isolate tested here were sensitive to streptomycin, demonstrating that this moderately high-level resistance is not ribosomally mediated. Moderately high-level resistance must, therefore, result from the failure of streptomycin to reach the ribosomes; our previous work indicates that streptomycin is prevented from entering the cell because of a natural permeability barrier (15). High-level streptomycin resistance, however, is clearly a property of the enterococcal ribosome, and this resistance appears to explain the failure of penicillin and streptomycin to act synergistically in certain strains of enterococci. Although the enzymatic inactivation of streptomycin might provide an additional mechanism of resistance, such a reaction has not been demonstrated in enterococci. Any effect of the soluble protein fraction from enterococcus was ruled out in the in vitro experiments reported here by using washed enterococcal ribosomes and a nonhomologous supernatant from *E. coli*. It therefore seems likely that the failure of synergism of penicillin and streptomycin which is seen in naturally occurring strains of enterococci (14) is due to ribosomal streptomycin resistance. If this proves to be the case in all strains, it would be of considerable interest since most clinically important resistance to streptomycin in gram-negative enteric bacteria has been thought to be due to resistance transfer factors or plasmids that code for enzymes capable of inactivating streptomycin (19). Studies are underway to determine whether ribosomal resistance accounts for all highly streptomycin-resistant strains that are found in clinical isolates.

ACKNOWLEDGMENTS

We thank L. Gorini for making the facilities of his laboratory available to us for part of this work. The technical assistance of Ruth Lehr and Christine Wenersten is acknowledged with appreciation. R.A.Z. was supported by a fellowship from the Helen Hay Whitney Foundation, and R.C.M. was supported by grants from the Medical Foundation and the King Trust.

LITERATURE CITED


