Identification and Sequence Analysis of Contact Sites between Ribosomal Proteins and rRNA in *Escherichia coli* 30 S Subunits by a New Approach Using Matrix-assisted Laser Desorption/Ionization-Mass Spectrometry Combined with N-terminal Microsequencing*

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Cross-linked peptide-oligoribonucleotide complexes derived from distinct regions of the rRNA and individual ribosomal proteins of the 30 S ribosomal subunits from *Escherichia coli* were isolated and purified. Cross-linking sites at the amino acid and nucleotide level were determined by N-terminal amino acid sequence analysis in combination with matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS). MALDI-MS analysis performed subsequent to a partial alkaline hydrolysis of cross-linked peptide-oligoribonucleotide complexes allowed for the first time the cross-linked rRNA moiety to be sequenced by this technique. In this manner Lys-44 in S4 was determined to be cross-linked to the oligoribonucleotide at positions 1531–1542 on the 16 S RNA (whereby either U-1541 or A-1542 is the actual cross-link site), Lys-75 in S7 to positions 1374–1379 (C-1378 cross-linked), Met-114 in S7 to 1234–1241 (U-1240 cross-linked), Lys-55 in S8 to 651–654 (U-653 cross-linked), and Lys-29 in S17 to 629–633 (U-632 cross-linked). The novel approach applied here promises to be useful for similar studies on other known protein-RNA complexes.

The quarternary structure of the ribosome from the eubacterium *Escherichia coli* has been studied intensively for many years, and several individual models concerning either the protein composition and the arrangement of proteins (e.g. Refs. 1–5) or the three-dimensional folding of 16 S RNA including the protein-rRNA interactions (e.g. Refs. 6–9) have been proposed. However, the resolution of these models at the molecular level is still limited, since the contact sites between adjacent proteins and between proteins and rRNA are not precisely known at the amino acid level with only a few exceptions of cross-linked protein pairs (e.g. Refs. 10–12), although a number of protein contact sites have been identified at the nucleotide level (for review, see Ref. 9). More recently, detailed protein-rRNA cross-linking studies at the peptide level (13) in combination with three-dimensional structures of isolated ribosomal proteins (e.g. Refs. 14–17) have provided more insight into the protein-RNA interactions and their functional implications within the prokaryotic ribosome. Additionally, site-directed hydroxyl radical probing of the rRNA neighborhood in reconstituted ribosomal particles has provided information relevant to the three-dimensional orientation of several proteins within the 30 S subunit (18, 19).

Even though discrete peptide regions of individual ribosomal proteins in close contact to the rRNA have been clearly established (13), the analysis of the corresponding sites on the rRNA has still remained a problem. Detailed modeling of ribosomal structures requires precise knowledge of protein-nucleotide and peptide-RNA contact sites concomitantly at the amino acid and nucleotide level. With the help of such information, the three-dimensional structures of ribosomal proteins can be placed into current models of the rRNA, and hence refined models can be adapted to the overall topography of the prokaryotic ribosome as derived from cryo-electron microscopy data (20, 21). For this purpose we have developed a new approach that enables us to determine simultaneously the contact sites at the peptide as well as at the nucleotide level within cross-linked peptide-oligoribonucleotide complexes isolated from ribosomal subunits. The strategy employed is based on N-terminal sequence analysis combined with matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS).1 MALDI-MS (22) has been successfully applied for e.g. the determination of phosphoproteins, glycoproteins, and oligonucleotides (for review, see Ref. 23–25). Furthermore, applications of MALDI-MS for sequencing of peptides (e.g. Refs. 26–28) and of oligodeoxynucleotides (e.g. Refs. 29 and 30) have been described. Here, we report for the first time the sequencing of oligoribonucleotides by MALDI-MS cross-linked to peptides. The strategy applied here should be a valuable tool for studying protein-RNA interactions, not only in ribosomes but also in other protein-RNA complexes.

EXPERIMENTAL PROCEDURES

Preparation of 30 S ribosomal subunits from *E. coli* (Eco 30S), chemical cross-linking of ribosomal subunits with 2-iminothiolane, and generation of cross-linked peptide-rRNA heteromers were performed according to Urlaub et al. (13).

C. Reversed Phase (RP) High Performance Liquid Chromatography (HPLC)—The rRNA pool derived from 150 *A. aerogenes* cross-linked ribosomal subunits treated with endopeptidases and RNase T1 (13) was subjected to a second incubation with endopeptidases Lys-C, Glu-C, or trypsin.

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1 The abbreviations used are: MALDI-MS, matrix-assisted laser desorption/ionization-mass spectrometry; Eco 30S, 30 S ribosomal subunit from *E. coli*; RP-HPLC, reversed phase high performance liquid chromatography; Lys-C, endopeptidase from *Lysobacter enzymogenes* (EC 3.4.21.50); Glu-C, protease V8 from *Staphylococcus aureus* V8 (EC 3.4.21.19).
Isolation of the cross-linked peptide-oligoribonucleotide complexes was achieved by RP-HPLC (13). Fractions that showed an absorbance at 220 and 260 nm were collected, and cross-linked peptide-oligoribonucleotide complexes were precipitated with 2 volumes of 98% ethanol in the presence of 1⁄10 volume of 3 M sodium acetate, pH 5.5, and 30 μg of glycogen (Boehringer Mannheim) for at least 2 h at −80 °C. After centrifugation at 13,000 rpm for 1 h at 4 °C the pellet was immediately redissolved in 25 mM Tris-HCl, pH 7.8, 1 mM EDTA, 10% (v/v) acetonitrile, and 0.1% (v/v) Triton® X-100 (hydrogenated, protein grade, Calbiochem). Precipitated and redissolved fractions were rechromatographed in the same buffer system as described in Ref. 13 using a gradient as follows: 15-min isocratic elution with 10% buffer B, then 10–45% buffer B for 120 min, and 45–90% buffer B for 10 min. Fractions with an absorbance at 220 and 260 nm were dried under vacuum and cross-linked peptide-ribo complexes were redissolved in 50% (v/v) acetonitrile, 0.1% (v/v) trifluoroacetic acid, divided into aliquots for further studies and stored at −80 °C.

N-terminal Sequence Analysis—For identification of the cross-linked peptide moiety, up to 5 pmol of cross-linked peptide-oligoribonucleotide complex in 50% (v/v) acetonitrile, 0.1% (v/v) trifluoroacetic acid was sequenced in a PROCISETM protein sequencer (Applied Biosystems Inc., Foster City, CA, USA). The SWISS-PROT data bank was used for comparison with the known E. coli protein sequences by the program FASTA (31).

MALDI-MS—Mass spectra of cross-linked peptide-oligoribonucleotide complexes were recorded in the linear mode of a time of flight VG-TofSpec (Fisons, Manchester, United Kingdom) equipped with a nitrogen laser (337 nm, pulse duration: 4 ns) according to Thiede et al. (28). The acceleration voltage was 22 kV, and the spectra were obtained either in the positive or in the negative mode by summing over 20–50 laser shots. For mass determination of untreated cross-linked complexes, a 0.8-μl sample in aqueous 50% (v/v) acetonitrile, 0.1% (v/v) trifluoroacetic acid (containing 1–10 pmol of cross-linked peptide-oligoribonucleotide complex) and 1.2 μl of matrix solution (a saturated solution of α-cyano-4-hydroxycinnamic acid in water:acetonitrile, 3:2; containing 0.1% (v/v) trifluoroacetic acid) were mixed on the target and air-dried. Alternatively, the sample was incubated with ammonium acetate activated ion exchange matrix (AG® 50W-X8, Resin, 100–200 mesh, Bio-Rad) as described by Nordhoff et al. (32) for 30 min at 37 °C. 5–10 ion exchange beads were sufficient for incubation. 0.8 μl of the supernatant was mixed with the matrix as above. Nucleotide compositions of the cross-linked oligoribonucleotides were determined from the mass spectra of untreated samples by the program NUCSEQ.2 Partial alkaline hydrolysis of the cross-linked oligoribonucleotide-peptide complex was carried out by treating 1–10 pmol of vacuum-dried sample with ammonium hydroxide solution at pH 10.0 for 10 min at 95 °C. The sample was dried again under vacuum and redissolved in an appropriate volume of aqueous 50% (v/v) acetonitrile, 0.1% (v/v) trifluoroacetic acid. Mass spectra were recorded as described above. Alternatively, vacuum-dried samples were redissolved in 5 μl of water and incubated with 5′→3′ phosphodiesterase (from calf spleen, Boehringer Mannheim) as described by Kirpekar et al. (29) and by Crain (33). For mass analysis samples were prepared as above.

RESULTS

Isolation of Cross-linked Peptide-Oligoribonucleotide Complexes Suitable for MALDI-MS—30 S ribosomal subunits from E. coli (Ec030S) were cross-linked with 2-iminothiolane followed by a short UV irradiation. Ribosomal proteins cross-linked to 16 S rRNA and the corresponding peptide-oligoribonucleotide complexes were precipitated with endoproteinase Lys-C and RNase T1, and cross-linked peptide-oligoribonucleotide heteromers derived from 100 A260 cross-linked Eco 30S after treatment with endoproteinase Lys-C and RNase T1, and cross-linked peptide-ribo complexes were redissolved in 25 mM Tris-HCl, pH 7.8, 1 mM EDTA, 10% (v/v) acetonitrile, and 0.1% (v/v) Triton® X-100 (hydrogenated, protein grade, Calbiochem) as described by Kirpekar et al. (28) and Crain (33). For mass analysis samples were prepared as above.

2 E.-C. Müller, unpublished data.
U-1240 could be identified as the site which cross-linked to Met-114 in S7. Fig. 2C shows the sequence of the T1-oligoribonucleotide cross-linked to Met-114 in S7 as determined by the mass fragments after partial alkaline hydrolysis.

**Sequence Analysis of an rRNA-Oligoribonucleotide Cross-linked to Lys-44 in S4**—The MALDI-MS analysis of the peptide-oligoribonucleotide complex derived from ribosomal protein S4 cross-linked to 16S rRNA (positions 35–56, Gln-Ala-Pro-Gly-Gln-His-Gly-Ala-Arg-Lys-Pro-Arg-Leu-Ser-Asp-Tyr-Gly-Val-Gln-Leu-Arg-Glu, with Lys-44 cross-linked, fraction 2 in Fig. 1B) is illustrated in Fig. 3. Fig. 3A shows the mass spectrum of the untreated cross-linked complex, and Fig. 3B shows the spectrum of the cross-linked complex after partial alkaline hydrolysis. Interestingly, the mass of the total complex (mass peak k, M_r: 6242 and 6243, respectively) as well as the mass of the cross-linked peptide derivatized with the 2-iminothiolane spacer (mass peak a, M_r: 2567 and 2568, respectively) occur in both spectra. Complexes in which the peptide is cross-linked to the oligoribonucleotide via 2-iminothiolane seem to be less stable under MALDI conditions than complexes derived after direct photoinduction (e.g. the S7 Met-114/U-1240 cross-link described above). After partial alkaline hydrolysis the mass spectrum revealed an almost perfect sequence ladder of the cross-linked T1-oligoribonucleotide (mass peaks b–j, Fig. 3B). The mass peaks a–k are listed and interpreted in Table II.

The sequence of the cross-linked T1-oligoribonucleotide obtained correlates exclusively with the T1 fragment of the 16S rRNA directly at its 3' terminus. Mass peak b corresponds to a fragment of the hydrolyzed complex consisting of the derivatized peptide cross-linked to UAOH or CAOH and thus corresponds to the extreme 3' end of the RNA. Mass peaks c–j differ by the masses of the nucleotide 3'-monophosphates shown, whereas mass peaks k and j differ by a dinucleotide of either AC or AU at the 5' end of the 12-mer oligoribonucleotide. Fig. 3C shows the sequence of the T1-oligoribonucleotide from positions 1531–1542, with either U-1541 or A-1542 directly cross-linked to Lys-44 within the Glu-C fragment of S4.

**Sequence Analysis of Peptide-Oligoribonucleotide Cross-links**
Fig. 2. MALDI mass spectra of the purified peptide-oligoribonucleotide complex derived from ribosomal protein S7 cross-linked to the 16 S RNA. A, mass spectrum of the untreated peptide-oligoribonucleotide complex from fraction 1 in Fig. 1A; B, mass spectrum of the peptide-oligoribonucleotide complex after treatment with ammonium hydroxide. Both spectra were recorded in the negative mode, the peaks between the masses 3300–4600 of spectrum B are magnified 5-fold. The base compositions and the sequences of the cross-linked oligoribonucleotides corresponding to the masses of the peaks a–k are outlined in Table I. C, sequence of the 16 S RNA T1-oligoribonucleotide cross-linked to Met-114 in S7. Positions on the 16 S RNA are shown as small numbers. The bold letter indicates the cross-linked nucleotide. Bars indicate either the masses of the total peptide-rRNA oligoribonucleotide complex (mass peak k) or fragments obtained from the total complex after treatment with ammonium hydroxide (mass peaks a–i). Designations of the bars correspond to those given in A and B and Table I. See text for further details.
Sequence Analysis of Peptide-Oligoribonucleotide Cross-links

The base composition and sequence were determined from the mass peaks a–k as shown in Fig. 3. The cross-linked S7 peptide sequence (fragment position 113–129, see Fig. 2A) is also listed. Cross-link sites within the rRNA-oligoribonucleotide and the peptide are shown in bold letters. Note that C and U differ by only 1 mass unit. Hence, different possible oligoribonucleotide compositions as well as different oligoribonucleotide sequences are listed, and the calculated mass (second column) is given as the average value of all possible combinations of C and U residues within the oligoribonucleotide concerned. See text and legend to Fig. 2 for details.

| Mass measured (M – H²O) | Mass calculated (M – H²O) | Peptide (molecular mass: 1833 Da) | Oligonucleotide composition sequence |
|-------------------------|---------------------------|-----------------------------------|-------------------------------------|
| 939 (peak a)            | 939 ± 1                   | A₁C₂₂, A₁C₂U₁, A₁U₂ –H₂O         |
| 1244 (peak b)           | 1244.5 ± 1                | A₁C₂₂, A₁C₂U₁, A₁C₂U₂, A₁U₂ –H₂O |
| 1574 (peak c)           | 1573.5 ± 1                | A₁C₂₂, A₁C₂U₁, A₁C₂U₂, A₁U₂ –H₂O |
| 2503 (peak e)           | 2500.5 ± 0.5              | SMALRLANELSDAENK                 |
| 2823 (peak f)           | 2829.5 ± 0.5              | SMALRLANELSDAENK                 |
| 3161 (peak g)           | 3158.5 ± 0.5              | SMALRLANELSDAENK                 |
| 3467 (peak h)           | 3464 ± 1                  | SMALRLANELSDAENK                 |
| 3794 (peak i)           | 3792 ± 1                  | SMALRLANELSDAENK                 |
| 4043 (peak j)           | 4041 ± 2                  | SMALRLANELSDAENK                 |
| 4405 (peak k)           | 4404 ± 2                  | SMALRLANELSDAENK                 |

The corresponding analysis of the S8 complex revealed a 4-mer T₁-oligoribonucleotide cross-linked to Lys-55 in S8, with either C or U directly 5’ to the 3’ Gp as the actual cross-linking site (mass spectra not shown). Because of its base composition (C₃Gp, C₂U₁Gp, C₁U₂Gp, or U₃Gp), no sequence could be clearly assigned. Comparison of these calculated compositions with the 16 S RNA sequence (34) shows sequence similarity with 13 possible T₁ fragments.

**DISCUSSION**

To determine and sequence the oligoribonucleotides cross-linked to peptides in 30 S ribosomal subunits, we have successfully applied MALDI-MS. To date, similar studies using MALDI-MS have only been carried out on cross-linked protein-DNA complexes (36), but without oligonucleotide sequence determination.

A novel strategy for isolation and purification of peptide-oligoribonucleotide complexes derived from cross-linked ribosomal subunits was recently established that led to the identification of peptides from 13 different ribosomal proteins cross-linked either to the 16 S or the 23 S RNA (13). However, in this previous study, only the cross-linking sites within the peptides, but not within the corresponding cross-linked oligoribonucleotides could be identified. A second proteolytic cleavage subsequent to the RNA fragmentation enabled us to obtain cross-linked complexes in sufficient amounts to be rechromatographed to purity and analyzed simultaneously by N-terminal amino acid sequencing and MALDI-MS. By combining these techniques, we were able to identify the cross-linked oligoribonucleotide moiety that contacts distinct regions of the ribosomal proteins S4, S7, S8, and S17 from *E. coli*. Moreover, the cross-linking sites could be analyzed at the nucleotide/uridine acid level.

The covalent linkage between the peptide and the oligoribonucleotide generated by the cross-linking reaction led to a series of complexes which resembled derivatized peptides. Thus, their masses could be measured by MALDI-MS under standard conditions for peptides by using α-cyano-4-hydroxycinnamic acid as a matrix (28). MALDI-MS has the advantage of being much less sensitive to salt contaminations than the ESI-MS technique (28, 29, 37) and generally generates data of...
mixtures that are easy to interpret. Accordingly, the cross-linked complexes could be analyzed by MALDI-MS directly after partial hydrolysis with ammonium hydroxide solution or treatment with phosphodiesterase, avoiding further purification steps. However, MALDI mass spectra of oligoribonucleotide-containing samples are often poorly resolved and show heterogeneity when compared with peptide or protein mass spectra. In particular, a variety of metal counterions that interact with the phosphate backbone of the oligoribonucleotides caused broad or multiple mass peaks of the total complex with

![Diagram A](image1.png)

![Diagram B](image2.png)

![Diagram C](image3.png)
increasing numbers of nucleotides. This is a well known fact from MALDI-MS analysis of oligodeoxynucleotides (32). In the case of the complexes derived from ribosomal proteins S7 (Met-114 cross-linked to U-1240; Lys-75 cross-linked to C-1378) and S4 (Lys-44 cross-linked to U-1541 or A-1542), the exact mass of each total cross-linked complex could be determined only after performing ion exchange according to Nordhoff et al. (32). Increased mass accuracy of the total complexes was also observed when the samples were incubated directly with saturated ammonium sulfate solution (37). The nucleotide compositions of all the cross-linked complexes were calculated from the mass differences between the sequenced peptides plus their iminothiolane spacer (except in the case of the S7 fragment cross-linked via Met-114 to the 16 S RNA; see “Results”) and the measured mass of the total complexes. Different compositions with respect to the nucleotides C and U were considered, due to the low mass resolution of the total complex (see above). Additionally, the samples could be analyzed only in the linear mode of the mass spectrometer, which has a decreased resolution in comparison with the reflector mode. Consequently, the calculated composition of cross-linked oligoribonucleotide moiety was not sufficient for an unambiguous identification of the corresponding positions in the 16 S RNA.

Despite the decreased mass accuracy, a partial alkaline hydrolysis of the total complexes followed by MALDI-MS analysis enabled us to obtain adequate sequence information of the cross-linked oligoribonucleotide for a localization of the corresponding positions in the 16 S RNA. By applying this sequencing strategy we identified Met-114 in S7 cross-linked to U-1240 on the 16 S RNA, thereby confirming previous results by Möller et al. (38) who determined Met-114 to be cross-linked to the oligoribonucleotide stretch 1238–1243 after direct UV irradiation. Furthermore, our data provide direct evidence for an additional interaction site between S7 and the 16 S RNA. We were able to isolate a second cross-linked peptide-oligoribonucleotide complex in which Lys-75 in S7 was cross-linked to U-1378 in the 16 S RNA. Nucleotides 1377–1378 have also been cross-linked with 2-iminothiolane in earlier studies to the intact S7 protein (39), but without determination of the cross-linked peptide. The two sites in S7 (Lys-75 and Met-114) which were cross-linked to nucleotides U-1378 and U-1240, respectively, are consistent with the data of Dragon et al. (40), who generated mutations in G-U base pairs in helices 41 and 43 (using here and in the following the helix nomenclature of Ref. 9). The mutation sites are close to the cross-linked oligoribonucleotide regions (positions 1234–1241 and 1374–1379, respectively) and alter the binding of S7 to the rRNA.

In S4 two sites which cross-linked to the 16 S RNA have now been established, namely the peptide stretch 77–90 with Lys-82 cross-linked (13) and 35–56 with Lys-44 cross-linked (this work). MALDI-MS analysis of the latter after partial alkaline hydrolysis of the cross-linked peptide-oligoribonucleotide complex revealed an almost perfect sequence ladder of the cross-linked oligoribonucleotide moiety. The cross-linking site to Lys-44 in S4 was found to be the extreme 3′ terminus of the 16 S RNA. Cross-linking studies with a different reagent (41) as well as studies with base probes (7) and free or directed hydroxyl radical probing (18, 42) have demonstrated an interaction of S4 with parts of helices 16, 17, 18, and 23a of the 16 S RNA. Nevertheless, the specificity of 2-iminothiolane as a cross-linking reagent has been demonstrated by the isolation of homologous peptide stretches derived from ribosomal proteins of related organisms cross-linked to the 16 S RNA (13). Therefore, our data show that the 3′ terminus of the 16 S RNA, which is known to be very flexible, is also able to contact the ribosomal protein S4.

MALDI-MS analysis of the S17 complex showed that four T1 fragments of the 16 S RNA have to be considered to be putative cross-linked oligoribonucleotides to Lys-29 in S17, namely positions 320–324, 629–633, 918–922, or 1339–1343. The site of cross-linking was identified to be either C or U directly 5′ to the 3′ Gp of the 5-mer T1 fragment. By comparison with previous cross-linking studies on the 16 S RNA, we conclude that U-632 of the 16 S RNA T1 oligoribonucleotide 629–633 is cross-linked to Lys-29 in S17, since exactly the same region was also identified to be cross-linked with 2-iminothiolane to the intact S17 protein (43). Other cross-linking studies with bis-(2-chloroethyl)dimethylamine (41) and chemical footprinting data (7) point to helix 11 of the 16 S RNA as a second interaction site to S17. In the three-dimensional structure of S17 from Bacillus stearothermophilus, two loop regions (loop 1 and 3, see Fig. 4A) were proposed to interact with the the 16 S RNA (17). Our results

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**Table II**

**Nucleotide sequence of the 16 S rRNA T1-oligoribonucleotide cross-linked to Lys-44 in ribosomal protein S4 as determined by MALDI-MS**

| Mass measured (M+H) | Mass calculated (M+H) | Peptide + IT (molecular mass: 2565 Da) | Oligonucleotide sequence |
|---------------------|-----------------------|---------------------------------------|-------------------------|
| 2567 (peak a)       | 2566                  | QAPGQHGAKPRLSDYGVQRLR + IT           | C\_CAOH\_U             |
| 3141 (peak b)       | 3140.5 ± 0.5          | QAPGQHGAKPRLSDYGVQRLR + IT           | C\_CAOH\_U             |
| 3447 (peak c)       | 3446.5 ± 0.5          | QAPGQHGAKPRLSDYGVQRLR + IT           | C\_CAOH\_U             |
| 3752 (peak d)       | 3751.5 ± 0.5          | QAPGQHGAKPRLSDYGVQRLR + IT           | C\_CAOH\_U             |
| 4058 (peak e)       | 4057 ± 2              | QAPGQHGAKPRLSDYGVQRLR + IT           | C\_CAOH\_U             |
| 4362 (peak f)       | 4362.5 ± 2.5          | QAPGQHGAKPRLSDYGVQRLR + IT           | C\_CAOH\_U             |
| 4665 (peak g)       | 4668 ± 3              | QAPGQHGAKPRLSDYGVQRLR + IT           | C\_CAOH\_U             |
| 4975 (peak h)       | 4973.5 ± 3.5          | QAPGQHGAKPRLSDYGVQRLR + IT           | C\_CAOH\_U             |
| 5305 (peak i)       | 5304.5 ± 3.5          | QAPGQHGAKPRLSDYGVQRLR + IT           | C\_CAOH\_U             |
| 5602 (peak j)       | 5608 ± 4              | QAPGQHGAKPRLSDYGVQRLR + IT           | C\_CAOH\_U             |
| 6243 (peak k)       | 6242.5 ± 4.5          | QAPGQHGAKPRLSDYGVQRLR + IT           | C\_CAOH\_U             |
is cross-linked to Lys-55 in S8. Very recently, the three-dimensional structure of S8 from *B. stearothermophilus* has been published (16). The cross-linked amino acid (whereby Lys-55 in *E. coli* corresponds to Gln-56 in *B. stearothermophilus*) is positioned within a loop structure of the protein (Fig. 4B), which has been suggested to be part of a more extended RNA binding site in S8 (17). As with the S17 complex discussed above, the corresponding cross-linked nucleotide in the S8 complex (U-653) and its adjacent area belong to a non-base-paired region of the 16 S RNA (Fig. 4B). Obviously, loop structures within ribosomal proteins together with looped or bulged structures within the rRNA are crucial for protein-RNA interactions in ribosomes (13, 47) similar to other protein-RNA complexes (e.g. Ref. 46). Additionally, it may be that only loop regions have sufficient flexibility to allow the bridging reaction by the cross-linking reagent to occur. The interaction of discrete loop regions in both proteins S8 and S17 with helix 21 is consistent with neutron scattering experiments which reveal a very close proximity of these proteins in the 30 S subunit (3). The data we present here should be very useful in combination with the three-dimensional structures of ribosomal proteins S8 and S17 and the current models of the 16 S RNA (9) and the 30 S ribosomal subunit (20, 21) for modeling a highly resolved S8-S17-16 S RNA complex.

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Identification and Sequence Analysis of Contact Sites between Ribosomal Proteins and rRNA in \textit{Escherichia coli} 30 S Subunits by a New Approach Using Matrix-assisted Laser Desorption/Ionization-Mass Spectrometry Combined with N-terminal Microsequencing

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