Cross-linked Amino Acids in the Protein Pairs L3-L19 and L23-L29 of Bacillus stearothermophilus Ribosomes after Treatment with Diepoxybutane*

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Treatment of native 50 S ribosomal subunits of Bacillus stearothermophilus with the homobifunctional cross-linking reagent diepoxybutane generated two cross-linked protein pairs, L3-L19 and L23-L29, which were isolated and identified. The analysis of the cross-linking sites at the amino acid level in both protein pairs is presented. Using a combination of sequence analysis and mass spectrometry it could be demonstrated that His-28 in protein L3 and the N-terminal amino acids Met-1, His-2, and His-3 in protein L19 are involved in forming the cross-link L3-L19. Within the pair L23-L29 Met-1 in protein L23 and Lys-4 in protein L29 were identified as cross-linking sites employing a similar approach. Comparison of our data with results derived from other cross-linking experiments showed that in general the structural organization of the ribosomes in eubacteria (the Gram-positive B. stearothermophilus and the Gram-negative Escherichia coli) has been conserved to quite an extent during evolution but that the fine structures differ slightly. By mass spectrometry the specificity of diepoxybutane and its cleaving mechanism using sodium periodate could be examined. In addition the complete amino acid sequence of protein L19 of B. stearothermophilus has been determined and revealed 58% identical amino acid residues to the homologous E. coli protein L19.

The construction of a reliable structural model of the ribosome is a prerequisite for understanding the translational process. Most topographical data so far available are derived from immune electron microscopy (Hackl and Stöfler-Meilicke, 1988; Stöfler-Meilicke and Stöffler, 1990), neutron scattering (Novotny et al., 1986; Capel et al., 1987, 1988), and chemical cross-linking of the different E. coli ribosomal proteins with respect to each other (Lutter et al., 1974; Traut et al., 1980; Walleczek et al., 1989) and to the ribosomal RNA (Brimacombe et al., 1990). Bifunctional reagents are applied whose characteristic applications have comprehensively been described (Ji et al., 1983; Kamp, 1988; Böhm and Fasold, 1989). The combination of the derived data has led to a substantial model of the 30 S subunit and for parts of the 50 S subunit which is still under investigation. In the last decade, sequence analysis of the ribosomal proteins derived from E. coli had been completed (Wittmann-Liebold, 1986) and for the Bacillus stearothermophilus ribosome 44 protein primary structures are available (Wittmann-Liebold et al., 1990; Arndt et al., 1991; Kruft et al., 1991). Therefore, it became possible to undertake topographical investigations by comparative cross-linking experiments in E. coli and in B. stearothermophilus ribosomes (Brockmölker and Kamp, 1986, 1988; Herwig, 1990). More recently these studies were extended to Haloarcula marismortui (Bernberg, 1992) since ribosomal particles from this organism are more suitable for x-ray crystallographic studies (Makowski et al., 1987; Von Böhlen et al., 1991; Vonath et al., 1980, 1991). In addition for this organism, the analysis of the primary structures is not far from completion (for overview, see Arndt et al., 1991). So far only a few cross-linked protein pairs have been identified at the amino acid level in E. coli (Allen et al., 1979; Maassen et al., 1981; Pohl and Wittmann-Liebold, 1988) as well as in B. stearothermophilus (Brockmölker and Kamp, 1988) and argue for an overall conserved structure within Gram-positive and Gram-negative organisms.

In this paper we describe the identification of the cross-linked amino acids in the protein pairs L3-L19 and L23-L29 of B. stearothermophilus ribosomes which have been formed after treatment of the native 50 S subunits with diepoxybutane. Using a combination of sequence analysis and mass spectrometry, we were able to exactly determine the cross-linking sites and to describe more precisely than known in the literature the specificity of diepoxybutane and the cleaving mechanism using sodium periodate. In order to assign the cross-linked amino acids in these protein pairs, the primary structures of proteins L3 and L19 from B. stearothermophilus were sequenced in the course of this work by protein-chemical sequence analysis (BstL19, this paper) and by sequencing of the corresponding genes, respectively (BstL3, Herwig et al., 1992).

EXPERIMENTAL PROCEDURES

Chemicals—DL-1,2,3,4-diepoxybutane and chymotrypsin were purchased from Serva (Heidelberg, Federal Republic of Germany [F. R. G.]). Endoproteinases Lys-C and Glu-C were obtained from Boehringer (Mannheim, F. R. G.). Sodium(meta)periodate was purchased from Aldrich (Milwaukee, Wisc., U.S.A.).

The abbreviations used are: Lys-C, Lysobacter enzymogenes protease (EC 3.4.21); DEB, diepoxybutane; Da, Dalton; DABITC, 4-(N,N-dimethylamino)azobenzene 4'-isothiocyanate; Glu-C, S. aureus protease V8 (EC 3.4.21.19); MS, mass spectrometry; PTH, phenylthiohydantoin; PVDF, polyvinylidene difluoride; PVP-40, polyvinylpyrrolidone (molecular mass 40,000 da); RP-HPLC, reversed-phase high performance liquid chromatography; MES, 4-morpholineethanesulfonic acid.
from Fluka (Buca, Switzerland). Polyvinylpyrrolidone (PVP-40) was obtained from Sigma (Deisenhofen, F. R. G.). Vydac C4 reversed-phase support was from The Separation Group (Hesperia, CA), and laboratory-filled columns were used. Lichrospher 100 CH-18/2 columns were from Merck (Darmstadt, F. R. G.). Immobilon PVDF membranes were from Waters, Millipore (Eschborn, F. R. G.) and PVDF-Sequelon AA membranes from Milligen (Burlington, MA). All other chemicals were of pro analysis grade or higher quality obtained from Merck.

Preparation of Ribosomes—Growth of B. stearothermophilus (strain 709) and separation of ribosomes and ribosomal subunits has already been described (Brockmoller and Kamp, 1986). Cross-linking of 50 S subunits with 1% (v/v) DEB was done as described (Brockmoller and Kamp, 1986). Total protein extracts were obtained by acetic acid extraction (Hardy et al., 1986).

Isolation of Proteins—Isolation of the protein pairs L3-L19 and L23-L29 has been described in detail (Herwig, 1990). Different to the described purification procedure for L23-L29 the protein pair was alternatively separated on preparative one-dimensional high Tri-SDS-gel electrophoresis (Fling and Gregerson, 1986) following the chromatography on laboratory packed Vydac C4 reversed-phase column (250 × 4.6 mm, 5-μm particle size, 300-Å pore size). Subsequent blotting onto Immobilon PVDF membranes (0.45-μm pore size) was carried out at 150 mA for 1 h and 650 mA for 6.5 h (Choli and Wittmann-Liebold, 1990). The membranes were stained with Ponceau S (0.5% w/v) in 1% (v/v) acetic acid (Salinovich and Montelaro, 1986) and destained with water.

Protein L19 was isolated to homogeneity from B. stearothermophilus 50 S total protein mixture by one-step reversed-phase chromatography on analytical Vydac C4 column using a gradient of 0.1% trifluoroacetic acid with acetonitrile as eluent.

Periodate Cleavage—Purified protein and peptide samples resulting from RP-HPLC separations were cleaved for 15 min in 100 ml 0.1% (v/v) trifluoroacetic acid at pH 2 containing 10 mM freshly dissolved sodium(meta)periodate. Oxidation was stopped by direct injection onto RP-HPLC columns.

Enzymatic Cleavages—Digestion with Staphylococcus aureus protease V8 (Glu-C) was performed in 50 mM amnomium acetate, pH 4.1, at an enzyme/substrate ratio of 1:50. Cleavage time was 12-24 h at 37 °C. Chymotryptic and Lysobacter enzymogenes protease digests were carried out for 4 h at 37 °C in 100 mM N-methyl-morpholine acetate buffer, pH 8.1, using an enzyme/substrate ratio of 1:50.

For digestion of blotted proteins, the corresponding bands were excised out of the PVDF membranes, cut into small pieces, and incubated for 30 min in 1 ml of PVP-40 (0.2% (w/v) in MeOH Uvasol) at room temperature. Excess PVP-40 was removed by washing the membranes three times with water. A final wash was done with the digestion buffer. Enzymatic cleavages were then carried out as described above. After cleavage the digestion supernatant was transferred to a vial and the polybicine membrane was washed twice with 80% formic acid and twice with water. All washing solutions were added to the digestion mixture dried by vacuum centrifugation and stored at −20 °C for further HPLC analysis.

Separation of Peptides—Peptides were separated by RP-HPLC on Lichrospher 100 CH-18/2-columns (particle size 5 μm, pore size 100 Å, 250 × 4.6 mm) with a linear gradient of acetonitrile in 0.1% trifluoroacetic acid.

N-terminal Sequencing—N-terminal sequence determination was performed on an Applied Biosystems pulsed liquid-phase sequencer, model 477A, equipped with a model 120 PTH amino acid analyzer. N-terminal cleavage was done in 100% trifluoroacetic acid and applied to a trifluoroacetic acid-treated polybicine-coated glass fiber that had been precycled, as described previously (Hewick et al., 1981).

Sequence analysis was also done in the Knauer Modular Protein Sequence model 510 (Knauer, Berlin) according to Wittmann-Liebold (1986) and Herfurth et al. (1991a). Glu-C-digested peptides were attached via their C-terminal glutamic acid onto alysyn deriva-
dized PVDF membranes (Sequelon AA, Herfurth et al., 1991b). For attachment the peptides (50–500 pmol) were dissolved in 50% (v/v) acetonitrile in water, applied onto the membrane, and dried for 5 min at 32 °C in a vacuum chamber. Coupling occurred after the addition of 10 μl of carbodiimide (10 mg/ml) dissolved in 0.1 M MES, pH 5, in 15% acetonitrile for 20 min at ambient temperature.

Mass Spectrometry—The mass spectra were recorded on a Biof-L 20 plasma desorption mass spectrometer (Applied Biosystems, Foster City, CA). Electrospray spectra were recorded in a 1 × 106 f.s. mass range corresponding to 10 min. For sample application a nitrocellulose matrix was employed prepared by electrospaying 50 μl of a nitrocel-

lulose solution (2 mg/ml) onto a Mylar foil. Samples were dissolved in 20 μl of 0.1% trifluoroacetic acid in 20% acetonitrile (v/v) and applied onto the target by the spin drying technique (Nielsen et al., 1988).

RESULTS

Treatment of 50 S Ribosomal Subunits with Diepoxybutane—After treatment of native 50 S ribosomal subunits of B. stearothermophilus with DEB, two new protein spots have been detected in the higher molecular mass area of the two-dimensional gel pattern of total protein extract corresponding to the protein pairs L3-L19 and L23-L29. L3-L19 is always detectable as a double spot, which both contain the cross-linked protein pair as deduced from sequence analysis. The isolation and identification of both protein pairs has been described in detail (Herwig, 1990; Brockmoller and Kamp, 1986). As given under “Experimental Procedures,” we had to slightly modify the purification procedure for L23-L29 which necessitated in situ enzymatic and chemical cleavages of the protein pair directly on blots.

Strategy for the Identification of Cross-linked Amino Acids—Preliminary identification of cross-linked peptides was done by HPLC analysis. Peaks exhibiting an altered elution behavior after periodate cleavage were candidates for cross-linked peptides and further investigated by sequence and MS analysis. The localization of the cross-linking site was based on the known sequence of the involved proteins. Due to the modification the cross-linked amino acids are not identifiable as PTH amino acids in the HPLC trace of the automatic Edman degradation since they are still linked to the other peptide chain via the cross-link. Monovaried modified amino acid residues also yielded gaps in the sequence because the modification has altered the elution position of the PTH derivative. Therefore, cross-linked peptides had to be confirmed by mass spectrometry.

The Cross-link L3-L19—5 nmol of L3-L19 was digested with S. aureus protease (Glu-C I digest) and the resulting peptide mixture separated by RP-HPLC (Fig. 1A). Periodate cleavage and chromatography of each single peak revealed one peak eluting at 34.5% acetonitrile (Fig. 1B) that was cleavable and resulted in five new peaks of shifted retention times (Fig. 1C). N-terminal sequencing of the peptides yielded fragments from both proteins, L3 and L19, resulting from this cleavage (Table I). These fragments were considered as candidates for cross-linked peptides.

Another glu-C digest of L3-L19 (Glu-C II digest), chromatographically analyzed prior to (Fig. 2a) and after periodate cleavage (Fig. 2b) of the whole mixture, showed altered elution behavior of two original peaks after periodate cleavage. The assignment of the resulting fragments (A = A1 + A2; B = B1 + B2) has been confirmed by periodate cleavage and chromatography of the single peaks A and B. Analysis of the origin peak A by sequencing, MS analysis and HPLC separation of chymotryptic peptides prior to and after periodate treatment (data not shown) revealed that both peptides merely coelute and that no cross-linking site exists between them. Sequence analysis of the original peak B gave two peptides already shown to elute together in the Glu-C I digest: L19 (positions 1–7), M(H)LH1QEl (L3 (positions 18–41), NGDLPVTVLaATPNVQLKKT). While histidine at position 11 of the L3 peptide could not be detected at all, both histidines of the L19 peptide were found in a very reduced rate. Additionally, both peptides were not detectable in equimolar amounts as would be expected for cross-linked peptides due to partial blockage of the L19 peptide. Periodate cleavage of peak B resulted in two new peaks (Fig. 2b). While the L3 peptide has scarcely changed its...
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Fig. 1. Separation of peptides derived from S. aureus protease (Glu-C) digest I of the cross-linked protein pair L3-L19. 5 nmol of the purified cross-link were digested with Glu-C enzyme as described under "Experimental Procedures" and the resulting peptide mixture separated by RP-HPLC (A). Each single peak was analyzed for cross-linked peptides by rechromatography prior to and after cleavage with 10 mM sodium periodate. Only one peak eluting at 34.5% acetonitrile (B) was cleavable and resulted in the elution of five new peaks of altered retention times (C).

Retention time (peak B2) elution of the L19 peptide shifted to 23% acetonitrile (peak B1). Due to the minute amount of cross-linked peptides we were not able to confirm the mass of the cross-linked peptide in the original peak B. Nevertheless, we analyzed the periodate-cleaved products B1 and B2 by mass spectrometry. In peak B1 we found the mass of the L19 peptide modified with one (949.5 Da) and two formylmethyl groups (991.9 Da), respectively, as is expected from periodate cleavage of the diepoxide bridge (Fig. 3). Further masses (931.6, 967.3, and 1010.6 daltons) result from uptake and release of H$_2$O. Additionally, partial fragmentation may have occurred (903.8, 860.3, and 799.5 daltons). In peak B2 the mass of the L3 peptide as well as the mass of the overlapping peptide, both modified by a formylmethyl group (2642.2 and
The results argue for a changing contribution of all three N-terminal amino acids to the cross-link formation.

The assignment of the putative cross-linking site, we reanalyzed the masses obtained from chymotryptic peptides of L23-L29. In fact we were now able to confirm the cross-linking site by mass analysis of the cross-linked peptide which is calculated to be 1221.36 Da and is in accordance with chymotryptic cleavage sites (data not shown).

Sequence Determination of Protein L19 from Bacillus stearothermophilus—Protein L19 was isolated from acetic acid protein extract of 50 S ribosomal subunits by one-step RP-HPLC on a Vydac C4 column and identified by two-dimensional gel electrophoresis (Geyl et al., 1981) and N-terminal sequencing. The whole sequence has been achieved by sequence analysis of overlapping peptides derived from enzymatic digests with Glu-C and Lys-C proteases (Fig. 7). The protein contains 116 amino acids and is typically basic with a net charge of +18. The positive charged domain is mainly located in the C-terminal half. The calculated molecular mass is 13446 Da. Comparison of the sequence with the corresponding protein from E. coli (Brosius and Arfsten, 1978) revealed 56% identical residues without introducing any gaps and gave an alignment score of 37.2 standard deviation units. Conserved regions are symmetrically distributed over the whole sequence (Fig. 8).

**TABLE I**

| Peptide no. (see Fig. 1C) | Peptide sequence | Protein |
|---------------------------|------------------|---------|
| 1                         | MHHLQFE          | L19 (position 1-7) |
| 2                         | TKGTLQIKGNTQIFAE| L3 as (position 1-17) |
| 3                         | GDIYDVTG1SKKGPGQA1KR . .  | L3 (position 104-166) |
| 4                         | QLRDLPDRFPGDTVRV . .  | L19 (12-34) |
| 5                         | NGDLIPVTVI (?) ATVPNVLQKKTIE | L3 (position 18-41) |

**Fig. 2.** Elution behavior of peptides resulting from S. aureus protease (Glu-C) digest II prior to and after periodate cleavage. 5 nmol of cross-linked protein pair L3-L19 were cleaved with Glu-C enzyme as described under “Experimental Procedures” and 1/10 of the whole mixture separated by RP-HPLC prior to (a) and after periodate cleavage (b), respectively. Only two peaks (A and B) were cleavable and resulted in peaks of altered retention times after periodate treatment (A1 and A2 are cleavage products of peak A; B1 and B2 result from cleavage of B). The assignment of the corresponding peptides was done by analyzing each single peak.

3220.2 daltons) could be confirmed (Fig. 4). These results strongly support that His-28 in protein L3 is cross-linked to one of the N-terminal amino acids Met-1, His-2, and His-3 of protein L19 (Fig. 5) and that an additional monovalent modification also exists in the N-terminal area of the L19 peptide. The results argue for a changing contribution of all three N-terminal amino acids to the cross-link formation.

At least analysis of all peptides resulting from Glu-C digest of L3-L19 by mass spectrometry revealed that no ε-amino groups of lysines have reacted with DEB since they are still protonated at pH 7.9. Only two sites, both located in the N-terminal region of the two proteins, displayed monovalent modification by DEB (Table II). One site could be identified as the N-terminal threonine of protein L3 due to blockage of the peptide. But parts of the corresponding peptide could be sequenced without exhibiting any modification at Thr-1 (see Table II). MS analysis revealed the occurrence of unmodified as well as monovalently modified peptide eluting in one single peak. Hence, only the unblocked portion was accessible to sequence analysis. The other site of DEB modification was located in the N-terminal part of L19 whereas at least two of the three N-terminal amino acids were shown to be monovalently modified. In both proteins no other site of monovalent modification, even of histidines, has been detected.

The cross-link L23-L29—3-5 nmol of L23-L29 were digested directly from blots with chymotrypsin and Glu-C enzyme, respectively. Sequence analysis of peptides resulting from Glu-C digest yielded all L23 peptides except the N-terminal fragment whereas L29 eluted as a bulk of undigested protein material at higher retention times. After periodate treatment of the whole mixture, the missing N-terminal fragment of L23 appeared in the HPLC chromatogram. Redigestion of the bulk material and sequencing of the resulting peptides revealed most peptides of protein L29, except the N-terminal and C-terminal fragments. As a result most peptides of L23-L29 could be detected as single peptides either by sequencing or by MS analysis (Fig. 6). Furthermore, both proteins were sequenced up to positions 33 (L29) and 34 (L23) in the cross-link. While lysine in position 4 of protein L29 could not be detected as PTH amino acid lysine of position 2 was found in protein L23 after the first coupling reaction instead of the N-terminal methionine. From these results we conclude that the N-terminal methionine of protein L23 is cross-linked to methionine in position 4 of L29. Based on the putative cross-linking site, we reanalyzed the masses obtained from chymotryptic peptides of L23-L29. In fact we were now able to confirm the cross-linking site by mass analysis of the cross-linked peptide which is calculated to be 1221.36 Da and is in accordance with chymotryptic cleavage sites (data not shown).

**Fig. 3.** Elution behavior of peptides resulting from S. aureus protease (Glu-C) digest I prior to and after periodate cleavage. 5 nmol of cross-linked protein pair L3-L19 were cleaved with Glu-C enzyme as described under “Experimental Procedures” and 1/10 of the whole mixture separated by RP-HPLC prior to (a) and after periodate cleavage (b), respectively. Only two peaks (A and B) were cleavable and resulted in peaks of altered retention times after periodate treatment (A1 and A2 are cleavage products of peak A; B1 and B2 result from cleavage of B). The assignment of the corresponding peptides was done by analyzing each single peak.

**DISCUSSION**

In order to obtain more precise data about the structural organization of the ribosome, we have made cross-linking experiments within the 50 S subunit of B. stearothermophilus ribosomes and identified the cross-linked amino acids in the two protein pairs L3-L19 and L23-L29. We chose diepoxy-
FIG. 3. Plasma desorption mass spectrum of the L19 peptide (eluting as peak B1 in Fig. 2b) resulting from periodate cleavage. Sample preparation and MS measurements have been described under "Experimental Procedures."

FIG. 4. Plasma desorption mass spectrum of the L3-peptide (eluting as peak B2 in Fig. 2b) after periodate cleavage.

L3-TKGLGRKIGMTQIFAIENGDLIPVTLHAVTNVVLQKKTIE(NDGYE)NGDLIPVTLHAVTNVVLQKKTIE

FIG. 5. Cross-linking site in the protein pair L3-L19. Presented are the two complete amino acid sequences of proteins L3 (Herwig et al., 1992) and L19 of B. stearothermophilus (this publication) and the cross-linked peptide sequences as obtained by S. aureus protease digest of the intact protein pair. Both peptides are linked by the butanediol group.

L3-GluC

18

NGDLIPVTLHAVTNVVLQKKTIE

| CH₂ | CH-OH | CH-OH | CH₂ | MHLIQE

L19-GluC

MHLIQE

L19-MHLIQEITKEQLRTDLPDFPGDTPVRH/VKVVVGNGRERIQVFEGVVKRRAGSETFTVRKVSYGVGVertFPVHTPKIAKLEIRGGVRRGKVRARKLYLLRELRGKAARIEKTAQ
TABLE II

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| Cross-linking reagents spanning distances between 4 and 12 Å, respectively (Trout et al., 1980; Walczek et al., 1989). The close location of both proteins was confirmed earlier by immunoelectron microscopy in combination with cross-linking data (Walczek et al., 1988) and positions both proteins in the seat region of the "crown projection" slightly displaced from the central point of the 50 S particle toward the L7/L12 stalk. These results are in agreement with RNA-protein binding- (Leffers et al., 1988) and cross-linking studies (Brimacombe et al., 1990) in which the L3-binding site lies close to the location of the 3'-end of the 23 S RNA. Recently, 50 protein distances have been determined by neutron scattering applying the strategy of the "glassy ribosome" (May et al., 1992). The protein distances between the corresponding mass centers was determined to be 74 Å for L3 and L19, which seems to be fairly high compared to our cross-linking data.

Cross-linking of L23-L29 has also been demonstrated in E. coli after treatment with the almost equally short cross-linking reagent o-phenylenediamine (5 Å) while no cross-link formation has been observed with DEB (Walczek et al., 1989). Interestingly, the same proteins could also be cross-linked in H. marismortui using DEB and di-thiobis(succinimidylpropionate) (12 Å) as cross-linking reagents (Bergmann, 1991). The same cross-linking site as for B. stearothermophilus could be identified in protein L23. These results display the close vicinity of proteins L23 and L29 in the different organisms and support the placement of protein L23 near the base of the 50 S subunit on the side of the L1 protuberance (Hackl and Stöffler, 1988). This result is in accordance with RNA-protein cross-linking data in which E. coli ribosomal proteins L23 and L29 are positioned to adjacent sites in the 23 S RNA (Wower et al., 1981; Brimacombe et al., 1990). Nevertheless, reconstitution experiments of E. coli ribosomal subunits containing puromycin-modified L23 also argue for the positioning of L23 close to the peptidyl-transferase center (Weitzmann and Cooperman, 1990). An elongated shape of L23 may therefore be assumed.

The combination of sequencing and MS analysis proved to be necessary because the digests of L3-L19 resulted in variable amounts of overlapping peptides. Glu-Arg and Glu-Lys bonds especially were relatively stable against Glu-C digest. In addition the high specificity of DEB was confirmed since the only monovalent modification occurred in the N-terminal part of both proteins. Taking into account that the C-terminal parts of proteins L3 and L19, which are predominantly basic, are rather involved in 23 S RNA binding the N-terminal part may be more accessible for the reagent.

As also deduced from MS analysis most of the methionines were present in their oxidized form after cross-linking. This effect is increased after treatment of the peptides with periodate (Yamasaki et al., 1982) and may explain slight displacements of unmodified peptides after periodate cleavage in the HPLC chromatogram (see Fig. 1C). This could also be proved by MS analysis of periodate-treated peptides: a formylmethyl group remained bound to the amino acid residue.

Within the protein pair L23-L29, Met-1 in protein L23 and Lys-4 in protein L29 were identified as cross-linking sites using the same approach as for L3-L19 except that enzymatic digests were carried out in situ on PVDF membranes. When Glu-C was used for fragmentation only peptides of protein L23 appeared in the HPLC chromatogram while L29 eluted as intact protein. Digestion of protein L29 failed when attached to the membrane due to the protein's high hydrophobicity.

The putative cross-linking site in the N-terminal area of L23-L29 was confirmed by N-terminal sequencing of the intact cross-link. While Lys-4 in L29 has not been detected...
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FIG. 7. Sequencing scheme for BstL19. Peptides from different fragmentations are denoted by L (digest with Lys-C) and G (digest with Glu-C). Bars indicate peptides identified by amino acid analysis (data not shown) and arrows amino acid residues identified by N-terminal sequencing.

as PTH amino acid the sequence of L23 started with Lys instead of the N-terminal Met. Such an abnormal degradation due to cyclization in alkaline conditions and partial degradation of the succeeding amino acid residue has already been described (Chang, 1978). For ribosomal proteins S11, L33, L16, and the initiation factor IF-3, the direct formation of the thiohydantoin ring at the coupling reaction (in alkaline solution) has been observed by reaction of DABITC (or phenylisothiocyanate) with the N-terminal N-monomethylated amino acid. Hence, the first amino acid was cleaved off at the coupling in the first degradation step and extracted into the n-heptane/ethylacetate solution together with excess DABITC/phenylisothiocyanate and by-products. The completeness of the cleavage obviously depends on the following sequence. Most likely, the same mechanism may explain the loss of N-terminal methionine during coupling when alkylated by DEB reaction. This phenomena requires further investigation.

Despite our findings that DEB does not tend to react with ε-amino groups of lysines at pH 7.9 Lys-4 in protein L29 has been identified as a cross-linking site. In this case, however, a glutamic acid which is deprotonated at pH 7.9 (pK₄ 4.25) is located at position 5 and may attract a proton of the lysine ε-amino group which thereupon becomes more nucleophilic.
The results demonstrate that the structural organization of the ribosomes in different organisms has been conserved to quite an extent during evolution although the fine structures may slightly differ. It has also been shown that cross-linking is a useful means for structural elucidation of the ribosome. As deduced from our data the cross-linked amino acid residues in the corresponding protein pairs are not more than 4 Å apart.

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