been identified in *Bacillus stearothermophilus*, and the amino acids involved in cross-linking have been determined (Herwig, 1991). Therefore, we were interested whether the same amino acids were cross-linked in the archaebacterial protein-pair as in the prokaryotic complex. After a two-step purification procedure including ion exchange chromatography and RP-HPLC, HmaL23-HmaL29 was obtained in purified form. A cross-linked peptide was isolated after endoproteolytic digestion of the protein-pair and by RP-HPLC of the resulting fragments. Automatic sequence analysis of this cross-linked peptide resulted only in one sequence, namely the C-terminal peptide from HmaL29 with Lys-57 missing in the sequence. Obviously, PTH-Lys-57 could not be detected in the HPLC-trace after degradation due to its reaction with the cross-linking reagent. On the other hand, the corresponding cross-linked region in HmaL23 could only be determined by mass spectrometry of the cross-linked HmaL23-HmaL29 peptide. These results clearly indicate that Ser-1 has reacted via its α-amino-group with DEB and has been cross-linked to Lys-57 in HmaL29. Due to this reaction, the peptide of HmaL23 was not accessible to Edman degradation. Comparison with the cross-linking data from *B. stearothermophilus* shows that in both protein-pairs the N-terminal region of protein L23 is engaged in cross-linking with protein L29, while the reactive site in L29 is different in both types of ribosomes. This indicates that at least parts, but not the entire fine structure, of these domains on the ribosome are conserved. The identification of reactive amino acids in covalently cross-linked protein-pairs is another example how efficiently mass spectrometry complements protein sequence information and enables contributions to structural studies.

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Naoki Morita, Minoru Yamaguchi, and Kiyoshi Nokihara. Routine Determination of Cysteine Residues and Disulfide Form by a Gas-Phase Sequencer with Isocratic Separation of PTH-Amino Acids. (Department of Biotechnology Instruments, Shimadzu Corporation, Nishino-kyo Kuwabaracho 1, Nakagyo-ku Kyoto, 604 Japan)

Determination of cysteine and cystine in the disulfide form is still a difficult task in structure analysis of proteins and peptides. Derivatization of cysteine residues before sequencing has been routinely carried out to identify cysteine residues, but disulfide forms cannot be determined.

The protocol for *in situ* *S*-pyridylethylationof cysteine residues in the reaction chamber of a gas-phase sequencer, Shimadzu Model PSQ-1, has been established (Nokihara *et al.*, 1992). In this method, *S*-alkylation can be performed *in situ* without interruption (Kruft *et al.*, 1991) of sequencing, however, no significant Nα-pyridylethylation, which generates overlapping sequences, was observed in our protocol using the PSQ-1.

Furthermore, we have established routine detection of intact cystinyl residues. No amino acids of phenylthiohydantoin derivatives (PTH-AA) can be seen in the first half-cystine residue cycle; on the other hand, PTH of the second half-cystine appears as a cysteine and a dithiothreitol adduct of dehydroalanine (ΔSer). These are eluted at specific positions and are not superimposed with other PTH-AA or by-products under the isocratic separation mode of PSQ-1. With the use of a gas-phase sequencer, cystinyl residues as well as serine residues are relatively stable during Edman degradation.

Sequence analyses of both intact and *S*-pyridylethylated cysteine can identify a disulfide form if one disulfide linkage exists in the sample. By sequencing the digests with an appropriate proteinase or chemical cleavage, two disulfide linkages can also be determined.

Recently solid-phase peptide synthesis has become a quite popular technique. However, disulfide formation in cystine-containing peptides is still problematic. The present paper also describes sequence analysis of synthetic peptides containing various *S*-protected cysteine, which allows examination of the correct chain assembly before disulfide bond formation.

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Development of a reliable C-terminal sequencing method has been expected from various aspects including sequenc-