*Proc. Natl. Acad. Sci. USA*
Vol. 87, pp. 5415–5419, July 1990
Microbiology

# A biological price of antibiotic resistance: Major changes in the peptidoglycan structure of penicillin-resistant pneumococci

(*Streptococcus pneumoniae*/penicillin binding proteins of resistant pneumococci/peptidoglycan stem peptides in pneumococci)

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*Communicated by Maclyn McCarty, May 4, 1990*

**ABSTRACT** **Pneumococcal strains with greatly elevated levels of resistance to penicillin have by now been described with increasing frequency worldwide. The mechanism of antibiotic resistance in these strains involves the molecular remodeling of cell wall synthetic enzymes (penicillin binding proteins). We have now analyzed the peptidoglycan structures of 10 penicillin-susceptible and 10 penicillin-resistant clinical isolates (4 of intermediate and 6 of high level resistance) with a high-resolution HPLC technique. Cell wall peptidoglycan of the susceptible strains contained monomeric and oligomeric forms of primarily (70% or more) linear stem peptides with the sequence of L-Ala-D-iGln-L-Lys-D-Ala (where iGln is isoglutamine). In contrast, the major peptide species (70% or more) of resistant cell walls were abnormal branched-stem peptides carrying Ala-Ser or Ala-Ala dipeptides on the ε-amino groups of the stem peptide lysine residues. The structural alteration in the peptidoglycan was not related to serotype, date, or site of isolation but showed strong correlation with penicillin resistance and was cotransformed with high-level penicillin resistance during genetic transformation. We suggest that the remodeling of the active site of penicillin binding proteins in the resistant bacteria, which results in the reduced affinity for penicillin, also changes the substrate preference of these enzymes for the more hydrophobic branched peptides (instead of linear peptides) for cell wall synthesis.**

Pneumococcal strains with greatly increased resistance to penicillin have been detected among clinical isolates in many parts of the world including South Africa, Australia, the United States, and Europe (1). The trait of resistance is stable and chromosomal. It is compatible with virulence, and resistant strains have been isolated from the nasopharyngeal flora of healthy carriers (2). Biochemical (3–5) and genetic studies (6, 7) have shown that resistance to penicillin involved alteration in the structural genes of several of the pneumococcal penicillin binding proteins (PBPs), leading to a remodeling of the active sites of these enzymes in such a way that their reactivity with penicillin was greatly decreased. PBPs are enzymes (transpeptidases or carboxypeptidases) that perform vital functions in the assembly of bacterial cell walls. The exquisite sensitivity of PBPs to penicillin is supposed to be related to the structural similarity between the β-lactam ring of penicillin and the carboxyl-terminal D-alanyl-D-alanine residues in the natural substrate(s) of these enzymes. It was of interest, therefore, to determine if the greatly decreased reactivity of PBPs in the resistant mutants with the substrate analog (penicillin) may not also be associated with altered reactivity of these proteins with their natural substrates. We have now compared the cell wall structure of a number of penicillin-susceptible and -resistant pneumococcal strains by using a method that is based on high performance liquid chromatography (HPLC) and mass spectrometry and allows a high degree of resolution of the peptide components of the pneumococcal cell wall (8). The 20 pneumococcal clinical isolates examined range in penicillin sensitivities from susceptible to highly resistant [minimal inhibitory concentration (MIC) of benzylpenicillin, 0.006 to 17.0 μg/ml]. Wall stem peptides of, resistant strains had strikingly different chemical structures from those found in susceptible strains.

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## MATERIALS AND METHODS

**Strains and Growth Conditions.** The wild-type strains used and their relevant properties are listed in Table 1. Laboratory strain R6 is a derivative of the unencapsulated strain R36A. Its penicillin-resistant transformant, Pen 4.0, was obtained by genetic transformation by using wild-type strain D20 as DNA donor according to published procedures (3). All strains were grown at 37°C in a casein-based semisynthetic medium (14). To introduce a radioactive label into the peptidoglycan, a small culture of each strain was grown in a chemically defined medium, Cden (15), modified by reducing the concentration of lysine to 10 μg/ml; and L-[4,5-$^3$H(N)]lysine was added to a final concentration of 5 μCi/ml (1 Ci = 37 GBq).

**Cell Wall Peptide Preparations.** The cell walls of bacteria from exponentially growing cultures were purified as described (8). All the preparations were analyzed for amino acids to ascertain the absence of protein peptides.

The peptidoglycan peptides were separated from the polysaccharide wall structures by digestion with pneumococcal amidase, and they were then extracted with organic solvents as described (16). Amidase digestion resulted in a quantitative (95–98%) release of stem peptides. After removal of the solvents by evaporation, the dry peptides were taken up in 0.1% trifluoroacetic acid and analyzed by reverse-phase HPLC with the conditions we have published (8). Briefly, the samples were separated in an octadecyl silica column (Vydac 218TP54) by using as eluent a linear gradient from 0 to 15% (vol/vol) acetonitrile in 0.1% trifluoroacetic acid at 0.5 ml/min for 80 min. The peptides were detected and quantified by their absorbance at 215 nm.

**Amino Acid Analysis.** Samples (1–5 nmol) were hydrolyzed with 200 μl of 6 M HCl at 110°C for 18 hr and analyzed in a Beckman system 6300 autoanalyzer.

**Mass Spectrometry.** Peptides (1–2 nmol) were applied in 0.1% trifluoroacetic acid to a thin layer of nitrocellulose electrosprayed onto an aluminized polyester support. After thorough drying of the sample at low pressure, its positive-ion mass spectrum was obtained by using a $^{252}$Cf fission fragment ionization/time-of-flight mass spectrometer as described (8).

Abbreviations: PBP, penicillin binding protein; MIC, minimal inhibitory concentration; iGln, isoglutamine.

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Table 1. Relevant properties of the wild-type pneumococcal strains used in this study

| Strain | Serotype | Isolation | | Pen MIC, μg/ml | Degree of crosslinkage, % | Source or ref. |
|---|---|---|---|---|---|---|
| | | Year | Country | | | |
| SP108 | 15B | 1939 | USA | 0.003 | 27 | CDC, Atlanta, GA |
| D39S | 2 | 1916 | USA | 0.01 | 35 | 9 |
| SIII | 3 | 1949 | USA | 0.01 | 34 | 9 |
| Br | 19 | 1985 | USA | 0.01 | 31 | 10 |
| SA23 | 6 | 1985 | South Africa | 0.01 | 35 | 11 |
| A101 | 23F | 1984 | USA | 0.02 | 32 | CDC, Anchorage, AK |
| SP96 | 10A | 1938 | USA | 0.03 | 34 | CDC, Atlanta, GA |
| MD10789 | 2 | 1985 | Spain | 0.03 | 29 | 12 |
| 34034 | 4 | 1984 | South Africa | 0.04 | 30 | 11 |
| A661 | 6B | 1985 | USA | 0.08 | 27 | CDC, Anchorage, AK |
| A112 | 6A | 1982 | USA | 0.12 | 30 | CDC, Anchorage, AK |
| F108 | 19 | 1986 | USA | 0.20 | 34 | 13 |
| A89 | 4 | 1985 | USA | 0.25 | 35 | CDC, Anchorage, AK |
| MD5229 | 14 | 1985 | Spain | 2.50 | 32 | 12 |
| 10760 | 6A | 1979 | South Africa | 4.00 | 24 | 11 |
| 140 | 19A | 1977 | South Africa | 4.00 | 27 | 3 |
| CO7 | 19A | 1977 | South Africa | 4.00 | 24 | 3 |
| 8249 | 19A | 1977 | South Africa | 6.00 | 26 | 3 |
| D20 | 19A | 1977 | South Africa | 12.0 | 28 | 3 |
| 29044 | 14 | ? | Czechoslovakia | 17.0 | 30 | CDC, Atlanta, GA |

Degree of crosslinkage was estimated by dividing one-half of the area under the dimers with the combined area of monomers plus dimers. This method underestimates the total crosslinkage (especially in the resistant strains) because trimers and higher oligomers are excluded. On the other hand, the method does allow a comparison between strains. Pen, penicillin; PG, peptidoglycan; CDC, Centers for Disease Control.

## RESULTS

Fig. 1*a* shows the HPLC profiles of the wall peptides from four representative penicillin-susceptible and four penicillin-resistant strains. The peptide patterns of resistant cells showed a dramatic shift toward the more hydrophobic region on the HPLC profile. Peaks numbered with roman or arabic numerals in Fig. 1 have been isolated and purified, and their

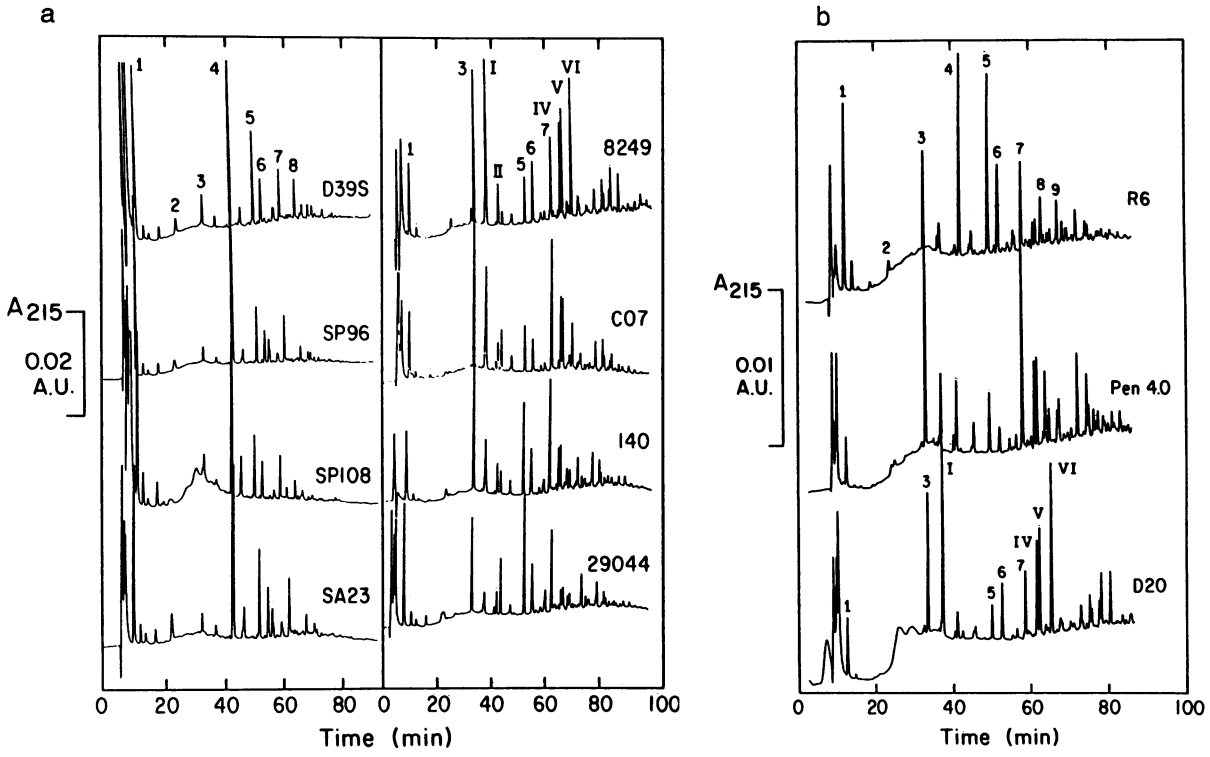


FIG. 1. Cell wall peptides from penicillin-susceptible and -resistant pneumococci. Approximately 20 nmol (*a*) or 10 nmol (*b*) of peptide material was analyzed by HPLC. The numbered peaks correspond to peptides that have been described structurally (6, 8). Peptides in peaks 1–3 and I–III are monomeric (made up of a single-stem peptide), those in peaks 4–7 and IV–VI are dimeric, and peaks 8 and 9 correspond to trimers. Peptides 5, 6 and 8 have one branched-stem peptide and peptide 9 has two. The peptides with roman numerals are composed exclusively of branched-stem peptides. The denomination of each strain illustrated is shown at the end of each chromatogram. For the structures of peptides, see Fig. 2. (*a*) Representative peptide patterns from wild-type pneumococci. The penicillin-susceptible strains are grouped on the left and the resistant ones are on the right. (*b*) Peptide patterns from a penicillin-resistant transformant (Pen 4.0), its parental strain (R6), and the DNA donor strain (D20). A.U., arbitrary unit(s).

chemical structures were determined by amino acid analysis, mass spectrometry, and sequencing (8, 16), and peaks in various strains were identified by cochromatography with a standard cell-wall peptide preparation (Fig. 2). In addition, the major peptide dimers, peak 4 of the susceptible bacteria and peak 7 of the resistant bacteria, were pooled and their identity was confirmed by amino acid analysis and mass spectrometry (Table 2).

The cell-wall peptide patterns obtained from susceptible strains were very similar; they contained primarily linear stem peptides, with the sequence L-Ala-D-iGln-L-Lys-D-Ala (where iGln is isoglutamine). By contrast, the patterns of wall peptides from resistant strains were more diverse, but they shared the common characteristics that the predominant molecular species of these walls were branched-stem peptides carrying Ala-Ser or Ala-Ala dipeptides on the $\varepsilon$-amino group of the stem peptide lysine residues.

Structural differences of the cell wall were not related to serotype, geographic site, or date of isolation, since penicillin-susceptible strains isolated in 1916 in the United States (D39S, serotype 2) or in 1985 in South Africa (SA23, serogroup 6) showed remarkably similar patterns of wall peptides (Table 3).

On the other hand, the altered wall peptide composition was clearly related to the penicillin resistance of the strains.

Transforming DNA isolated from the South African strain D20 (MIC = 12 $\mu$g/ml) was used to introduce penicillin resistance (and the altered PBP pattern of the DNA donor) into the penicillin-susceptible laboratory strain R6 (MIC = 0.006 $\mu$g/ml) by a series of successive genetic transformations (3). Cell walls were purified from a transformant that had an MIC of 4.0 $\mu$g/ml and a PBP pattern resembling that of strain D20. Cell walls were also purified from the recipient and DNA donor strains and the cell wall peptide patterns of the three strains were compared (Fig. 1*b*). Although the pattern of peptides in the transformant was not identical with that of the donor of the DNA, the predominance of branched-stem peptides is quite evident. The particular transformant analyzed had an MIC value (4.0 $\mu$g/ml) that was lower than that of the DNA donor strain D20 (MIC = 12 $\mu$g/ml). When the peptidoglycan structure of this transformant was compared to the wall peptide patterns of strains with comparable levels of resistance (e.g., strains CO7 or 140 in Fig. 1*a*), the similarity was even more pronounced. Penicillin resistance and a different peptidoglycan structure, therefore, were cotransformed, as demonstrated for a different genetic cross (8).

Table 2. Analysis of pooled peaks 4 and 7

| Peak | Residue, mol/mol of Glx | | | | $M_r$ |
|---|---|---|---|---|---|
| | Ala | Glx | Lys | Ser | |
| 4 | 1.6 | 1.0 | 1.3 | 0.0 | 746.3 |
| 7 | 2.0 | 1.0 | 1.0 | 0.3 | 1059.8 |

Peaks 4 and 7 were pooled from chromatographies like those illustrated in Fig. 1 and analyzed. Molecular weights were obtained by time-of-flight mass spectrometry as described (8).

A detailed quantification of the stem peptide composition of the walls of resistant and susceptible strains is shown in Table 3. A virtual inversion in the ratio of linear to branched peptides is evident when comparing the peptidoglycans of resistant to susceptible strains. A particularly clear illustration of this finding may be seen in peptides 1, 3, and 4. In susceptible cells, 18–30% of the stem peptides were represented by the monomeric tripeptide L-Ala-D-iGln-L-Lys (peptide 1); the concentration of this peptide dropped to 10% or less in resistant bacteria. Peptide 3, the monomeric tripeptide L-Ala-D-iGln-L-Lys, carrying the Ala-Ala dipeptide branch

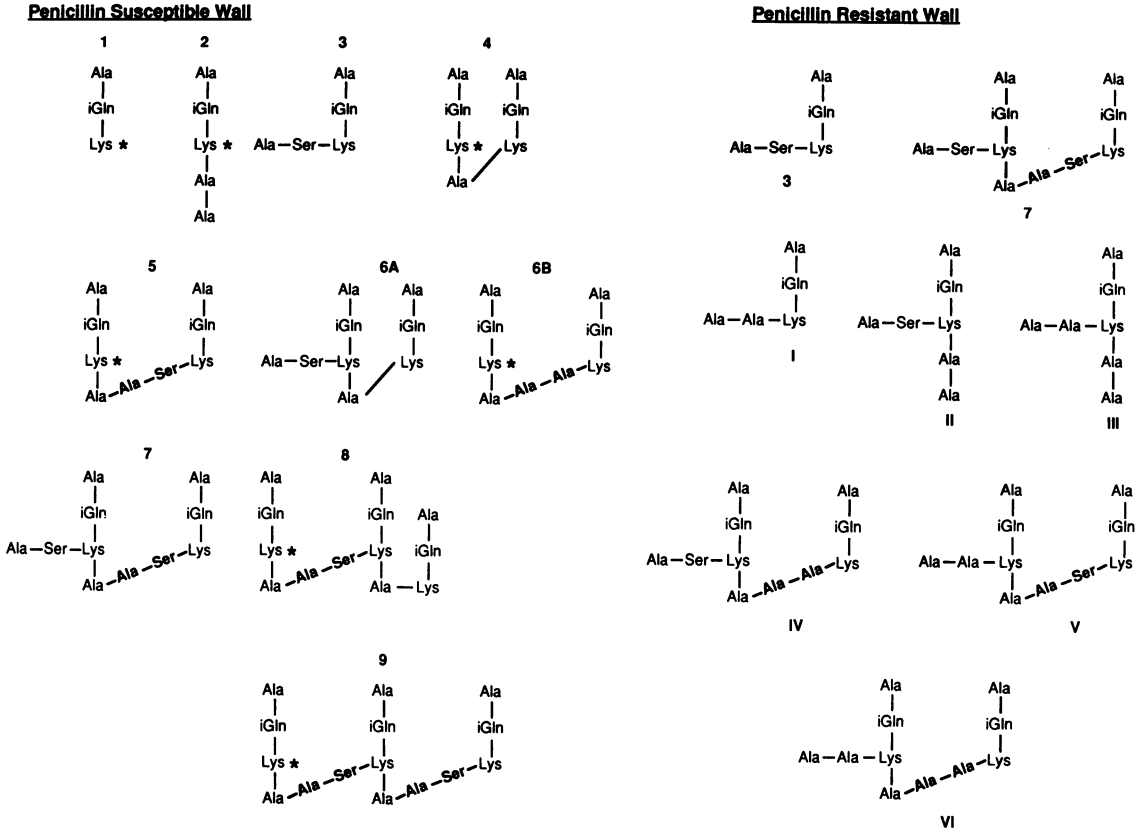


FIG. 2. Structures of the peptidoglycan stem peptides isolated from pneumococcal cell walls. Arabic and roman numerals identify the peptides in the HPLC elution profile in Fig. 1 and in Table 1. The alanylserine and alanylalanine substituents are attached to the $\varepsilon$-amino group of lysine. Free (unsubstituted) lysine residues are marked with an asterisk.

Table 3. Main peptide species in peptidoglycan from penicillin-susceptible and penicillin-resistant pneumococci

| Strain | Penicillin phenotype | Peptide material, % | | | | | | | | | | | | | | | Recovery, % | Peptides, % | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | I | II | III | IV | V | VI | | Linear | Branched |
| D39S | S | 20.5 | 1.9 | 4.1 | 37.6 | 12.3 | 7.3 | 6.4 | 4.9 | 1.5 | 0.9 | 2.4 | — | — | — | — | 79 | 73 | 27 |
| SP96 | S | 27.5 | — | 1.5 | 49.0 | 7.1 | 5.2 | 6.2 | 2.3 | — | — | — | 1.2 | — | — | — | 94 | 84 | 16 |
| SP108 | S | 33.8 | — | 0.9 | 40.6 | 9.5 | 4.9 | 7.5 | 3.0 | — | — | — | — | — | — | — | 90 | 83 | 17 |
| SA23 | S | 24.1 | 4.5 | 1.6 | 45.6 | 7.9 | 5.0 | 6.0 | 2.1 | — | 1.0 | — | 2.3 | — | — | — | 84 | 82 | 18 |
| 8249 | R | 7.0 | — | 14.4 | — | 4.2 | 6.4 | 9.1 | 14.8 | — | 20.6 | 2.6 | — | 9.3 | 11.6 | — | 74 | 20 | 80 |
| CO7 | R | 7.6 | — | 25.5 | 3.8 | 4.7 | 4.1 | 15.5 | — | — | 16.7 | — | — | 7.9 | 8.3 | 5.8 | 76 | 16 | 84 |
| 140 | R | 10.5 | 2.2 | 18.2 | 2.1 | 11.8 | 7.1 | 14.5 | 1.7 | 4.3 | 9.0 | 3.4 | 1.7 | 5.5 | 5.9 | 2.2 | 76 | 27 | 73 |
| 10760 | R | 9.7 | — | 12.0 | — | 3.1 | 4.9 | 6.9 | — | — | 22.2 | 3.9 | 2.4 | 7.9 | 8.8 | 11.7 | 87 | 14 | 86 |
| R6 | Susceptible recipient | 19.1 | 2.4 | 8.1 | 19.4 | 17.4 | 12.0 | 8.1 | 4.3 | 4.2 | 1.8 | — | — | 0.3 | 2.0 | — | 93 | 60 | 40 |
| Pen 4.0 | Resistant transformant | 4.4 | — | 19.5 | — | 5.4 | 3.4 | 20.1 | 7.2 | 4.2 | 6.8 | 7.1 | 2.1 | 8.6 | 8.2 | 3.0 | 71 | 14 | 86 |
| D20 | Resistant DNA donor | 6.4 | — | 13.3 | 0.8 | 3.4 | 6.1 | 5.6 | — | — | 22.6 | 2.6 | — | 9.6 | 11.1 | 18.4 | 79 | 12 | 88 |

Percent of peptide material, as determined by absorbance at 215 nm, present in each of the major peptide species (peptides I–VI) in cell wall peptidoglycan is shown. Peptide numbering corresponds to that in Fig. 1. Arabic numbers indicate peptides typically found in penicillin-susceptible cells. Roman numerals represent peptides more abundant in the walls of resistant strains. The amounts of peptides were quantitated by their absorbance at 215 nm and the numbers represent the relative amounts of individual peptides expressed as percent of the sum of all the peptides identified. S, Susceptible; R, resistant. The total peptide material (i.e., sum of absorbance at 215 nm) recovered in the identified peptides numbers 1–9 and I–VI is shown. Linear peptides and branched-stem peptides carrying Ala-Ala or Ala-Ser branches on the ε-amino group of the lysine residues are shown as percent of total peptides.

on the ε-amino group of the lysine residue, was a minor component in susceptible cells (1–4%), but it became a major peptide (12–26%) in resistant bacteria. Peptide 4, the directly crosslinked tritetrapeptide dimer, which was a major peptide species (38–49% of total peptides) in susceptible walls, dropped to 4% or less (not detectable) in the peptidoglycan of resistant cells. The compositional differences were so large that, classified on the basis of crosslinking mode (17), highly penicillin-resistant and -susceptible pneumococci would belong to different taxonomic groups. For instance, in Table 1, the percentage of dimers crosslinked directly was more than 50% in strains 1–12 (crosslinking mode A1 α, ref. 17). In the highly resistant isolates 13–20, more than 50% of the crosslinks in the peptide dimer occurred indirectly through dipeptide bridges (A3 α). Yet, in the two types of cell walls, the average degree of crosslinking was practically the same.

When the abundance of branched-stem peptides in all the peptide species was plotted against the degree of penicillin resistance (expressed as the MIC of penicillin), the data tended to fall into two groups (Fig. 3*A*). At an MIC of less than 0.25 μg/ml, all strains had peptidoglycan in which less than 30% of the stem peptides were branched. In strains with an MIC of greater than 4.0 μg/ml, the peptidoglycan was composed predominantly of branched-stem peptides (greater than 70%). Strains 14 and 20 occupied intermediate positions between these two groups.

When the percentage of indirectly crosslinked dimers (dimers crosslinked through dipeptide branches) was plotted as a function of the MIC, the proportion of indirect crosslinks increased gradually in parallel with the increasing MICs of penicillin (Fig. 3*B*). This plot clearly indicates that cell wall synthetic enzymes (transpeptidases) in resistant pneumococci prefer to use branched peptides for crosslinking of the peptidoglycan.

## DISCUSSION

All the penicillin-resistant pneumococci studied here (both the intermediate and the highly resistant bacteria) had abnormal (low affinity) PBPs, while the susceptible isolates had the common PBP pattern characteristic of the penicillin-susceptible laboratory strain R36A of *Streptococcus pneumoniae* (18). The findings described suggest that the decreased affinity for penicillin of PBPs in penicillin-resistant strains of pneumococci also alters the reactivity of these proteins with their natural substrates. Recent biochemical

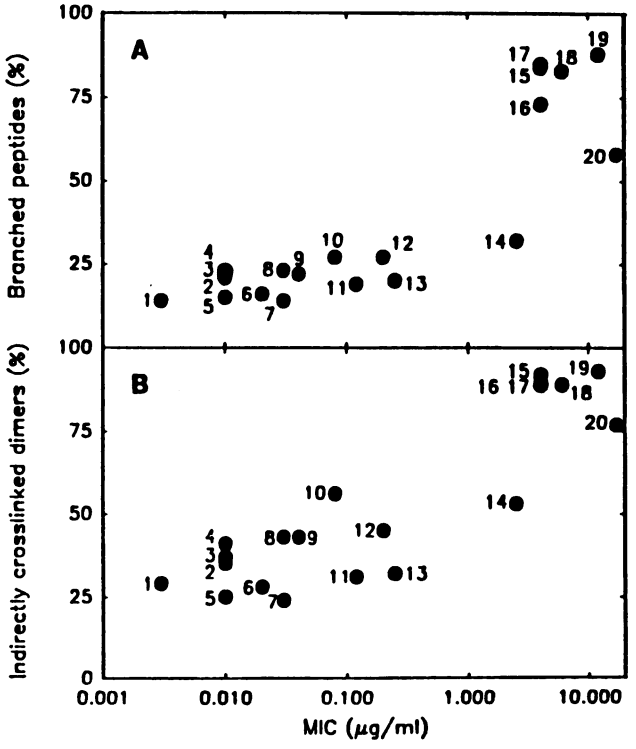


FIG. 3. Relationship between penicillin MIC and peptidoglycan structure in wild-type pneumococci. The areas of the peaks corresponding to all the major peptide species from penicillin-susceptible and -resistant cell walls (peaks with arabic or roman numerals in Fig. 1) were quantified. (*A*) The fraction (%) of stem peptides carrying dipeptide branches on the lysine ε-amino group was plotted against the MIC values of the strains. (*B*) The amounts of dimers crosslinked through dipeptide bridges (i.e., peptide dimers in which the acceptor stem peptide was branched) are expressed as the percentage of all dimers and are plotted against the MIC value of the strains. Numbers next to the data points correspond to the strain numbers in Table 1. Note that the scale of the *x* axis is logarithmic.

and genetic studies have shown that, in at least some of the PBPs, resistance-related structural alteration occurred close to the β-lactam binding site (5, 6). We suggest that these alterations also affect the catalytic efficiency of these proteins toward their normal substrates, the linear disaccharide peptide wall precursors, since PBPs of resistant strains appear to prefer the use of the more hydrophobic branched peptides as substrates for cell wall synthesis. We do not know anything about the mechanism of this preference. Possibly, wall precursors containing linear and branched peptides compete for binding to the PBPs and, in the resistant cells, wall precursors with the less preferred (linear) peptides may be "recycled" back to the cytoplasmic face of the membrane where they may become available for dipeptide addition. Although penicillin-susceptible strains are clearly capable of producing branched peptides, adequate supply of these precursors in resistant strains may require additional mutation(s) in the early steps of wall synthesis. Genetic studies (6, 7) suggest that the structural determinant of at least one of the low-affinity PBPs (PBP-2B) in resistant pneumococci contains "foreign" DNA, and it is conceivable that the altered substrate preference of the PBPs is related to this.

The observations described here are in full agreement with and also extend the basic tenets of the Tipper–Strominger model for the molecular mechanism of penicillin action, in which the β-lactam antibiotic is considered to be a suicide substrate: an acylating agent directed at the active site of cell wall synthetic enzymes (19). Apparently, the particular mechanism of resistance that involves remodeling of the active site of these enzymes also exacts a biological price from the bacteria, in forcing them to remodel their cell wall structure as well and to produce a peptidoglycan of altered chemical structure.

We acknowledge the excellent technical assistance of M. Chung and the help of T. Chaudhary with the mass spectrometer and M. Pospischil with the amino acid analyzer. These investigations have been supported by a grant from the U.S. Public Health Service.

1. Klugman, K. P. (1990) *Clin. Microbiol. Rev.* **3,** 171–196.
2. Jacobs, M. R., Koornhof, H. J., Robins-Browne, R. M., Stevenson, C. M., Vermaak, Z. A., Freiman, I., Miller, G. B., Witcomb, M. A., Isaacson, M., Ward, J. I. & Austrian, R. (1978) *N. Engl. J. Med.* **299,** 735–740.
3. Zighelboim, S. & Tomasz, A. (1980) *Antimicrob. Agents Chemother.* **17,** 434–442.
4. Handwerger, S. & Tomasz, A. (1986) *Antimicrob. Agents Chemother.* **30,** 57–63.
5. Hakenbeck, R., Ellerbrok, H., Briese, T., Handwerger, S. & Tomasz, A. (1986) *Antimicrob. Agents Chemother.* **30,** 553–558.
6. Dowson, C. G., Hutchison, A. & Spratt, B. G. (1989) *Mol. Microbiol.* **3,** 95–102.
7. Dowson, C. G., Hutchison, A., Brannigan, J. A., George, R. C., Hansman, D., Linares, J., Tomasz, A., Smith, J. M. & Spratt, B. G. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 8842–8846.
8. Garcia-Bustos, J. F., Chait, B. C. & Tomasz, A. (1987) *J. Biol. Chem.* **262,** 15400–15405.
9. Ravin, A. W. (1959) *J. Bacteriol.* **77,** 296–309.
10. Tuomanen, E., Pollack, H., Parkinson, A., Davidson, M., Facklam, R., Rich, R. & Zak, O. (1988) *J. Infect. Dis.* **158,** 36–43.
11. Klugman, K. P. & Koornhof, H. J. (1988) *J. Infect. Dis.* **158,** 956–964.
12. Latorre, C., Juncosa, T. & Sanfeliu, I. (1985) *Antimicrob. Agents Chemother.* **28,** 357–359.
13. Simberkoff, M. S., Lukazewski, M., Cross, A., Al-Ibrahim, M., Baltch, A. L., Smith, R. P., Geiseler, P. J., Nadler, J. & Richmond, A. S. (1986) *J. Infect. Dis.* **153,** 78–82.
14. Lacks, S. & Hotchkiss, R. D. (1960) *Biochim. Biophys. Acta* **39,** 506–517.
15. Tomasz, A. (1964) *Bacteriol. Proc.*, 29 (abstr.).
16. Garcia-Bustos, J. F., Chait, B. T. & Tomasz, A. (1988) *J. Bacteriol.* **170,** 2143–2147.
17. Schleifer, K. H. & Kandler, O. (1972) *Bacteriol. Rev.* **36,** 407–477.
18. Jabes, D., Nachman, S. & Tomasz, A. (1989) *J. Infect. Dis.* **159,** 16–23.
19. Tipper, D. J. & Strominger, J. L. (1965) *Proc. Natl. Acad. Sci. USA* **54,** 1133–1141.