before and after the irradiation showed the increase in the absorption band around 3,300-2,500 cm^{-1} and 1,699 cm^{-1}, indicating the formation of carboxylic acid. The decrease of the absorption band at 1,780 cm^{-1} indicates the photodecomposition of a C-N bond in the imide group. The increase of absorption band at 1,577 cm^{-1} indicates the formation of primary aromatic amine by photodecomposition of the urethane bond. The increase of the absorption band at 670 cm^{-1} is due to the photosplitting of cyclobutane ring to form maleimide derivatives.

The results presented so far show that the PUIs containing cyclobutane rings in the main chain are somewhat thermally stable and soluble in aprotic organic solvents unlikely known polyimides. It can be developed in an alkali after photolysis with 254 nm light. Photodecomposition of imide and urethane bonds as well as photosplitting of cyclobutane rings are considered to be the major photodegradation process for these PUIs.

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Structure Elucidation of Methicillin-Resistant Peptidoglycan Monomer by Tandem Mass Spectrometry

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Antibiotics which interrupt peptidoglycan synthesis have been effective since the peptidoglycan heteropolymer surrounding the cytoplasmic membrane is unique to bacterial cell walls.[1] However, many *beta*-lactam antibiotics are no longer effective due to the acquired resistance.[2] This resistance has been known to be related to the altered structure of peptidoglycan, even though the functional relationship is not yet clear.[3-5] Peptidoglycan is composed of repeating units of 1,4-linked N-acetylglucosaminyl-N-acetylmuramic acid. From the latter monosaccharide residue is typically attached a short peptide chain that can vary from species to species; there can be significant heterogeneity in the peptide sequences and further, the peptide chains can be linked to one another directly.

The fragments of the individual peptidyl disaccharide residues can be obtained by treating the peptidoglycan with muramidase to hydrolyze the 1,4-glycosyl bond between the muramic acid and N-acetylglucosamine, followed by purification using HPLC.[6] In this way the individual muropeptide monomers, dimers, trimers and higher oligomers (*i.e.*, monomers connected *via* their peptide chains) have been isolated. The fine structures of bacterial cell wall are then studied by tandem mass spectrometry (MS/MS).[7] The role of tandem mass spectrometry here is to provide precise determinations of molecular size and also sequence on the same ultrafine scale of resolution.[8] An important aspect of a high performance tandem mass spectrometer is the ability to select a single mass from MS 1 for collisionally induced dissociation. The resulting sequencing-characteristic fragment ions are then mass analyzed by scanning MS 2.

Previously, we have reported our mass spectral results on several highly purified peptidoglycans isolated from various bacteria strains.[9-13] Analysis by MS/MS yielded sufficient information to provide the complete structure of monomers. Assignments for some of the peaks were confirmed by amino acid analysis. Inferences based on the monomer structures and molecular weights allowed most of the dimers and higher oligomers to be tentatively identified. The question addressed was to determine the nature of the peptidoglycan modification in reduced resistance transposon mutants of a methicillin resistant strain of the bacterium *Staphylococcus aureus*, a major pathogen in hospital-born infections. We describe, in this paper, the complete structure determination of peptidoglycan monomer of a new strain, Tn551 mutant of *Staphylococcus aureus* (RUSA208) selected for reduced methicillin resistance by tandem mass spectrometry.

Peptidoglycan of RUSA208 was isolated and enzymatically

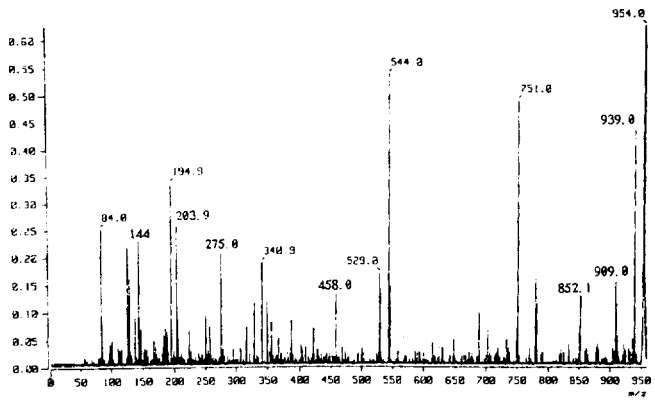

Figure 1. The FAB tandem mass spectrum of the $(M+H)^+$ ion of G-M-Ala-Gln-Lys-Gly-Ala, m/z 954.

hydrolyzed, and the muropeptides were separated by HPLC as described earlier.[14] The monomeric muropeptide was identified by HPLC retention time and secondary lysostaphin digestion, and further confirmed by molecular weight determination. Amino acid analysis was performed to determine the type of amino acids present and their molar ratios. The results show that a glycine, a glutamine, a lysine and two alanines are present. Our concern then is to determine the sequence of the amino acids in this monomeric muropeptide. Note that since this peptide is N-terminally blocked and the sample size is very limited, the conventional techniques such as Edman sequencing or NMR could not be used for fine structural determination. Nevertheless, as mentioned previously, once the structure of monomer is identified, it is much easier to assign structure of their higher oligomers, simply by comparing their molecular masses.

Tandem mass spectrometry was performed for the protonated molecular ions and the MS/MS spectrum shows abundant ions at m/z 751, 544 and 204 which are characteristic of the loss of carbohydrate moieties (Figure 1). Unlike the disaccharide portion, the majority of the fragment ions associated with the cleavage of peptide portion in the MS/MS mode are those which retain charge on the COOH terminus, *i.e.* m/z 529, 458, 275, 144, and etc. Some N-terminal fragment ions, for example at m/z 852 and 909, are also observed in the MS/MS spectrum. Among them, however, the most important ions are of m/z 458 and 852 which correspond to the cleavage of NH-CHR bonds. This information allows us to distinguish the locations of alanine and glycine. Consequently, it clearly confirms the monomeric sequence as G-M-**Ala**-Gln-Lys-**Gly**-Ala, not as G-M-**Gly**-Gln-Lys-**Ala**-Ala.

Further evidence for this structure is the absence of the corresponding ions of m/z 472 and 838 which, otherwise, should be observed in the MS/MS spectrum if the sequence was G-M-Gly-Gln-Lys-Ala-Ala. Note that two possible sequences could have same molecular masses (954 Da) and amino acid composition. A summary of the sequence ions observed in the MS/MS spectrum and their related structural components are shown in Figure 2. The elemental composition of peptidoglycan monomer also could be determined by exact mass measurement with the high resolution mass spectrometer as a further confirmation of its structure.

In conclusion, molecular weight determination and amino

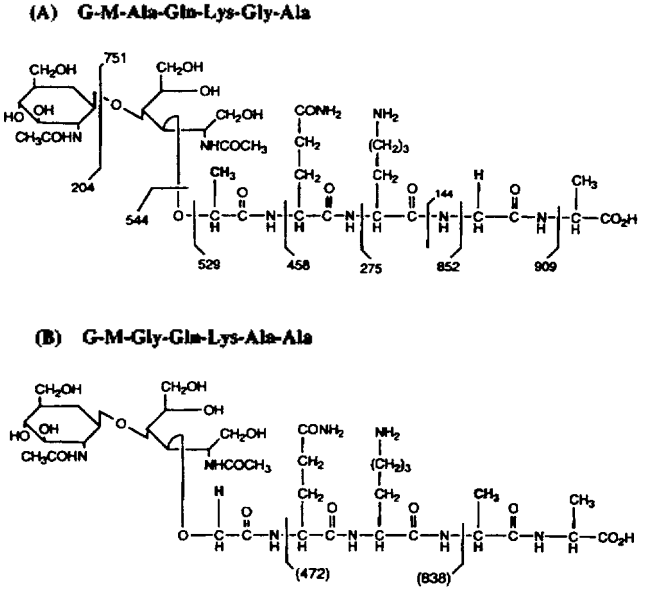

Figure 2. The structures of (A) G-M-Ala-Gln-Lys-Gly-Ala and (B) G-M-Gly-Gln-Lys-Ala-Ala, indicating fragmentation exhibited in the spectrum shown in Figure 1.

acid analysis of HPLC purified monomer yield some structural information. However, analysis by tandem mass spectrometry provides unambiguous primary sequence data for the peptidoglycan monomer and defines the positions of glycine and alanine. Nevertheless, the functional relationship between the modified peptidoglycan composition and the reduced methicillin resistance is not yet clear. In the future, analysis of more peptidoglycans of the heterogeneous transposon mutants will be performed to gain more insight in this phenomenon.

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14. The muropeptides were isolated and purified in the Rockefeller University, Microbiology Lab. (New York, USA). Briefly, bacteria were sonicated and crude cell wall was precipitated in boiling 10% sodium dodecyl sulfate. The peptidoglycan-associated proteins were removed with α-amylase and Pronase and the peptidoglycan was reprecipitated with *Streptomyces globisporus muramidase*. The material was reduced with sodium borohydride at conditions known to preserve the O-acetyl groups, if present. HPLC analysis was carried out at room temperature under the established conditions in a 50 mM sodium phosphate buffer in water and a linear gradient from 0 to 16% methanol (v/v). Percent cross-linkage and chain length were calculated using established formulas from the relative abundance of each glycopeptide. For the final comparative analysis, the relative abundance of each peak was corrected for the number of peptide bonds as described. For the mass analysis, individual glycopeptides were desalted by HPLC using a gradient from 0 to 50% acetonitrile in 0.05% trifluoroacetic acid/water.
15. The FAB-MS and MS/MS experiments were carried out on a JEOL HX110A/HX110A high resolution tandem mass spectrometer at the Korea Basic Science Center. The accelerating voltage was 10 kV with a mass resolution of 1 : 1000 (10% valley). The JEOL Cs^+ ion gun was operated at 25 kV. The collision cell potential was held at 3.0 kV and the ion collision energies were 7.0 kV. Helium collision gas was introduced into the collision chamber at a pressure sufficient to reduce the precursor ion signal by 50%. The sample was dissolved in water/acetonitrile (1 : 1) solution and a volume of 1 μL of this solution (100 pmol) was mixed with 1 μL of glycerol matrix prior to the mass spectral analysis.