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Occurrence of Glucosamine Residues with Free Amino Groups in Cell Wall Peptidoglycan from Bacilli as a Factor Responsible for Resistance to Lysozyme

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Analysis by dinitrophenylation techniques revealed the occurrence of significant amounts of glucosamine residues with free amino groups in the peptidoglycan component of cell walls isolated from *Bacillus cereus*, *Bacillus subtilis*, and *Bacillus megaterium*. A close correlation was demonstrated between the content of *N*-unacetylated glucosamine residues in the peptidoglycan component and the resistance of the cell walls to lysozyme. These lysozyme-resistant cell walls and peptidoglycan were converted into a lysozyme-sensitive form by means of *N*-acetylation with acetic anhydride. Thus, the occurrence of the *N*-unacetylated glucosamine residues in the peptidoglycan component accounts for the resistance of these cell walls to lysozyme. The *N*-unacetylated glucosamine residues were not found in a significant amount in the cell walls of *Micrococcus lysodeikticus*, *Staphylococcus aureus*, *Streptococcus faecalis*, *Lactobacillus casei*, or *Lactobacillus arabinosus*.

Resistance of bacterial cell wall peptidoglycan to lysozyme (EC 3.2.1.17) has so far been accounted for by the presence of *O*-acetyl groups (6), attachment of other polymers such as teichoic acid (13) and polysaccharide (12), the occurrence of free amino groups probably in the peptide portion (16), and a high degree of peptide cross-linking (19). In 1971, Araki et al. (2, 3) reported that in the cell wall peptidoglycan from three strains of *Bacillus cereus* the majority of glucosamine residues have free amino groups, and that resistance of these cell walls to lysozyme is accounted for by the mine whether the *N*-unacetylated glucosamine residues. Thus, it became of interest to determine whether the *N*-unacetylated glucosamine residues are a general occurrence in cell walls of various bacteria, or not. This paper reports that the *N*-unacetylated glucosamine residues occur also in the cell wall peptidoglycan from *Bacillus megaterium* and *Bacillus subtilis* and are a factor responsible for resistance of the cell walls to lysozyme.

MATERIALS AND METHODS

Bacteria and cell walls. *B. cereus* AHU 1030 and AHU 1356, *B. megaterium* AHU 1240 and AHU 1375, *B. subtilis* AHU 1031 and AHU 1037, *Lactobacillus casei* AHU 1060, and *Lactobacillus arabinosus* AHU 1413 were furnished by Y. Sasaki, Hokkaido University. Another strain of *B. megaterium* (strain is unknown and tentatively referred to as strain J) was given by J. L. Strominger, Harvard University. *B. subtilis* IAM 1069 and *Streptococcus faecalis* IAM 1262 were supplied by the Institute of Applied Microbiology of University of Tokyo. *Staphylococcus aureus* H and 209P and *Micrococcus lysodeikticus* ATCC 4698 were also used. *B. cereus*, *B. megaterium*, *S. aureus*, and *B. subtilis* were grown under the same conditions as those described for growth of *B. cereus* in the previous report (3). *L. arabinosus* and *L. casei* were grown as described by Baddiley et al. (5). *S. faecalis* was grown in a medium containing 1% tryptone, 0.5% yeast extract, and 1% K_2HPO_4 at *p*H 7.0. *M. lysodeikticus* was grown as described in a previous paper (3). Cells harvested at half-maximal growth were disrupted with glass beads in a sonic oscillator, and cell walls were isolated by the method of Cummins and Harris (7) with a small modification as described previously (3).

Peptidoglycan. Cell walls were treated in 0.1 N HCl at 60 C for 24 hr to remove acid-labile components such as teichoic acid and polysaccharide (11). The insoluble fraction was recovered by centrifugation, washed repeatedly with water, lyophilized, and used as peptidoglycan.

O-deacylation and N-acetylation. The *O*-acyl

groups were removed from cell walls by treatment in 0.01 N NaOH at 37 C for 1 hr (16). The cell walls were recovered by centrifugation, washed three times in water, and lyophilized. *N*-acetylation of cell walls and peptidoglycan was carried out by shaking 8 mg of sample in a solution containing 0.3 ml of acetic anhydride and 0.42 g of $NaHCO_3$ in 5 ml of water overnight at 0 C as described by Heymann et al. (8). Then the insoluble material was collected by centrifugation, washed three times in water, and lyophilized.

Lysozyme digestion. Sensitivity of cell walls and peptidoglycan to lysozyme was examined as follows unless otherwise indicated. Samples (0.4 mg) were incubated at 37 C in a mixture containing 30 μg of egg white lysozyme, 0.5% sodium azide, and 40 mM tris(hydroxymethyl)aminomethane (Tris)-chloride (*p*H 7.2) in a final volume of 740 μliters. In the turbidity assay, the absorbance at 500 nm was measured at frequent intervals with a Hitachi Perkin-Elmer 139 spectrophotometer. In the reducing group assay, liberation of reducing groups was measured by the method of Park and Johnson (15) with *N*-acetylglucosamine as a reference standard. Some samples were incubated with *N*-acetylmuramyl-L-alanine amidase prior to lysozyme digestion. Incubation with the amidase was carried out in 40 mM Tris-chloride, *p*H 8.2, by using the amount of enzyme that caused 50% decrease in the absorbance at 500 nm per hour. When the decrease in turbidity had finished, the *p*H was adjusted to 7.2 with HCl, and the measurement of reducing group liberation was started with the addition of lysozyme.

Analytical methods. Amino acids and amino sugars were estimated in a Shibata AA100 autoanalyzer after hydrolysis in 4 N HCl for 12 hr at 95 C. Since the occurrence of *N*-unacetylated glucosamine residues interferes with the analysis (3), samples were *N*-acetylated prior to the hydrolysis. The amino acid analyzer integration constants were calculated from analysis of dissaccharide *N*-acetylglucosaminyl-$\beta(1 \rightarrow 4)$-*N*-acetylmuramic acid (3) which had been hydrolyzed under the conditions used for the assay of amino sugars. The integration constants were 52.6 for muramic acid and 72.4 for glucosamine, as compared to 96.2 for glutamic acid. The free amino groups were determined by the dinitrophenylation techniques as described previously (3). The dinitrophenylated (DNP-) material was hydrolyzed in 4 N HCl for 12 hr at 95 C, and the resulting DNP-amino acids and DNP-amino sugars were separated on two-dimensional paper chromatography in 1-butanol-pyridine-water (6:4:3) and 1.5 M potassium phosphate, *p*H 6.0. The spots of the DNP-derivatives were eluted with 40 mM NH_4OH. The eluates were evaporated to dryness, and the absorbance at 360 nm was measured in a 1% $NaHCO_3$ solution.

Other materials. Egg white lysozyme (twice crystallized) was purchased from Sigma. *N*-acetylmuramyl-L-alanine amidase was prepared by diethylaminoethyl-cellulose column chromatography from *Flavobacterium* L-11 enzyme (10) kindly supplied by S. Kotani, Osaka University. The amidase preparation had virtually no activity of hydrolyzing glycosidic linkages of peptidoglycan.

RESULTS

Analysis of cell walls and peptidoglycan. Table 1 summarizes data on analysis of the cell wall and peptidoglycan preparations. Each preparation had alanine, glutamic acid, muramic acid, and either lysine or diaminopimelic acid in an approximate molar ratio of 2:1:1:1. In addition, the peptidoglycan from *M. lysodeikticus* and *S. aureus* contained glycine, and the peptidoglycan from *L. casei* and *S. faecalis* contained aspartic acid. The content of other amino acids was less than 30 nmoles per mg. These data indicate homogeneity of the cell wall and peptidoglycan preparations. The excess content of glucosamine over that of muramic acid and glutamic acid in some cell wall preparations is accounted for by the presence of polysaccharide or teichoic acid which contains glucosamine.

Determination of free amino groups revealed that all the cell wall preparations from the strains of *B. cereus*, *B. subtilis*, and *B. megaterium* contained significant amounts of *N*-unacetylated glucosamine residues. Most of the excess glucosamine residues were removed from the cell walls on the acid treatment carried out to isolate peptidoglycan, whereas some peptidoglycan preparations, particularly that from *B. cereus* AHU 1356, still had excess glucosamine, which is believed to be due to the presence of a residual amount of the polysaccharide component. The values of the molar ratio of *N*-unacetylated glucosamine to glutamic acid do not differ significantly between the cell wall and peptidoglycan preparations from each bacterial strain (Table 1). This suggests that the *N*-unacetylated glucosamine residues are present only in the peptidoglycan component, although the possibility that some minor proportions of these residues may also be distributed in other components than peptidoglycan cannot be excluded (1, 3). Therefore, the molar ratio of *N*-unacetylated glucosamine to glutamic acid was adopted as a measure of the extent of *N*-unacetylation at the glucosamine residues in the peptidoglycan component of cell walls.

On the basis of this value, the cell walls examined seem to be classifiable into three groups. In the cell walls of the first group, which includes the cell walls of AHU 1356 and two other strains (3) of *B. cereus*, the majority of the amino groups in the glucosamine residues of the peptidoglycan component are free.

TABLE 1. *Analysis of cell walls and peptidoglycan*

Strain and preparation	Component[a]							Molar ratio of $GlcNH_2$ to glutamic acid[b]
	Alanine	Glutamic acid	Lysine	Diamino-pimelic acid	Gluco-samine	Muramic acid	$GlcNH_2$[b]	
B. cereus								
AHU 1356								
Cell walls	630	358		363	1,690	358	264	0.74
Peptidoglycan	941	569		568	1,180	537	476	0.84
AHU 1030								
Cell walls	609	394		404	555	367	63	0.16
Peptidoglycan	675	551		540	659	513	128	0.23
B. subtilis								
AHU 1031								
Cell walls	1,010	615		650	750	568	117	0.19
Peptidoglycan	1,073	707		785	712	614	165	0.23
AHU 1037								
Cell walls	1,040	383		460	486	372	90	0.23
Peptidoglycan	1,155	680		815	638	467	142	0.21
IAM 1069								
Cell walls	733	382		378	423	304	77	0.20
Peptidoglycan	1,192	740		813	742	628	136	0.18
B. megaterium								
AHU 1240								
Cell walls	887	455		485	779	476	104	0.23
Peptidoglycan	1,134	596		777	642	549	141	0.24
AHU 1375								
Cell walls	797	455		414	733	413	109	0.24
Peptidoglycan	1,037	588		690	635	454	140	0.24
J								
Cell walls	667	370		381	937	366	113	0.31
Peptidoglycan	897	575		756	678	572	155	0.27
M. lysodeikticus								
ATCC 4698								
Peptidoglycan[c, d]	1,412	623	694		722	637	7	0.01
L. casei								
AHU 1060								
Peptidoglycan[e]	1,600	953	727		784	818	13	0.01
L. arabinosus								
AHU 1413								
Peptidoglycan	1,480	1,010		778	764	968	14	0.01
S. aureus								
H								
Peptidoglycan[d]	1,790	602	687		865	572	6	0.01
209P								
Peptidoglycan[d]	1,774	778	577		865	594	5	0.006
Streptococcus faecalis								
IAM 1262								
Peptidoglycan[e]	1,442	836	580		738	649	5	0.006

[a] Amino sugar and amino acid were assayed as described under Materials and Methods by using samples which had been *N*-acetylated prior to hydrolysis. The values for glucosamine include both *N*-acetylated and *N*-unacetylated glucosamine. All the values are expressed in nanomoles per milligram of the samples.

[b] $GlcNH_2$ represents *N*-unacetylated glucosamine. The values for *N*-unacetylated glucosamine were not corrected for the loss of DNP-glucosamine sustained during hydrolysis and separation. The recovery of *N*-unacetylated glucosamine, as measured in the analysis of glucosaminyl-$\beta(1 \rightarrow 4)$-muramic acid under the conditions used for the assay of free amino groups, was about 70%.

[c] *N*-unacetylated muramic acid (31 nmoles per mg) was present.

[d] The following amounts of glycine (nanomoles per milligram) were present: *M. lysodeikticus*, 692; *S. aureus* H, 3,600; *S. aureus* 209P, 3,530.

[e] The following amounts of aspartic acid (nanomoles per milligram) were present: *L. casei*, 553; *Streptococcus faecalis*, 524.

The cell walls of all the strains of *B. subtilis* and *B. megaterium*, together with the cell walls of *B. cereus* AHU 1030, make the second group. In the cell walls of this group, minor proportions of the glucosamine residues in the peptidoglycan component are *N*-unacetylated. Another group of cell walls, namely those from *M. lysodeikticus*, *L. casei*, *L. arabinosus*, *S. aureus*, and *S. faecalis*, do not contain significant amounts of glucosamine with a free amino group.

Sensitivity of cell walls to lysozyme. The cell walls of *B. cereus* AHU 1356 exhibited unusually strong resistance to lysozyme as reported previously (2, 3). Removal of the *O*-acyl groups, polysaccharide component, and peptide moiety from these cell walls had no effect on their resistance to lysozyme. On the other hand, *N*-acetylation caused drastic decrease in the resistance of the cell walls and peptidoglycan to lysozyme as shown in Fig. 1. *B. cereus* AHU 1030 cell walls, which are *N*-unacetylated at a minor proportion of *N*-acetylglucosamine residues in the peptidoglycan component, had moderate sensitivity to lysozyme. *N*-acetylation increased the rate and extent of lysozyme digestion of the cell walls and peptidoglycan from this strain as measured either by turbidity reduction or reducing group liberation. The effect of *N*-acetylation on the extent of reducing group liberation was evident even when the lysozyme concentration and incubation time were increased. Thus, reducing group liberation on digestion with 250 μg of lysozyme per ml for 40 hr was 281 nmoles/mg for *B. cereus* AHU 1030 cell walls, as compared to 483 nmoles/mg for the cell walls after *N*-acetylation.

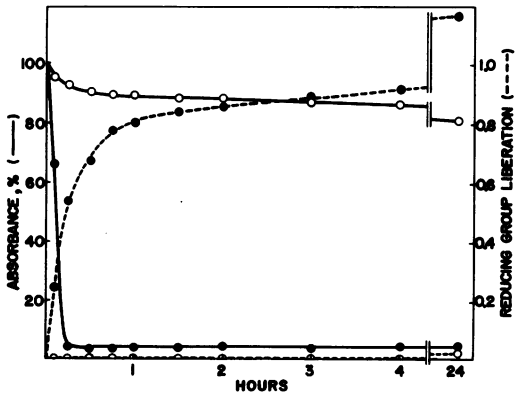

FIG. 1. *Lysozyme digestion of intact and N-acetylated peptidoglycan from B. cereus AHU 1356 cell walls. Symbols:——, the absorbance expressed as percentage of the initial value; ---, reducing group liberation expressed as micromoles per milligram; ○, peptidoglycan; ●, N-acetylated peptidoglycan.*

The results obtained with the cell walls of *B. subtilis* (Fig. 2) and *B. megaterium* were essentially the same as those with the cell walls of *B. cereus* AHU 1030 in respect to the effect of *N*-acetylation. The lysozyme resistance of this group of cell walls was partly accounted for by the presence of the polysaccharide or teichoic acid component. The peptidoglycan preparations obtained after removal of these components by acid treatment still had considerable lysozyme resistance, which declined following *N*-acetylation.

Table 2 summarizes the data on the extent of reducing group liberation during lysozyme digestion of the peptidoglycan preparations and their *N*-acetylation products. These data indicate that the *N*-acetylation-dependent increase in lysozyme sensitivity correlates closely with the extent of *N*-unacetylation at *N*-acetylglucosamine residues in peptidoglycan. It seems, therefore, most likely that the occur-

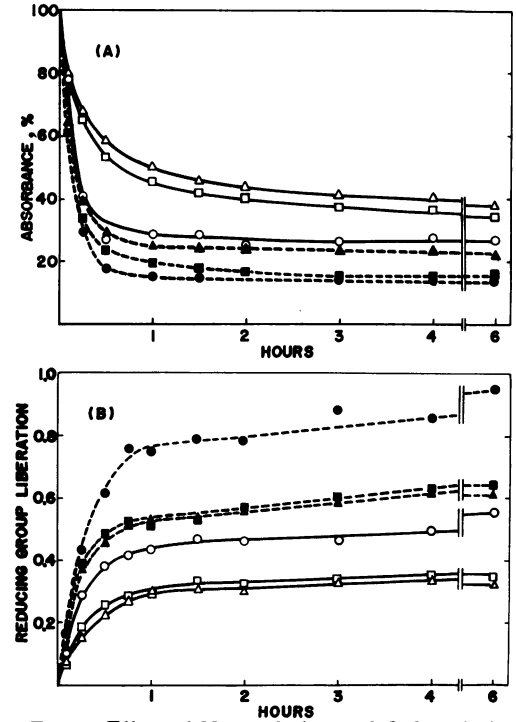

FIG. 2. *Effect of N-acetylation and O-deacylation on lysozyme sensitivity of B. subtilis AHU 1031 cell walls and their peptidoglycan component. (A) The absorbance expressed as percentage of the initial value. (B) Reducing group liberation expressed as micromoles per milligram.* △——△, *Walls;* □——□, *O-deacylated walls;* ○——○, *peptidoglycan;* ▲---▲, *N-acetylated walls;* ■---■, *walls N-acetylated and then O-deacylated;* ●---●, *N-acetylated peptidoglycan.*

TABLE 2. *Reducing group liberation on lysozyme digestion of peptidoglycan*

Peptidoglycan	Reducing group liberation[a]		
	Before *N*-acetylation (μmoles/mg)	After *N*-acetylation (μmoles/mg)	*N*-acetylation-dependent fraction[b] (%)
B. cereus			
AHU 1356	0.01	0.86	99
AHU 1030	0.60	1.04	42
B. subtilis			
AHU 1031	0.50	0.87	43
AHU 1037	0.56	0.85	34
IAM 1069	0.62	0.93	33
B. megaterium			
AHU 1240	0.60	1.03	42
AHU 1375	0.30	0.50	40
J	0.34	0.58	41
M. lysodeikticus			
ATCC 4698	0.75	0.69	−9
L. casei			
AHU 1060	0.40	0.43	7
L. arabinosus			
AHU 1413	0.77	0.69	−11
S. aureus			
H	0.65	0.78	17
209P	0.29	0.84	61
S. faecalis			
IAM 1262	0.63	0.67	6

[a] Reducing group liberation was assayed after incubation with lysozyme for 6 hr under the conditions described in the text.

[b] The values were calculated as 100(B−A)/B, where A and B refer to reducing group liberation with peptidoglycan before and after *N*-acetylation, respectively.

rence of the *N*-unacetylated glucosamine residues in peptidoglycan is responsible for the resistance of the first and second groups of cell walls to lysozyme. The alternative possibility, that the lysozyme resistance of the cell wall peptidoglycan of *B. subtilis* and *B. megaterium* is due to the presence of the free amino groups in the peptide moiety, is unlikely since the rate and extent of reducing group liberation on lysozyme digestion of these peptidoglycans were not influenced by prior treatment with *N*-acetylmuramyl-L-alanine amidase as shown in Fig. 3.

In contrast to the cell walls in the first and second groups, those in the third group, with the exception of *S. aureus* cell walls, have lysozyme-sensitive peptidoglycan. The lysozyme digestion of these peptidoglycans was not influenced by *N*-acetylation as shown in Table 2. The resistance of these cell walls to lysozyme should be accounted for in terms of some other factors. For example, the resistance of the cell walls of *L. casei* and *L. arabinosus* to lysozyme appeared to depend partly on the occurrence of *O*-acyl groups and mostly on the occurrence of polysaccharide or teichoic acid. The *N*-acetylation-induced increase in lysozyme sensitivity of staphylococcal peptidoglycan may be ascribed to either blocking of the amino groups of the peptide moiety or partial degradation of the peptide cross-linkage in alkali (4). As shown in Fig. 4, *S. aureus* peptidoglycan and its *N*-acetylation product were digested similarly

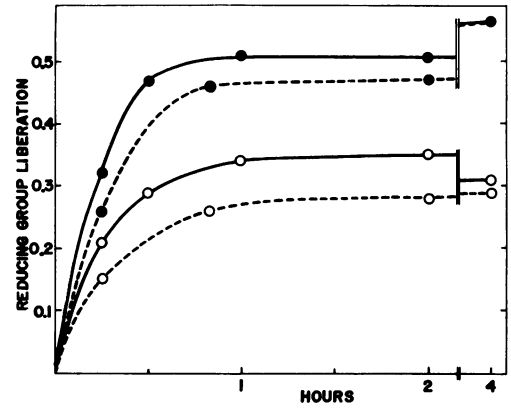

FIG. 3. *Effect of N-acetylmuramyl-L-alanine amidase treatment on lysozyme sensitivity of cell wall peptidoglycan of B. megaterium J. Reducing group liberation is epxressed in micromoles per milligram.* O——O, *Peptidoglycan;* ●——●, *N-acetylated peptidoglycan;* O---O, *peptidoglycan after preincubation with amidase;* ●---●, *N-acetylated peptidoglycan after preincubation with amidase.*

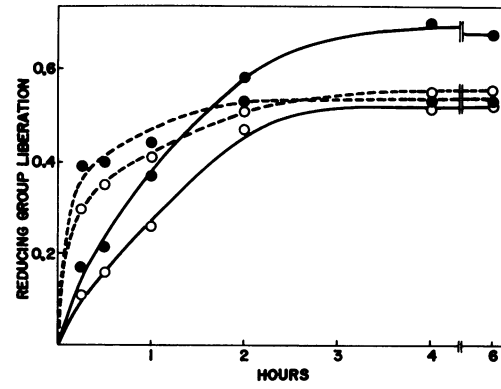

FIG. 4. *Effect of N-acetylmuramyl-L-alanine amidase treatment on lysozyme sensitivity of cell wall peptidoglycan of S. aureus H. Reducing group liberation is expressed in micromoles per milligram. Symbols are the same as for Fig. 3.*

when they were treated with lysozyme after preincubation with N-acetylmuramyl-L-alanine amidase.

DISCUSSION

The finding of N-unacetylated glucosamine residues in the cell walls of *B. subtilis* and *B. megaterium*, together with the previous demonstration of these residues in *B. cereus* cell walls (2, 3), suggests the possibility that the N-unacetylated glucosamine residues may be distributed somewhat widely among cell walls of bacilli. Ingram and Salton (9) have described the occurrence of muramic acid residues with free amino groups in lysozyme digests of *M. lysodeikticus* cell walls, and Mirelman and Sharon (14) have isolated a disaccharide with an N-unacetylated muramic acid residue from the lysozyme digests of the *M. lysodeikticus* cell walls. Unlike the N-unacetylated glucosamine residues, the N-unacetylated muramic acid residues were not found in the cell walls of the examined strains other than *M. lysodeikticus*. The presence of the N-unacetylated muramic acid residues in the *M. lysodeikticus* cell walls is in agreement with the observation by Ingram et al. and Sharon et al.

The importance of the acetamido groups of N-acetylglucosamine residues in the formation of the lysozyme-substrate complex was predicted by Phillips and his collaborators (17) on the basis of the X-ray crystallographic model. On the other hand, Shockman et al. (18) and Perkins (16) have reported that N-acetylation induces significant increase in lysozyme sensitivity of cell walls from *M. lysodeikticus*, *Corynebacterium tritici*, *B. megaterium*, and *S. faecalis*. This effect of N-acetylation seemed to be explained in terms of the blocking of the free amino groups in the peptide portion of the peptidoglycan component. The present result indicates that the effect of N-acetylation on the lysozyme sensitivity of the cell walls from the strains of *B. cereus*, *B. subtilis*, and *B. megaterium* is accounted for by the blocking of the free amino groups of the glucosamine residues in the peptidoglycan component.

With the intact and the N-acetylated cell walls of *B. cereus*, Araki et al. (3) previously demonstrated that the sensitivity of these cell walls to lysozyme depends upon the degree of N-acetylation at the glucosamine residues in the peptidoglycan component. In the present investigation, the extents of lysozyme digestion were compared between the cell wall polymers from different sources in terms of reducing group liberation, which appeared to be a more quantitative measure of digestion than turbidity decrease. The N-unacetylated glucosamine contents shown in Table 1 were not corrected for the loss of DNP-glucosamine during analysis. The recovery of N-unacetylated glucosamine, as measured in the analysis of glucosaminyl-$\beta(1 \rightarrow 4)$-muramic acid under the conditions used for the assay of free amino groups, was about 70%. Taking account of this percentage, the actual values of the extent of N-unacetylation at N-acetylglucosamine residues in the examined cell wall peptidoglycan are believed to be 26 to 33% for *B. subtilis*, 34 to 40% for *B. megaterium*, and 33 to 100% for *B. cereus*. These corrected values of the extent of N-unacetylation at N-acetylglucosamine residues coincide approximately with the percentages of the N-acetylation-dependent fraction in reducing group liberation, that is, 33 to 43% for *B. subtilis*, 40 to 42% for *B. megaterium*, and 42 to 99% for *B. cereus*, as shown in Table 2.

From this result, it seems most likely that lysozyme cannot hydrolyze the glycosidic linkages adjacent to the glucosamine residues with free amino groups. Thus, on lysozyme digestion, the peptidoglycan partially N-unacetylated at N-acetylglucosamine residues is expected to yield oligosaccharides, which resist further digestion. Studies on the isolation and characterization of such oligosaccharides will be described elsewhere. Furthermore, the above evidence suggests the presence of a specific enzyme which catalyzes deacetylation of the N-acetylglucosamine residues in peptidoglycan. Such an enzyme has been reported in a previous paper (1).

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LITERATURE CITED

1. Araki, Y., S. Fukuoka, S. Oba, and E. Ito. 1971. Enzymatic deacetylation of N-acetylglucosamine residues in peptidoglycan from *Bacillus cereus* cell walls. Biochem. Biophys. Res. Commun. **45**:751–758.
2. Araki, Y., T. Nakatani, H. Hayashi, and E. Ito. 1971. Occurrence of non-N-substituted glucosamine residues in lysozyme-resistant peptidoglycan from *Bacillus cereus* cell walls. Biochem. Biophys. Res. Commun. **42**:691–697.
3. Araki, Y., T. Nakatani, K. Nakayama, and E. Ito. 1972. Occurrence of N-nonsubstituted glucosamine residue in peptidoglycan of lysozyme-resistant cell walls from *Bacillus cereus*. J. Biol. Chem. **247**:6312–6321.
4. Archibald, A. R., J. Baddiley, and J. Gundry. 1970. The action of alkali on the glycyl residues of staphylococcal peptidoglycan. Biochem. J. **116**:313–315.
5. Baddiley, J., and A. L. Davison. 1961. The occurrence

and location of teichoic acid in lactobacilli. J. Gen. Microbiol. **24**: 295–299.
6. Brumfitt, W., A. C. Wardlaw, and J. T. Park. 1958. Development of lysozyme-resistance in *Micrococcus lysodeikticus* and its association with an increased *O*-acetyl content of the cell wall. Nature (London) **181**:1783–1784.
7. Cummins, C. S., and H. Harris. 1956. The chemical composition of the cell wall in some gram-positive bacteria and its possible value as a taxonomic character. J. Gen. Microbiol. **14**:583–600.
8. Heymann, H., J. M. Manniello, and S. S. Barkulis. 1964. Structure of streptococcal cell walls. III. Characterization of alanine-containing glucosaminyl muramic acid derivative liberated by lysozyme from streptococcal glycopeptide. J. Biol. Chem. **239**:2981–2985.
9. Ingram, V. M., and M. R. J. Salton. 1957. The action of fluorodinitrobenzene on bacterial cell walls. Biochim. Biophys. Acta **24**:9–14.
10. Kato, K., and S. Kotani. 1962. Lysis of *Staphylococcus aureus* cell walls by a lytic enzyme purified from culture supernatants of *Flavobacterium* species. Biken J. **5**:155–180.
11. Knox, K. W., and E. A. Hall. 1965. The linkage between the polysaccharide and mucopeptide components of the cell wall of *Lactobacillus casei*. Biochem. J. **96**:302–318.
12. Krause, R. M., and M. McCarty. 1961. Studies on the chemical structure of the streptococcal cell wall. J. Exp. Med. **114**:127–141.
13. Mandelstam, M., and J. L. Strominger. 1961. On the structure of the cell wall of *Staphylococcus aureus* (Copenhagen). Biochem. Biophys. Res. Commun. **5**:466–471.
14. Mirelman, D., and N. Sharon. 1967. Isolation and study of the chemical structure of low molecular weight glycopeptide from *Micrococcus lysodeikticus* cell walls. J. Biol. Chem. **242**:3414–3427.
15. Park, J. T., and M. J. Johnson. 1949. A submicrodetermination of glucose. J. Biol. Chem. **181**:149–151.
16. Perkins, H. R. 1965. The action of hot formamide on bacterial cell walls. Biochem. J. **95**:876–882.
17. Phillips, D. C. 1967. The hen egg-white lysozyme molecule. Proc. Nat. Acad. Sci. U.S.A. **57**:484–495.
18. Shockman, G. D., J. S. Thompson, and M. J. Conover. 1967. The autolytic enzyme system of *Streptococcus faecalis*. II. Partial characterization of the autolysin and its substrate. Biochemistry **6**:1054–1065.
19. Strominger, J. L., and J.-M. Ghuysen. 1968. Mechanism of enzymatic bacteriolysis. Science **156**:213–221.