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Cross-Linking of Streptomycin to the 16S Ribosomal RNA of *Escherichia coli*†

Michel Gravel, Pierre Melançon, and Lêa Brakier-Gingras*
Département de Biochimie, Université de Montréal, Montréal, Québec, Canada H3C 3J7
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ABSTRACT: [^{3}H]Dihydrostreptomycin was cross-linked to the 30S ribosomal subunit from *Escherichia coli* with the bifunctional reagent nitrogen mustard. The cross-linking primarily involved the 16S RNA. To localize the site of cross-linking of streptomycin to the 16S RNA, we hybridized RNA labeled with streptomycin to restriction fragments of the 16S RNA gene. Labeled RNA hybridized to DNA fragments corresponding to bases 892–917 and bases 1394–1415. These two segments of the ribosomal RNA must be juxtaposed in the ribosome, since there is a single binding site for streptomycin. This region has been implicated both in the decoding site and in the binding of initiation factor IF-3, indicating its functional importance.

The aminoglycoside antibiotic streptomycin binds to the 30S subunit of *Escherichia coli* at a single binding site (Chang & Flaks, 1972; Schreiner & Nierhaus, 1973; Grisé-Miron & Brakier-Gingras, 1982). We have shown (Melançon et al., 1984) that streptomycin could be covalently linked to the 30S subunit, using the bifunctional cross-linking agent phenyldiglyoxal. Some cross-linking occurred between streptomycin and ribosomal proteins S1, S5, S11, and S13, but most of the cross-linking involved the 16S RNA.

The purpose of this study was to determine which parts of 16S RNA are involved in the interaction with streptomycin. We have cross-linked [^{3}H]dihydrostreptomycin to the 30S subunit or to the 70S ribosome of *E. coli*, and to define the sites of the 16S RNA which are cross-linked to the antibiotic, we have formed hybrids between restriction fragments of the 16S RNA gene and 16S RNA cross-linked to [^{3}H]dihydrostreptomycin, using a procedure developed by Hall et al. (1985). Single-stranded RNA and DNA were digested with a single-strand-specific nuclease, the hybrids were fractionated by gel electrophoresis, and the radioactivity in the hybrid bands was counted. Knowing the sequence of the 16S RNA gene, it is relatively simple to identify the regions of labeling of the 16S RNA from the distribution of the radioactivity among the hybrid bands. The use of various restriction enzymes, alone or in combination, allows the localization of the labeling to limited regions of 16S RNA. The method is applicable even with a very low level of labeling. An analogous procedure based on the hybridization of modified 16S RNA with DNA restriction fragments has also been used by Van Stolk and Noller (1984) to probe the 16S RNA conformation.

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Table I: Plasmids Used in This Study

designation	comments
pKK3535	derivative of pBR322 where the *Bam*HI fragment of phage λ *rif*^{d} 18, containing the complete *rrn*B operon coding for the 16S, 23S, and 5S RNA of *E. coli*, has been cloned into the *Bam*HI site of pBR322 (Brosius et al., 1981)
pCP28	derivative of pBR327 (a pBR322 derivative; Soberon et al., 1980), where the unique *Pvu*I site has been replaced by an *Xba*I site
pPM1	derivative of pCP28, carrying the entire coding sequence for the 16S RNA from *E. coli*, inserted between the *Pst*I and the *Xba*I site; the 16S RNA sequence originates from pKK3535 and was contained in a *Dra*I–*Xba*I fragment where the *Dra*I end has been converted to a *Pst*I site (this laboratory)
pMG1	derivative of pUC18 with an inserted *Hin*dIII–*Xba*I fragment, originating from pKK3535, which contains the 3′ part of the 16S RNA gene, starting at base 648 (this laboratory)
pMG3	derivative of pPM1, which contains the 5′ part of the 16S RNA gene extending to base 679 (this laboratory); this plasmid was constructed by removing from pPM1 the *Eco*RI fragment extending from the middle of the 16S RNA gene to the unique *Eco*RI site of the vector

Since the phenyldiglyoxal link between streptomycin and 16S RNA is labile, we have selected for this study another cross-linking reagent, nitrogen mustard [bis(2-chloroethyl)-methylamine]. This nucleophilic reagent has already been shown to cross-link proteins to RNA and also to generate intra-RNA cross-links in the ribosome (Ulmer et al., 1978; Stiege et al., 1982; Atmadja et al., 1986).

Our results show that streptomycin can be linked to two sites in the 16S RNA: one in the 892–917 region, at the junction between the central domain and the 3′ major domain, and the other in the 1394–1415 region, at the junction between the 3′ major domain and the 3′ minor domain.

Materials and Methods

Reagents. Streptomycin sulfate, nitrogen mustard [bis(2-chloroethyl)methylamine], and ethidium bromide were from Sigma Chemical Co. Restriction enzymes *Hha*I, *Nco*I, *Rsa*I, and *Taq*I and mung bean nuclease were from Pharmacia. Ribonuclease T1 was purchased from Calbiochem-Behring and ribonuclease A from Boehringer-Mannheim. Proteinase K (ribonuclease free) was from Bethesda Research Laboratories. Aquasol was obtained from New England Nuclear. [^{3}H]-Dihydrostreptomycin was prepared by reduction of streptomycin with sodium [^{3}H]borohydride as described by Chang and Flaks (1972). Its specific activity was 2 Ci/mmol.

Cross-Linking Reactions. The 70S ribosomes and 30S subunits were isolated from *E. coli* K12A19 (ribonuclease I$^-$) (Grisé-Miron & Brakier-Gingras, 1982; Melançon et al., 1984). In a typical cross-linking reaction, the 70S ribosomes or the 30S subunits, at 15 and 5 A_{260} units/mL, respectively (1 A_{260} unit equals 25 pmol of 70S ribosomes and 74 pmol of 30S subunits), were first incubated for 15 min at 37 °C in the cross-linking reaction buffer (100 mM sodium cacodylate, 200 mM potassium chloride, 10 mM magnesium acetate, and 6 mM β-mercaptoethanol, pH 7.2) with [^{3}H]dihydrostreptomycin added at a molar ratio of 2 per ribosomal particle. Nitrogen mustard was then added at a final concentration of 1 mM. The mixture was incubated for 2 h at 37 °C. Unlabeled dihydrostreptomycin (in a 100-fold molar excess over labeled dihydrostreptomycin) was added to displace the [^{3}H]dihydrostreptomycin which was not covalently bound to the ribosomal particles, and the reaction mixture was further incubated for 15 min at 37 °C. The ribosomes or the 30S subunits were then immediately either filtered on Millipore filters to determine the extent of the cross-linking reaction (Melançon et al., 1984) or precipitated with ethanol, resuspended, and purified by centrifugation through a 10–30% sucrose gradient in a high-salt buffer (cross-linking buffer made 1 M in potassium chloride). The 30S subunits treated with nitrogen mustard cosedimented with untreated control 30S subunits in a sucrose gradient (data not shown), indicating that the treatment with nitrogen mustard did not induce gross structural changes in the ribosomal particles.

The distribution of the covalently bound [^{3}H]dihydrostreptomycin between RNA and proteins in the ribosomal particles was determined after digesting either the RNA or the proteins with a mixture of ribonucleases or with proteinase K, respectively (Melançon et al., 1984).

Partial Ribonuclease T1 Digestion of 16S RNA Cross-Linked to [^{3}H]Dihydrostreptomycin. [^{3}H]Dihydrostreptomycin was cross-linked to either the 30S subunits or the 70S ribosomes, and the 30S subunits were then isolated by centrifugation through a sucrose gradient. Ribosomal proteins were digested with proteinase K, and the labeled 16S RNA (about 45 μg) was incubated 30 min at 40 °C in 50 μL of reconstitution buffer [30 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl),[1] pH 7.5, 20 mM magnesium chloride, and 350 mM potassium chloride], chilled on ice, and treated with RNase T1 at an enzyme:substrate ratio of 1:400 (w/w) for 15 min at 0 °C. The treatment cleaves the 16S RNA into two fragments, a 12S fragment corresponding to the 5′ two-thirds of the RNA and an 8S fragment representing the 3′ one-third (Carbon et al., 1979). The fragments of RNA were then fractionated by gel electrophoresis in 1.2% (w/v) agarose in Tris–borate buffer (90 mM Tris base, 90 mM boric acid, pH 8.3, and 2.5 mM Na_2EDTA). Slices of the gels were melted, diluted with water, and counted in Aquasol.

Standard Procedure for Hybridization of 16S RNA Labeled with [^{3}H]Dihydrostreptomycin to Restriction Fragments of the 16S RNA Gene and Analysis of the Resulting Hybrids by Gel Electrophoresis. The plasmids used in this study, which contain either the entire 16S RNA gene or part of it, are listed in Table I.

The procedure used to hybridize RNA labeled with [^{3}H]-dihydrostreptomycin to restriction fragments of the 16S RNA gene is adapted from Hall et al. (1985). Restriction fragments were obtained by treatment of the plasmids with the restriction enzymes, following the protocols given by the manufacturers. They were deproteinized by phenol extraction, precipitated with ethanol, and resuspended in hybridization buffer (80% formamide, 40 mM PIPES, pH 6.4, 0.4 M sodium chloride, and 1 mM Na_2EDTA).

Ribosomal RNA cross-linked with [^{3}H]dihydrostreptomycin was obtained from either 30S subunits or 70S ribosomes after digestion of the ribosomal proteins with proteinase K, as described above. Hybridization reactions were performed in hybridization buffer with a 10-fold molar excess of 16S RNA over its complementary part in the plasmid. In a typical hybridization reaction, 100 μg of restriction fragments ori-

[1] Abbreviations: DHSm, dihydrostreptomycin; Na_2EDTA, disodium ethylenediaminetetraacetate; PIPES, 1,4-piperazinediethanesulfonic acid; RNase, ribonuclease; Tris, tris(hydroxymethyl)aminomethane.

Table II: Cross-Linking of [^{3}H]Dihydrostreptomycin with Nitrogen Mustard to the 30S Subunit or the 70S Ribosome from *E. coli*[a]

ribosomal particle	conditions of reaction: nitrogen mustard	conditions of reaction: addition of unlabeled DHSm	binding of [^{3}H]DHSm (cpm)
70S ribosome	absent	no	49656
70S ribosome	absent	yes	913
70S ribosome	present	no	51219
70S ribosome	present	yes	7531
30S subunit	absent	no	44468
30S subunit	absent	yes	674
30S subunit	present	no	46902
30S subunit	present	yes	3868

[a] The binding of [^{3}H]dihydrostreptomycin was assessed by Millipore filtration as described under Materials and Methods. Unlabeled dihydrostreptomycin, added in excess, chases the [^{3}H]dihydrostreptomycin which was not covalently linked to the ribosomal particles with nitrogen mustard. Six A_{260} units of 70S ribosomes or 2 A_{260} units of 30S subunits were used in each assay. Results are the means of four independent experiments. The standard deviation on the means was ±15%. The efficiency of counting was 18%. The specific activity of [^{3}H]dihydrostreptomycin was 4400 dpm/pmol.

ginating from plasmid pMG1 was hybridized for 4 h at 50 °C with 225 μg of 16S RNA or 675 μg of total ribosomal RNA in 375 μL of hybridization buffer. The temperature of hybridization was chosen so as to favor the formation of DNA–RNA hybrids but to allow only trace amounts of DNA–DNA duplexes to be re-formed. After the hybridization reaction, unhybridized single-stranded DNA and RNA were digested as described by Hall et al. (1985), except that mung bean nuclease (at 2 units/μg of nucleic acids) was substituted for nuclease S1. Digestion was allowed to proceed for 20 min at 37 °C. Limited RNase A digestion followed the action of the mung bean nuclease, in order to reduce the size of the residual RNA fragments liberated by the mung bean nuclease.

The hybrids were fractionated by gel electrophoresis in a 2% (w/v) agarose gel in the Tris–borate buffer. Bands were visualized by ethidium bromide staining. The gels were cut into slices; the slices were melted, diluted with water, and counted in Aquasol, as described above. Because of the presence of small residual fragments of labeled RNA in the lower part of the gels, the radioactivity in hybrid bands smaller that 100 base pairs could not be accurately measured.

Results

Cross-Linking of Streptomycin to the 30S Subunit and the 70S Ribosome. [^{3}H]Dihydrostreptomycin was bound to the 30S subunit and the 70S ribosome from *E. coli* K12A19 in the absence or the presence of nitrogen mustard, and non-cross-linked streptomycin was chased by addition of an excess of unlabeled antibiotic. As in our previous study with phenyldiglyoxal (Melançon et al., 1984), we used Millipore filtration as a rapid assay to demonstrate that streptomycin can be linked covalently to the ribosomal particles with nitrogen mustard and to assess the amount of cross-linked antibiotic. The results are presented in Table II. They show that nitrogen mustard can link streptomycin to the 30S subunit or the 70S ribosome since, in the presence of the cross-linking agent, a significant amount of [^{3}H]dihydrostreptomycin cannot be chased by an excess of unlabeled dihydrostreptomycin. Under our conditions of reaction, the cross-linking amounted to about 7% and 13% of the bound antibiotic for the 30S subunit and the 70S ribosome, respectively.

To verify that the cross-linking took place at the specific streptomycin binding site, the cross-linking reaction was carried out under conditions which prevent the interaction of streptomycin with the ribosome, by raising the KCl concentration above 500 mM or by adding bluensomycin, a competitive inhibitor for the streptomycin binding site. The cross-linking was abolished under both of these conditions (data not shown).

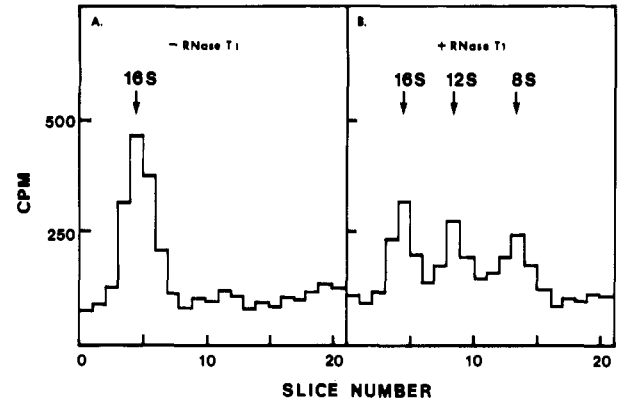

FIGURE 1: Partial digestion with ribonuclease T1 of 16S RNA labeled with [^{3}H]dihydrostreptomycin. Digestion was as described under Materials and Methods, and the RNA fragments (about 45 μg) were fractionated by electrophoresis in a 1.2% (w/v) agarose gel in Tris–borate buffer. The gel was stained with ethidium bromide, and the radioactivity across the gel was determined as described under Materials and Methods. (A) Sample untreated with ribonuclease T1. (B) Sample digested with ribonuclease T1. The arrows indicate the position of the stained bands.

The distribution of the cross-linked [^{3}H]dihydrostreptomycin between 16S RNA and the ribosomal proteins indicated that 70% (±5%) of the cross-linking involved the RNA. An identical distribution had been obtained with phenyldiglyoxal as the cross-linking agent (Melançon et al., 1984).

Localization of the Streptomycin Binding Site after Partial Digestion of 16S RNA with Ribonuclease T1. Partial digestion of 16S RNA with RNase T1 cleaves the RNA in two fragments: a 12S fragment, corresponding to the 5′ two-thirds of 16S RNA, and an 8S fragment, corresponding to the remaining 3′ one-third, except that it does not contain the 50 3′-terminal bases. From the known sequence of the 16S RNA, it has been inferred that the fragmentation occurs after nucleotide 917 [see Carbon et al., (1979)].

The 16S RNA labeled with [^{3}H]dihydrostreptomycin was treated with RNase T1, as described under Materials and Methods, and the fragments were separated by gel electrophoresis. Figure 1 shows that the radioactivity is distributed between the 12S and the 8S fragment, indicating that the antibiotic interacts with at least two regions in the 16S RNA. Approximately the same amount of radioactivity was bound to each of the two fragments.

Localization of the Streptomycin Binding Site by Formation of Hybrids between Restriction Fragments of the 16S RNA Gene and 16S RNA Labeled with Streptomycin. Preliminary assays of hybridization followed by nuclease treatment were done with plasmid pMG3, corresponding to a fragment of the 16S RNA gene encompassing nucleotides 1–679, and plasmid pMG1, containing nucleotides 648–1542 of the 16S RNA gene. The portion of 16S RNA which is labeled with streptomycin was found to hybridize exclusively with pMG1 (data not shown).

Further localization of the sites of interaction of streptomycin with 16S RNA was therefore performed with restriction fragments originating from pMG1. Table III summarizes the sequences of the different fragments which are obtained when pMG1 is digested with three different restriction enzymes, *Rsa*I, *Hha*I, and *Taq*I.

The determination of the radioactivity in the hybrid fragments obtained with these enzymes is shown in Figure 2 and 3, using 16S RNA labeled with streptomycin in the 70S ri-

Table III: Restriction Fragments Obtained with Plasmid pMG1

restriction enzyme	sequence of restriction fragments	band no.[a]
*Rsa*I	892–1393	1
	648–891	2
	1394–1542	3
*Taq*I	964–1323	1
	1324–1542	2
	648–824	3
	878–963	4[b]
	825–877	5[b]
*Hha*I	648–1106	1
	1243–1459	2
	1107–1242	3
	1460–1542	4[b]
*Hha*I + *Nco*I	648–1106	1
	1243–1415	2
	1107–1242	3
	1460–1542	4[b]
	1416–1459	5[b]

[a] Hybrids were formed between 16S RNA and the restriction fragments listed in the table, and they were fractionated according to size by agarose gel electrophoresis. The band number corresponds to the position of the hybrid bands on the agarose gel by order of decreasing size. [b] Hybrids smaller than 100 base pairs have been listed, but they are not included in the analysis since their position of migration coincides with that of small fragments of labeled RNA.

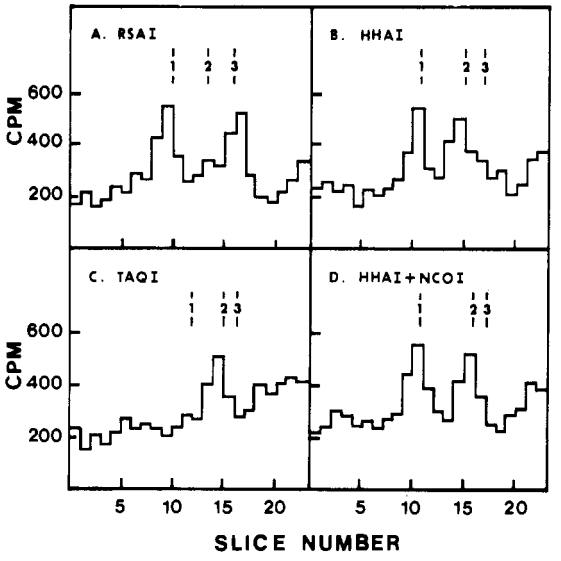

FIGURE 2: Radioactivity profiles of gel electrophoresis analysis of 16S RNA labeled with [^{3}H]dihydrostreptomycin in the 30S subunit associated to the 50S subunit and hybridized to restriction fragments from plasmid pMG1. (A) Hybrids with *Rsa*I fragments. (B) Hybrids with *Hha*I fragments. (C) Hybrids with *Taq*I fragments. (D) Hybrids with *Hha*I + *Nco*I fragments. Hybridization conditions were as described under Materials and Methods, with 100 μg of DNA and 675 μg of total RNA in 375 μL of hybridization buffer. The position of ethidium bromide stained hybrid bands (numbered as in Table III) is indicated.

bosome or the 30S subunit, respectively. The distribution of the radioactivity varied somewhat from one experiment to the other, but on the average, it was similar whether the 16S RNA had been labeled with streptomycin in the free or in the associated 30S subunit. Figures 2 and 3 (A–C) show that there are two regions where the 16S RNA is labeled with streptomycin, in the patterns of restriction fragments generated by the endonucleases *Rsa*I or *Hha*I. Only one region of labeling was observed after hybridization to the fragments resulting from digestion with nuclease *Taq*I. However, the extent of the labeling detected with the *Taq*I fragments was about half of that observed with the other restriction nucleases. This suggests that one site of labeling to the *Taq*I fragments falls in a band too small to be counted.

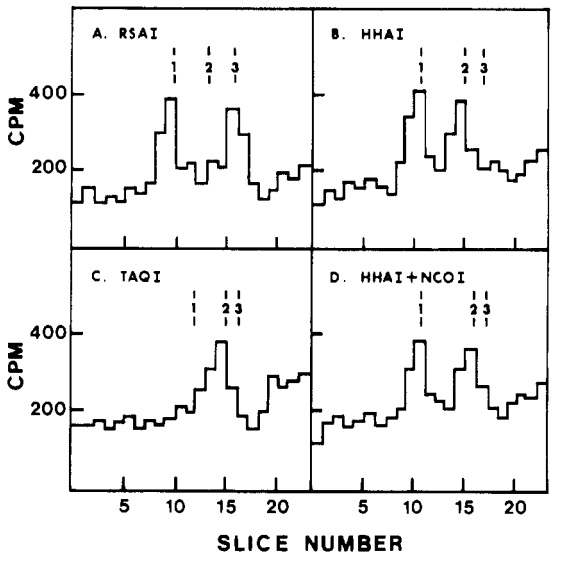

FIGURE 3: Radioactivity profiles of gel electrophoresis analysis of 16S RNA labeled with [^{3}H]dihydrostreptomycin in the free 30S subunit and hybridized to restriction fragments from plasmid pMG1. See details in the legend to Figure 2. Hybridization was done with 100 μg of DNA and 225 μg of 16S RNA in 375 μL of hybridization buffer.

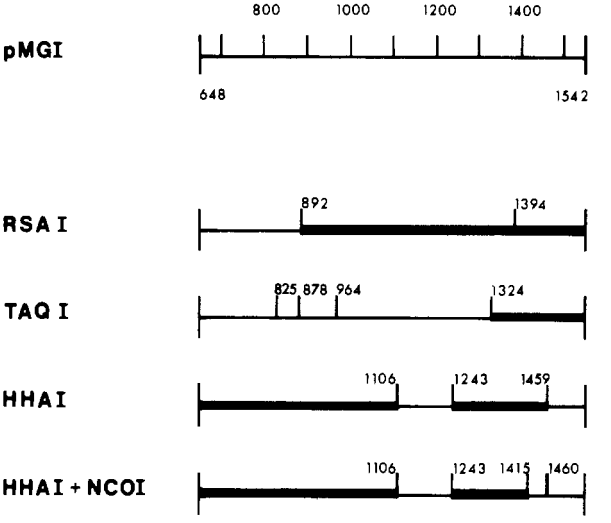

FIGURE 4: Localization of the ^{3}H-labeled 16S RNA–DNA hybrid fragments. The vertical bars show the sites of fragmentation by the restriction enzymes, and the black boxes indicate the hybrid fragments which are radioactive except for those fragments which are less than 100 base pairs and cannot be analyzed. This identifies two regions as the sites of cross-linking. One encompasses nucleotides 892–963 but can be further narrowed to the segment between nucleotides 892 and 917 (site I) on the basis of data obtained with labeled 16S RNA partially digested with ribonuclease T1 (see the text). The second region extends from nucleotides 1394 to 1415 (site II). There is no labeling in the region extending from nucleotides 1416 to 1542, as shown by fragmentation studies with nuclease *Nco*I (see the text).

From the sequences of the restriction fragments generated by the nucleases *Rsa*I, *Taq*I, and *Hha*I (Table III), it can be deduced that labeling of the 16S RNA with streptomycin occurs in two regions: one falling between nucleotides 892 and 963 and the other between nucleotides 1394 and 1459 (Figure 4). The size of these two regions can be further narrowed. Indeed, when the 16S RNA labeled with streptomycin was cut into a 12S and an 8S fragment, radioactivity was found in both fragments. Since the 12S fragment extends from the 5′ end of 16S RNA to nucleotide 917, the site of interaction which comprises nucleotides 892–963, as determined by the hybridization studies, can be narrowed to a segment encompassing nucleotides 892–917 (site I). To limit the size of the

other region of labeling, 16S RNA cross-linked to streptomycin was hybridized to restriction fragments resulting from the digestion of pMG1 with a mixture of nucleases *Hha*I and *Nco*I. The nuclease *Nco*I introduces an additional cut in band 2 generated by nuclease *Hha*I (see Table III). The distribution of radioactivity between band 1 and the original band 2 (1243–1459) generated by nuclease *Hha*1 was identical with the distribution of radioactivity between band 1 and the new band 2 (1243–1415) generated by nucleases *Hha*I and *Nco*I (Figures 2 and 3D), implying that the second region of labeling of the 16S RNA with streptomycin can be limited to a segment encompassing nucleotides 1394–1415 (site II). One could argue that an additional labeling site could be present in band 5 (1416–1459) generated by the mixture of nucleases, but not be detected because of the small size of the fragment. However, if this had been the case, the distribution of radioactivity between band 1 and band 2 would not have been identical with the restriction fragments generated either by nuclease *Hha*I or by the mixture of nucleases *Hha*I and *Nco*I. A similar type of reasoning can be used to exclude the possibility that a third site of labeling could occur in segment 918–963 or in segment 1460–1542. The distribution of radioactivity between the two regions of the 16S RNA has been found approximately equal, whether the analysis was based on the fragmentation of 16S RNA with RNase T1 or on the hybridization of 16S RNA to restriction fragments generated by nucleases *Rsa*I or *Hha*I. A third site of labeling between nucleotides 918 and 963 or between nucleotides 1460 and 1542 could not account for this situation. The absence of labeling in the 3′-terminal region of the 16S RNA was further confirmed by hybridization assays where pMG1 was cut into two fragments with nuclease *Nco*I, one containing nucleotides 648–1415 and the other, nucleotides 1416–1542. The labeling was found exclusively in the fragment 648–1415 (data not shown).

Discussion

Previous reports from Gorini and his collaborators (Biswas & Gorini, 1972; Garvin et al., 1974) suggested that the 16S RNA provides the binding site for streptomycin. We have recently shown that streptomycin can be cross-linked primarily to the 16S RNA, using phenyldiglyoxal as a cross-linking agent (Melançon et al., 1984). The present study, using nitrogen mustard as a cross-linking agent, confirms and extends this observation by demonstrating that streptomycin can be cross-linked to two sites in the 16S RNA. Site I, which encompasses the region from nucleotides 892 to 917, is located at the junction between the central and the 3′ major domain of the 16S RNA. Site II, which is found in an area extending from nucleotides 1394 to 1415, is located at the junction between the 3′ major and the 3′ minor domain (Figure 5). These two regions correspond to sequences which are highly conserved throughout evolution (Woese et al., 1983), as can be expected for regions involved in important ribosomal functions.

Since streptomycin is known to bind to a single area in the ribosome (see the introduction), sites I and II must be close neighbors in the 16S RNA tertiary structure. This agrees with recent data on that structure [reviewed by Expert-Bezançon and Wollenzien (1985)], as exemplified by the occurrence of a cross-link between nucleotides 930 and 1420. Two of the four proteins which we found to be linked to streptomycin with phenyldiglyoxal (Melançon et al., 1984) interact with 16S RNA in the same regions: protein S1 interacts near site I (Golinska et al., 1981), and protein S13 interacts with both site I and site II (Hajnsdorf et al., 1986).

In addition, interestingly, Montandon et al. (1985) have characterized a streptomycin-resistant chloroplast mutant of

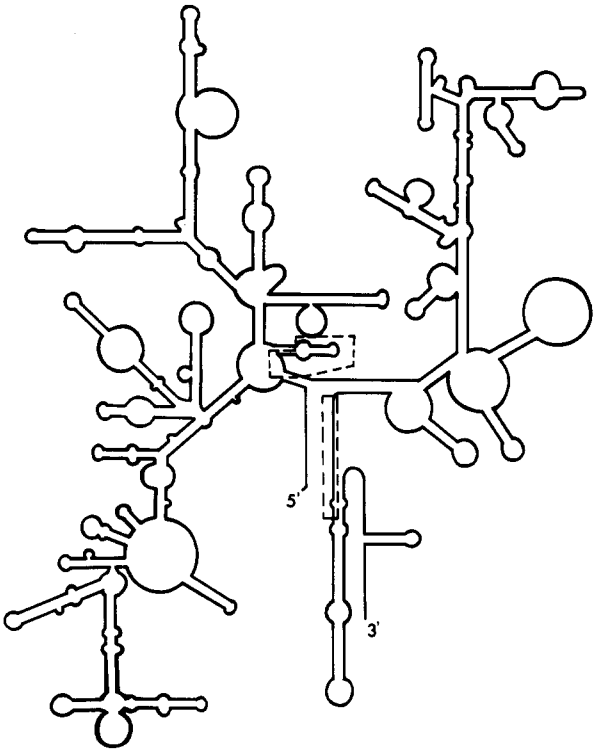

FIGURE 5: Localization of the streptomycin binding domain. The dashed boxes delineate the segments corresponding to site I and site II in the skeleton of the 16S RNA secondary structure model of Woese et al. (1983).

Euglena gracilis, where the mutation is located in a position equivalent to position 912 of the *E. coli* 16S RNA. More recently, Montandon et al. (1986) have shown that introducing a mutation in the cloned *rrn*B operon of *E. coli* at position 912 of the 16S RNA and transforming *E. coli* cells with a recombinant plasmid harboring this mutated operon confer resistance to streptomycin. This position 912 falls within binding site I of streptomycin.

The binding domain of streptomycin appears to be located in an important functional area, since the sequence around nucleotide 1400, which is a prominent component of the decoding site, is situated within this domain (Prince et al., 1982; Ciesiolka et al., 1985). Such a location can directly account for the well-known interference of streptomycin with the accuracy of translation of the message [reviewed in Vazquez (1979), Wallace et al. (1979), and Brakier-Gingras and Phoenix (1984)]. Recent studies with initiation factor IF-3 (Ehresmann et al., 1986) have identified two sites of interaction between this factor and the 16S RNA: one in the region extending from nucleotides 819 to 859 and the other in the area encompassing nucleotides 1506–1529 [see also Wickstrom (1983)]. These two sites of interaction are near the streptomycin binding domain. The irregular helix containing region 819–859 is a neighbor of the helix containing part of the streptomycin binding site I, and region 1506–1529 has been shown by cross-linking with psoralen to be contiguous to segment 919–923, which is also very close to streptomycin binding site I (Hui & Cantor, 1985). This proximity supports our previous observations, based on cross-linking studies involving ribosomal proteins (Melançon et al., 1984), that there is some overlap between the binding domain of streptomycin and that of IF-3. This finding also agrees with the known interference of streptomycin with the action of IF-3 [see Vazquez (1979) and Wallace et al. (1979)].

The technique of DNA hybridization electron microscopy has been recently applied to locate the sequence around nu-

cleotide 1400, which lies within the streptomycin binding domain, on the surface of the 30S subunit (Oakes et al., 1986). This sequence was mapped at the interface with the 50S subunit, at the level of the neck of the small subunit, near the cleft. This location is fully consistent with our previous location of the streptomycin binding domain, as inferred from cross-linking studies between streptomycin and 30S ribosomal proteins (Melançon et al., 1984).

All our data thus support the location of the streptomycin binding domain in a functionally important region of the ribosome. They also stress the involvement of the ribosomal RNA in the interaction between the ribosome and the antibiotic. To further characterize the binding domain of streptomycin in the 16S RNA, site-directed mutations can be introduced in the regions which were labeled with streptomycin. They can then be assessed for their effect on the response of the ribosome to the antibiotic. Experiments along this line are under way in our laboratory.

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