Peptide Environment of the Peptidyl Transferase Center from Escherichia coli 70 S Ribosomes as Determined by Thermoaffinity Labeling with Dihydrospiramycin*

(Received for publication, June 16, 1995)

Oliver Bischof, Henning Urlaub, Volker Kruft, and Brigitte Wittmann-Liebold§
From the Max-Delbrück-Centrum für Molekulare Medizin, Robert-Rössle-Strasse 10, Abteilung Protechnic, 13125 Berlin-Buch, Germany

In an attempt to gain information about the peptidyl transferase center at the peptide level we cross-linked the spiramycin derivative dihydrospiramycin to its functional binding site in the 70 S ribosome of Escherichia coli. In this manner ribosomal proteins S12, S14, L17, L18, L27 and L35 were found specifically affinity-labeled. Proteolytic fragmentation of these proteins, separation by C18 reversed-phase high performance liquid chromatography of the peptide mixtures, and subsequent sequence analysis of labeled peptides revealed peptide regions at positions Ala1-Lys6 and Tyr119-Lys119 of S12, Leu47-Asp53 of protein S14, Ser4-Lys35 of protein L17, Ala67-Lys63 of protein L18, Ala35-Lys31 and Val186-Lys71 of protein L27, and Thr5-Lys11 of protein L35. This approach is a valuable tool to characterize the binding site of spiramycin as well as the peptidyl transferase center at the molecular level.

Studies on ribosomal structure and function have been greatly aided by the use of antibiotics acting as inhibitors of protein biosynthesis. Such studies have shed light on functional domains of the ribosome and also have helped to characterize the antibiotic binding sites (Cundliffe, 1990; Cooperman et al., 1990).

The location of greatest functional and structural interest in the ribosome is the peptidyl transferase center, since it is the site where peptide bond formation takes place (Monro et al., 1969). To date, only limited data exist on the fine structure of the peptidyl transferase center at the molecular level. Though detailed structural information is available on the rRNA moiety (Brimacombe et al., 1990), a more minute insight into the topography of the peptidyl transferase center can be obtained by cross-linking ribosomal proteins to their functional sites (Cooperman et al., 1990).

Prominent among these antibiotics are macrolides. They can be roughly classified structurally and functionally into two main groups represented by spiramycin and erythromycin, respectively (Vazquez, 1979). The erythromycin group has a 13-carbon lactone ring that is glycosylated in two positions by different monosaccharides. The second group consists of a 15-carbon lactone ring system, usually derivatized at only one position by a disaccharide. Members of the erythromycin family appear to block peptide synthesis only after the formation of several peptide bonds due to steric hindrance by the drug molecule (Moazed and Noller, 1987; Contreras and Vazquez, 1977). The spiramycin group of macrolides exerts its effects in the very early stage of peptide bond formation by competing for the ribosomal A-site with the incoming charged tRNA as determined by kinetic analysis with puromycin (Dinos et al., 1993).

In this respect the effect is comparable with that of typical peptidyl transferase inhibitors such as chloramphenicol. The dissimilar modes of action of macrolides have mainly been attributed to the different sugar moieties attached to the lactone ring. So far, the question remains to be answered whether the two groups of macrolides have overlapping or in fact different binding sites (Vazquez, 1979).

A direct way to test both alternatives is to cross-link these molecules to their functional sites. Previous affinity labeling has revealed protein L22 as the major cross-linking site for erythromycin (Are ´valo et al., 1988) and protein L27 for spiramycin (Tejedor and Ballesta, 1986), thus favoring the notion of different binding sites for the two groups. The amino acid residues involved in the respective cross-links have not been characterized so far but are of great interest in terms of elucidating the topography of the peptidyl transferase center.

In this communication we report peptide regions of the small and large ribosomal subunit proteins cross-linked to spiramycin in the Escherichia coli ribosome that were identified by direct sequence analysis. The results present details of the peptide environment close to the peptidyl transferase center. The cross-linking data are integrated with the available data concerning the peptidyl transferase center topography.

MATERIALS AND METHODS

The following buffers were used: BKM10, 10 mM borate (pH 7.0 at 37 °C), 100 mM KCl, and 10 mM MgCl2; and BKM-β, as above plus 6 mM 2-mercaptoethanol.

All chemicals were of highest quality, obtained from Merck (Darmstadt, Germany). Endoproteinase Lys-C was purchased from Boehringer Mannheim (Germany).

[13125 Berlin-Buch, Germany

† Present address: Applied Biosystems GmbH, Brunnenweg 13, 64331 Weiterstadt, Germany.

§ To whom correspondence and reprint requests should be addressed: Tel.: 30-9406-2875; Fax: 30-9406-3869.

* This work was supported by Grant SFB 344 TP YE 6 from the Deutsche Forschungsgemeinschaft (to B. W.-L.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ Present address: Applied Biosystems GmbH, Brunnenweg 13, 64331 Weiterstadt, Germany.

§ To whom correspondence and reprint requests should be addressed: Tel.: 30-9406-2875; Fax: 30-9406-3869.

* The abbreviations used are: RP-HPLC, reversed-phase high performance liquid chromatography; Lys-C, Lysobacter enzymogenes protease (EC 3.4.21.50); TP70, total protein of 70 S ribosomes.
Specificity of Spiramycin Binding—70 S ribosomes (0.2 μM) were incubated at 37°C for 15 min in the presence of 0.4 μM [3H]dihydrospiramycin (0.14 Ci/mmol) in BKM-B and increasing amounts of spiramycin. The ribosomes were precipitated for 3 h with acetone at −20°C, filtered through glass fiber filters (size 0.45 μm; Millipore) and washed with 60% acetone, 100 mM KCl. Filters were monitored for radioactivity by scintillation counting.

Thermal Incorporation of [3H]dihydrospiramycin into 70 S Ribosomes—70 S ribosomes were activated at 37°C for 15 min prior to labeling (Bischof et al., 1994). Thermal incorporation of 1 μM [3H]dihydrospiramycin (0.14 Ci/mmol) into activated 70 S ribosomes (50 A260 units/ml) was performed in BKM10 buffer at 37°C for 30 min under constant mild agitation in a waterbath. Immediately after radiolabeling, the ribosomes were precipitated with 2 volumes of ethanol, 2-mercaptoethanol (9:1) for 2 h at −20°C. Reprecipitation of the ribosomes four times removed more than 90% of nonincorporated spiramycin (data not shown). The final pellet was redissolved in BKM10 buffer (340 A260 units/ml), and proteins were extracted for 5 min with 50% acetic acid (Bischof et al., 1994). The precipitated RNA was pelleted for 20 min at 13,000 rpm in an HB6-Sorvall rotor at 4°C. Supernatants were portioned into 30 A260 units and stored at −80°C. Radioactivity of ribosomal proteins and rRNA was monitored by liquid scintillation counting.

Preparation and Purification of Labeled Ribosomal Proteins—Total proteins of 70 S ribosomes were separated by RP-HPLC on a laboratory-packed Vydac C4 column (5 μm, 300 Å) employing a gradient of acetonitrile in water both containing 0.1% trifluoroacetic acid. An aliquot of each protein fraction was counted in a liquid scintillation counter, the remainder lyophilized and stored at −80°C.

Peptide Mapping—Protein fractions containing radioactivity were digested with endoproteinase Lys-C at an enzyme/substrate (total protein) ratio of 1:50 in 400 μl of 50 mM NH4HCO3, pH 7.8, for 12 h. The digests were stopped by injection of the reaction mixture into the HPLC system. All peptide separations were performed on a Vydac C4 column as described by Kamp (1986) employing buffer A, 0.1% trifluoroacetic acid in water, and buffer B, 0.2% trifluoroacetic acid in acetonitrile. Peak fractions were monitored for radioactivity. Fractions containing labeled peptides were dried under vacuum and sequenced in a model 477A pulsed liquid phase sequencer equipped with a model 120A phenylthiohydantoin-derivative analyzer (Applied Biosystems, Foster City, CA). Sequences were identified by comparison with sequences in the NBRF, Swiss Prot and RIBO data banks.2

RESULTS

Binding Specificity of Dihydrospiramycin—Specificity of dihydrospiramycin binding to 70 S ribosomes was tested in a saturation experiment. As is demonstrated in Fig. 1, increasing amounts of spiramycin competed with [3H]dihydrospiramycin binding, indicating a specific binding of dihydrospiramycin to the ribosome.

Identification of Dihydrospiramycin-labeled Ribosomal Proteins and Peptides—Incorporation of dihydrospiramycin into 70 S ribosomes is a rather fast reaction. Approximately 50% of the total incorporation takes place within the first 2 min. The reaction reaches saturation after about 60 min. 2.6% of total radioactivity was specifically incorporated into ribosomes. 72% of this radioactivity was found in ribosomal proteins, and 17% was found in rRNA. [3H]Dihydrospiramycin-labeled total protein extracted from 70 S ribosomes was fractionated by C4 RP-HPLC. Five major radiolabeled peaks were detected in the chromatogram (Fig. 2). Only one peptide was labeled in the case of peaks 3, 4, and 13, while two and three peptides were found radiolabeled in peaks 20 and 2, respectively. Two examples of RP-HPLC separations of Lys-C-digested protein pools are presented in Fig. 3.

Sequence Analysis of [3H]Dihydrospiramycin-labeled Peptides—During sequence analysis of the purified affinity-labeled peptides a gap at the position of the modified amino acid is expected. However, for all residues determined the predicted phenylthiohydantoin-derivatives were found. Therefore, the af-

---

2 B. Wittmann-Liebold, D. Dziorna, A. Beck, A. K. Köpcke, and Boeckh, T., unpublished results.
finity cross-link product is obviously not stable under the harsh conditions of Edman chemistry, and indeed acid lability of the dehydrospiramycin cross-link was suggested by Tejedor and Ballesta (1986). We proved this assumption correct by extended exposure of affinity-labeled ribosomes in 20% trifluoroacetic acid, 10% trichloroacetic acid, and 2% acetic acid at sequence-like temperatures (i.e. 48°C). Here, we found that most of the originally incorporated radioactivity was subsequently lost during the heat procedure (data not shown).

We digested the cross-linked ribosomal proteins with endoproteinase Lys-C, and the resulting peptide mixtures were separated by C18 RP-HPLC employing the following gradient: isocratic elution for 5 min at 6% solvent B (acetonitrile in 0.1% trifluoroacetic acid) followed by a linear gradient from 6 to 12% solvent B in 25 min, 12–35% solvent B in 270 min, 35–55% solvent B in 30 min, and 75–99% solvent B in 10 min. The absorbance range was 0.04 absorbance units at full scale, and the flow rate 0.03 ml/min. The eluate was monitored at 220 nm. Peptides were monitored for radioactivity by scintillation counting. The radioactivity contained in single fractions is depicted as bars. The dashed line indicates the background radioactivity. Resulting peptides are indicated by numbers.

Approximately 72% of dehydrospiramycin incorporated into ribosomal proteins. Extraction of labeled 70 S ribosomes and separation of ribosomal proteins by RP-HPLC resulted in 5 (dihydro)spiramycin-labeled protein fractions. Proteolytic digestion of these fractions and subsequent peptide sequence analysis of labeled peptides yielded peptide sequences from six different ribosomal proteins i.e. proteins S12, S14, L17, L18, L27, and L35 (Table II).

The proteins thus identified to be in close contact with the spiramycin molecule have been implicated to be close to or at the peptidyl transferase center in the ribosome. Similar to spiramycin, dehydrospiramycin inhibits peptidyl bond formation and is competing with puromycin for an overlapping binding site (Dinos et al., 1993). Our experimental approach was designed to identify peptide-(dihydro)spiramycin cross-links by direct peptide sequencing, thereby establishing data concerning the molecular environment of (dihydro)spiramycin in its functional binding site.

In our affinity-labeling approach with (dihydro)spiramycin the N-terminal region of S12 (positions Ala1-Lys9) was found to be labeled with the highest intensity. Additionally, we obtained a second cross-link with medium intensity in the C terminus of the protein (positions Tyr116-Lys119). These findings confirm speculations of Tejedor and Ballesta (1986) who proposed that S12 is involved in spiramycin binding, but these authors were not able to identify the second cross-link since the 3S subunit was not available for their analysis.

**Table II** lists the sequences of all [3H]dihydrospiramycin cross-linked peptides together with the parental ribosomal proteins. It should be noted at this point that the sequence of the labeled S14 peptide (peak 13 in Table II) abruptly terminated at position Asp-53. Eventually, this might be an indication for a modification. The peptides obtained in this study can be roughly divided into three classes according to the degree of their incorporated radioactivity: (i) high label, peptide region Ala1-Lys9 of protein S12; (ii) medium label, peptide region Thr5-Lys11 of protein L35, Ser6-Lys35 of protein L17, Leu47-Lys18 of protein S14, Tyr116-Lys119 of S12, and Ala37-Lys63 of protein L18; and (iii) low label, peptide region Ala57-Lys63 of protein L18.

**DISCUSSION**

Affinity labeling with (dihydro)spiramycin is ideally suited to investigate structural components of the peptidyl transferase center in the ribosome. Similar to spiramycin, dehydrospiramycin inhibits peptide bond formation and is competing with puromycin for an overlapping binding site (Dinos et al., 1993). Our experimental approach was designed to identify peptide-(dihydro)spiramycin cross-links by direct peptide sequencing, thereby establishing data concerning the molecular environment of (dihydro)spiramycin in its functional binding site.

Approximately 72% of dehydrospiramycin incorporated into ribosomal proteins. Extraction of labeled 70 S ribosomes and separation of ribosomal proteins by RP-HPLC resulted in 5 (dihydro)spiramycin-labeled protein fractions. Proteolytic digestion of these fractions and subsequent peptide sequence analysis of labeled peptides yielded peptide sequences from six different ribosomal proteins i.e. proteins S12, S14, L17, L18, L27, and L35 (Table II).

The proteins thus identified to be in close contact with the spiramycin molecule have been implicated to be close to or at the peptidyl transferase center in the ribosome. Similar to spiramycin, dehydrospiramycin inhibits peptidyl bond formation and is competing with puromycin for an overlapping binding site (Dinos et al., 1993). Our experimental approach was designed to identify peptide-(dihydro)spiramycin cross-links by direct peptide sequencing, thereby establishing data concerning the molecular environment of (dihydro)spiramycin in its functional binding site.

In our affinity-labeling approach with (dihydro)spiramycin the N-terminal region of S12 (positions Ala1-Lys9) was found to be labeled with the highest intensity. Additionally, we obtained a second cross-link with medium intensity in the C terminus of the protein (positions Tyr116-Lys119). These findings confirm speculations of Tejedor and Ballesta (1986) who proposed that S12 is involved in spiramycin binding, but these authors were not able to identify the second cross-link since the 3S subunit was not available for their analysis.

**Table II** lists the sequences of all [3H]dihydrospiramycin cross-linked peptides together with the parental ribosomal proteins. It should be noted at this point that the sequence of the labeled S14 peptide (peak 13 in Table II) abruptly terminated at position Asp-53. Eventually, this might be an indication for a modification. The peptides obtained in this study can be roughly divided into three classes according to the degree of their incorporated radioactivity: (i) high label, peptide region Ala1-Lys9 of protein S12; (ii) medium label, peptide region Thr5-Lys11 of protein L35, Ser6-Lys35 of protein L17, Leu47-Lys18 of protein S14, Tyr116-Lys119 of S12, and Ala37-Lys63 of protein L18; and (iii) low label, peptide region Ala57-Lys63 of protein L18.

**DISCUSSION**

Affinity labeling with (dihydro)spiramycin is ideally suited to investigate structural components of the peptidyl transferase center in the ribosome. Similar to spiramycin, dehydrospiramycin inhibits peptide bond formation and is competing with puromycin for an overlapping binding site (Dinos et al., 1993). Our experimental approach was designed to identify peptide-(dihydro)spiramycin cross-links by direct peptide sequencing, thereby establishing data concerning the molecular environment of (dihydro)spiramycin in its functional binding site.

Approximately 72% of dehydrospiramycin incorporated into ribosomal proteins. Extraction of labeled 70 S ribosomes and separation of ribosomal proteins by RP-HPLC resulted in 5 (dihydro)spiramycin-labeled protein fractions. Proteolytic digestion of these fractions and subsequent peptide sequence analysis of labeled peptides yielded peptide sequences from six different ribosomal proteins i.e. proteins S12, S14, L17, L18, L27, and L35 (Table II).

The proteins thus identified to be in close contact with the spiramycin molecule have been implicated to be close to or at the peptidyl transferase center in the ribosome. Similar to spiramycin, dehydrospiramycin inhibits peptidyl bond formation and is competing with puromycin for an overlapping binding site (Dinos et al., 1993). Our experimental approach was designed to identify peptide-(dihydro)spiramycin cross-links by direct peptide sequencing, thereby establishing data concerning the molecular environment of (dihydro)spiramycin in its functional binding site.

**DISCUSSION**

Affinity labeling with (dihydro)spiramycin is ideally suited to investigate structural components of the peptidyl transferase center in the ribosome. Similar to spiramycin, dehydrospiramycin inhibits peptide bond formation and is competing with puromycin for an overlapping binding site (Dinos et al., 1993). Our experimental approach was designed to identify peptide-(dihydro)spiramycin cross-links by direct peptide sequencing, thereby establishing data concerning the molecular environment of (dihydro)spiramycin in its functional binding site.

**DISCUSSION**

Affinity labeling with (dihydro)spiramycin is ideally suited to investigate structural components of the peptidyl transferase center in the ribosome. Similar to spiramycin, dehydrospiramycin inhibits peptide bond formation and is competing with puromycin for an overlapping binding site (Dinos et al., 1993). Our experimental approach was designed to identify peptide-(dihydro)spiramycin cross-links by direct peptide sequencing, thereby establishing data concerning the molecular environment of (dihydro)spiramycin in its functional binding site.

**DISCUSSION**

Affinity labeling with (dihydro)spiramycin is ideally suited to investigate structural components of the peptidyl transferase center in the ribosome. Similar to spiramycin, dehydrospiramycin inhibits peptide bond formation and is competing with puromycin for an overlapping binding site (Dinos et al., 1993). Our experimental approach was designed to identify peptide-(dihydro)spiramycin cross-links by direct peptide sequencing, thereby establishing data concerning the molecular environment of (dihydro)spiramycin in its functional binding site.
not able to provide unambiguous experimental proof for their assumption. The results presented here for S12 are a bit surprising since macrolides were thought to bind only to the 50 S subunit (Vazquez, 1979). However, previous experiments with a macrolide-resistant L4 mutant in E. coli have demonstrated that additional mutations in proteins S12 and S5 of the 30 S subunit can mask this effect, thereby leading to a macrolide-sensitive phenotype again (Saltzman and Apirion, 1976). These findings shed light on the intricate influence both subunits have on the binding site as well as on the mode of action of macrolides. In the case of S12 this influence can be easily explained since the N-terminal region that we found labeled in our approach was also observed to be in close contact with the 16 S tRNA (Urlaub et al., 1995), most likely with the 530 loop or 900 stem loop (Stern et al., 1988). In addition, Alexander et al. (1994) identified S12 as a major neighbor to 16 S rRNA nucleotides 518–526. Accordingly, conformational changes in the N-terminal region of the protein can be transferred through the 16 S rRNA molecule, and by this means the overall topography of the macrolide binding site can be modulated.

Immunoelectron microscopy (Stöffler-Meilicke and Stöffler, 1990) as well as neutron-scattering data (Capel et al., 1987) have placed S12 on the cytosolic side, close to the neck of the 30 S subunit. However, since the functional binding site of spiramycin is located directly in the peptidyl transferase center, which is believed to be located at the interface side of the 30 and 50 S subunit close to the base of the central protuberance (Oakes et al., 1990), at least the N-terminal as well as the C-terminal region of the protein have to be exposed at the peptidyl transferase center. This notion is supported by intraprotein cross-linking results of S12 and L16 (Traut et al., 1986). The latter is a well known constituent of the peptidyl transferase center and forms an intraprotein cross-link to L27, a protein that was also labeled in our approach. Another consequence that arises from the above results is that the 530 loop and/or the stem loop 900 of the 16 S rRNA must be exposed at the luminal side of the ribosome.

The second constituent of the spiramycin binding site on the 30 S subunit is S14. This finding further demonstrates the importance of the small subunit on the macrolide binding site. The peptide stretch that was labeled (positions Leu47, Asp53) is adjacent to a peptide region that we found cross-linked to puromycin. This confirms that the midsection of S14 is a major component of the peptidyl transferase center.

The main constituents of the spiramycin binding site on the 50 S subunit are proteins L17, L27, and L35. The location of L17 in the ribosome has been a subject of controversy. Wallaczek et al. (1988) place it distant from the peptidyl transferase center, while Traut et al. (1986) position it closer to the peptidyl transferase center since it can be cross-linked to protein L16. Our results favor the Traut location of L17, at least of the N-terminal region (positions Ser8–Lys25). The discrepancies of the L17 location in both models could be brought into accordance if L17 possesses a more elongated structure. The finding that protein L17 is a major neighbor of nucleotides 2497–2505 of domain V supports this assumption.3

In the case of L27 there is no doubt about the localization of this protein in the ribosomal models. Protein-protein cross-linking studies (Traut et al., 1986) as well as immunoelectron microscopy (Stöffler-Meilicke and Stöffler, 1990) all place it slightly beyond and left of the base of the central protuberance toward the L1 arm. Since L27 is affinity-labeled by several photoreactive tRNA derivatives (Wower et al., 1993) as well as by chloramphenicol (Cooperman et al., 1990) and by tiamulin (Högenauer et al., 1981) it must be considered a central element of the tRNA binding site. In addition, it can be cross-linked to numerous nucleotides in domain VII of the 23 S rRNA, which is in close neighborhood to domain V, the latter being an essential constituent of the peptidyl transferase center (Brimacombe et al., 1990).

A fair amount of labeling was found in L35. So far, no structural or functional information is available for this protein. However, it is obvious from our results that the N-terminal part of the protein must be part of the spiramycin binding pocket and therefore be exposed at the peptidyl transferase center. Additional data are necessary to clarify the significance of our results.

The protein that was labeled to the weakest extent in our approach was L18 (positions Ala57–Lys63). It binds to the 5 S rRNA-complex and is implicated to be part of the peptidyl transferase center as proved by affinity-labeling (Cooperman et al., 1990) as well as by reconstitution studies (Hampl et al., 1981). Furthermore, L18 was one of the proteins we recently identified as being cross-linked to puromycin (Bischof et al., 1994). Both cross-link positions are adjacent to each other (puromycin, Tyr64–Lys68). Taking the S14 data into account this result indicates that at least part of the spiramycin and puromycin binding sites are close to each other.

Another discussion point is noteworthy. As briefly mentioned in the discussion of the S12 results, a correlation of antibiotic and rRNA binding sites in ribosomal proteins was found (Urlaub et al., 1995). This general idea is supported by the existence of various other peptide-antibiotic cross-links that

### Table III

| Ribosomal protein | Peptide region of antibiotic interaction | Antibiotic | Peptide region of rRNA interaction |
|-------------------|----------------------------------------|------------|----------------------------------|
| Eco S7            | Pro2-Lys10                             | Puromycin  | Pro2-Lys10                        |
| Bst S7            | Ala1-Lys9                              | Spiramycin | Ala1-Lys14                        |
| Eco S12           | Ala26-Lys46                            | Puromycin  | Lys46-Asp3                        |
| Eco S14           | Bst S14                                |            | Glu5-Thr21                        |
| Eco L27           | Val66-Lys71                            | Spiramycin | Val66-Lys71                       |
| Eco L29           | Thr55-Lys66                            | Puromycin  | Thr55-Lys66                       |
| Bst L29           |                                        |            |                                  |

3 R. Alexander, personal communication.

![Fig. 4. Localization of dihydrospiramycin-labeled proteins (black circles) in the 70 S ribosome of Stöffler-Meilicke and Stöffler (1990). The view presents the 30 S subunit from the cytosolic side and the 50 S subunit from the luminal side. The circle indicates the most probable position of L35 (the most likely location of L35 in the circle is depicted by arrows). Dihydrospiramycin-labeled peptide regions of the respective ribosomal proteins have to be placed toward the interface gap. The two-way arrow depicts the discrepancy in the positioning of L17 in the Traut (Traut et al., 1986) and Wallaczek model (Wallaczek et al., 1988).](http://www.jbc.org/)
are found to be affinity-labeled in the same peptide region that is also in close neighborhood to the rRNA (Table III). Most of the peptide regions listed in Table III are highly conserved and predominantly contain basic residues and/or aromatic residues that can contribute to a protein-rRNA interaction that is prone to disruption by the respective antibiotic. These findings shed light on the importance of rRNA sequences and the need for rRNA modification for the antibiotic binding site. Similar studies on the binding site of other antibiotics are also described, which provide insights into the functional differences among antibiotics.

The results discussed above prove that the labeled peptide stretches of proteins S12, S14, L17, L18, L27, and L35 are part of the spiramycin binding site in the ribosome (Fig. 4).

Cross-linking of antibiotics to their functional site in combination with isolation and sequencing of the peptide-antibiotic cross-links is a direct approach to establish data concerning the peptide environment of these antibiotics in their functional binding site. Similar studies on the binding site of other antibiotics and ribosomal substrates at the peptide level, as recently described for puromycin (Bischof et al., 1994), lead to a more detailed insight into ribosomal structure-function correlation.

REFERENCES

Alexander, R., Muralikrishna, P., and Cooperman, B. S. (1994) Biochemistry 33, 12109–12118
Arevalo, M. A., Tejedor, F., Polo, F., and Ballesta, J. P. G. (1988) J. Biol. Chem. 263, 58–63
Bischof, O., Kruft, V., and Wittmann-Liebold, B. (1994) J. Biol. Chem. 269, 18315–18319
Brimacombe, R., Greuer, B., Mitchell, P., Osswald, M., Rinkle-Appel, J., Schüller, D., and Stade, K. (1990) in The Ribosome: Structure and Function (Hill, W. E., Dahlberg, A., Garrett, R. A., Moore, P. B., Schlessinger, D., and Warner, J. S., eds) pp. 93–106, American Society for Microbiology, Washington, D.C.
Brodömüller, J., and Kamp, R. M. (1986) Biochimie (Paris) 69, 879–884
Contreras, A., and Vazquez, D. (1979) in The Ribosome: Structure and Function (Hill, W. E., Dahlberg, A., Garrett, R. A., Moore, P. B., Schlessinger, D., and Warner, J. S., eds) pp. 123–133, American Society for Microbiology, Washington, D.C.
Dinos, G., Synetos, D., and Coutsogeorgopoulos, C. (1993) Biochemistry 32, 10638–10647
Funatsu, G., Yaguchi, M., and Wittmann-Liebold, B. (1977) FEBS Lett. 73, 12–17
Hampl, H., Schulze, H., and Nierhaus, K. H. (1981) J. Biol. Chem. 256, 2284–2288
Högenauer, G., Egger, H., Ruf, C., and Stumper, B. (1981) Biochemistry 20, 546–552
Kamp, R. M. (1986) in Advanced Methods in Protein Microsequence Analysis (Wittmann-Liebold, B., Salnikow, J., and Erdmann, V., eds) pp 8–20, Springer Verlag, Berlin
Moazed, D., and Noller, H. F. (1987) Biochimie (Paris) 69, 879–884
Monro, R. E., Stachelin, T., Celma, M. I., and Vazquez, D. (1969) in Cold Spring Harbor Symp. Quant. Biol. 34, 357–368
Oakes, M. J., Scheinman, A., Atha, T., Shankweiler, G., and Lake, J. A. (1990) in The Ribosome: Structure and Function (Hill, W. E., Dahlberg, A., Garrett, R. A., Moore, P. B., Schlessinger, D., and Warner, J. S., eds) pp. 180–193, American Society for Microbiology, Washington, D.C.
Rombauds, W., Feytons, V., and Wittmann-Liebold, B. (1982) FEBS Lett. 149, 320–327
Sacerdoli, R., Fayat, G., Dessen, P., Springer, M., Plumbridge J. A., Grunberg-Manago, M., and Blanquet, S. (1982) EMBO J. 1, 311–315
Saltzman, L., and Apirion, D. (1976) Mol. & Gen. Genet. 143, 301–306
Stern, S., Weiser, B., and Noller, H. F. (1988) J. Mol. Biol. 204, 447–461
Stöffler-Mellicks, M., and Stöffler, G. (1990) in The Ribosome: Structure and Function (Hill, W. E., Dahlberg, A., Garrett, R. A., Moore, P. B., Schlessinger, D., and Warner, J. S., eds) pp. 123–133, American Society for Microbiology, Washington, D.C.
Tejedor, F., and Ballesta, J. P. G. (1986) Biochimie (Paris) 69, 7725–7731
Traut, R. R., Tewari, D. S., Sommer, A., Gavino, G. R., Olson, H. M., and Glitz, D. (1988) in Structure, Function and Genetics of Ribosomes (Hardesty, B., and Kramer, G., eds) pp. 286–308, Springer Verlag, Berlin
Urbahl, H., Kruft, V., Bischof, O., Müller, E. C., and Wittmann-Liebold, B. (1995) EMBO J. 14, 101–111
Vazquez, D. (1979) in Inhibitors of Protein Synthesis, pp. 120–126, Springer Verlag, New York
Wallacez, J., Schüller, D., Stöffler-Mellicks, M., Brimacombe, R., and Stöffler, G. (1988) EMBO J. 7, 3571–3576
Wower, J., Sylvers, L. A., Rosen, K. V., Hixson, and Zimmermann, R. A. (1993) in The Translational Apparatus: Structure, Function, Regulation, Evolution (Nierhaus, K. H., Franceschi, F., Subramaniam, A. R., Erdmann, V. A., and Wittmann-Liebold, B., eds) pp. 455–464, Plenum Press, New York
Yaguchi, M. (1975) FEBS Lett. 59, 217–220

Downloaded from http://www.jbc.org/ on July 24, 2018
