ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Mar. 1992, p. 566–572
0066-4804/92/030566-07$02.00/0

Vol. 36, No. 3

Autolysis of Methicillin-Resistant and -Susceptible *Staphylococcus aureus*

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Received 16 July 1991/Accepted 24 December 1991

The autolytic activities, including unstimulated, Triton X-100-stimulated, and daptomycin-induced, of various sets of methicillin-resistant and related methicillin-susceptible strains were compared. Faster rates of autolysis were noted in two heterogeneous methicillin-resistant transductants than in their methicillin-susceptible parental recipients, in a heterogeneous resistant strain than in a susceptible derivative created by chemical mutagenesis, and in a homogeneous resistant strain than in a derivative that had decreased methicillin resistance and was created by transposon Tn*551* mutagenesis. These results suggest that the presence of the methicillin resistance region, *mec*, either directly or indirectly through an interaction with other host genes, confers a faster rate of autolysis on strains. Various auxilliary genes are known to affect methicillin resistance expression, and one of these genes, *femA*, was necessary for the expression of this faster rate of autolysis. These differences in autolytic activities were not observed in isolated crude cell walls retaining autolytic activities, suggesting different modes of regulation of autolysins in intact cells and isolated walls. In contrast, one homogeneous, highly resistant strain, DU4916, had a lower autolytic activity than did derived heterogeneous resistant and susceptible strains created by chemical mutagenesis and a strain that had decreased resistance and was created by transposon mutagenesis. Our observations suggest that methicillin resistance expression is associated with an enhanced rate of autolysis, in heterogeneous resistant strains at least.

There are two major classes of methicillin-resistant *Staphylococcus aureus* strains—heterogeneous and homogeneous. Only rare cells in the population of heterogeneous strains grow in the presence of high concentrations of methicillin (34), whereas most cells in the population of homogeneous strains grow in the presence of high methicillin concentrations (18). A very important aspect of methicillin resistance is the production of an additional low-affinity penicillin-binding protein (PBP), PBP 2a (12, 26). PBP 2a is encoded by a gene, designated *mecA* (23, 33), in the *mec* region of the chromosome, which also carries other antibiotic resistance determinants (15).

The methicillin resistance mechanism is more complex than can be accounted for by PBP 2a alone. There is a poor correlation between the cellular amounts of PBP 2a in and the methicillin MICs for heterogeneous strains (13). Under certain growth conditions, the sensitivities of growth and peptidoglycan synthesis were not clearly related to the level of PBP 2a (19). The synthesis of PBP 2a is subject to a variety of regulatory influences. The production of PBP 2a is altered by growth conditions and is inducible in some strains by β-lactams, and the inducibility may be affected by the presence of a penicillinase plasmid (5, 27, 39). Recently, Tesch et al. (35) described a region in *mec*, called *mecR*, that affects the expression of PBP 2a.

Genetic studies have revealed the existence of genes other than *mecA* and located elsewhere on the chromosome that influence methicillin resistance. Transposon Tn*551* mutants carried the intact *mecA* gene and often produced normal amounts of PBP 2a, but the MICs for these mutants were close to those for susceptible strains (3, 17). Transformational and transductional analyses of *mec* have demonstrated the importance of auxiliary genes in determining the heterogeneous or homogeneous characteristics of the transformants or transductants (18, 24). A factor essential for the expression of methicillin resistance, *femA*, has recently been cloned and characterized (2). *femA* resides on chromosomal segment 18, which is very distant from *mec*. The product of *femA* is a 48-kDa protein that has no influence on the synthesis of PBP 2a but is needed for growth in the presence of β-lactam antibiotics.

There are a number of good reasons to examine the autolytic properties of methicillin-resistant *S. aureus* for their possible involvement in methicillin resistance. The classic response of penicillin-susceptible *S. aureus* to β-lactams is the inhibition of growth followed by cell lysis and killing (4, 28, 38). Smith and Wilkinson (32) and Wyke et al. (45) noted that homogeneous resistant strains were not lysed and killed by high methicillin concentrations, despite the presence of the four high-affinity PBPs found in susceptible strains. Growth in the presence of methicillin leads to poorly cross-linked peptidoglycan (25, 45), but resistant strains are able to control this potential for increased lysis (25). Seligman (30) reported that methicillin-resistant *S. aureus* had an unusual increased autolytic activity compared with that of methicillin-susceptible strains and that this increased autolytic activity was manifested as a decrease in turbidity in Trypticase soy broth cultures shaken at 33°C but not in standing cultures or cultures shaken at 37°C. Tomasz (36) proposed that a hypothetical autolysin regulator, termed factor X, is particularly important in homogeneous strains. Gustafson and Wilkinson (11) found that a homogeneous resistant strain had a lower autolytic activity than did derived heterogeneous and susceptible strains.

Our purpose in this work was to examine the autolytic properties of methicillin-resistant *S. aureus* in a variety of ways. We studied a range of strains, including homogeneous and heterogeneous strains and genetically related suscepti-

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TABLE 1. Strains of *S. aureus* used and their rates of autolysis

Strain	Relevant genotype	Relevant phenotype[a]	Reference	Rate of autolysis[b] in cells that were:	
				Unstimulated	Triton X-100 stimulated
Set 1					
BB255	8325	Mc^s	1, 2	0.06 ± 0.01	0.45 ± 0.001
BB270	8325 *mec*	Mc^r He	1, 2	0.22 ± 0.02	0.49 ± 0.03
BB308	8325 *mec* Ω(*femA*::Tn*551*)*2003*	Mc^s Em^r	1, 2	0.08 ± 0.01	0.23 ± 0.02
BB314	8325 *mec* deletion at 14.7 kb Ω(*femA*::Tn*551*)*2003*	Mc^r Em^s	3	0.12 ± 0.02	0.42 ± 0.01
BB330	8325 Ω(*femA*::Tn*551*)*2003*	$Mc^{(s)}$ Em^r	3	0.04 ± 0.01	0.40 ± 0.01
BB586	8325 *mec* Ω(*femA*::Tn*551*)*2003* pBBB31	Mc^r He Em^r Cm^r	3	0.07 ± 0.01	0.22 ± 0.01
BB568 (COL)	COL *mec*	Mc^r Ho	17	0.06 ± 0.002	0.29 ± 0.01
BB403	COL *mec* Ω(*femA*::Tn*551*)*2003*	$Mc^{(s)}$ Em^r	1, 2	0.08 ± 0.01	0.17 ± 0.01
BB399 (DU4916)	DU4916 *mec*	Mc^r Ho	1, 2	0.15 ± 0.004	0.25 ± 0.002
BB401	DU4916 *mec* Ω(*femA*::Tn*551*)*2003*	Mc^s Em^r	1, 2	0.12 ± 0.01	0.49 ± 0.01
Set 2					
ANS46	*mec*	Mc^r He	22	0.09 ± 0.01	0.45 ± 0.02
ANS62		Mc^s	22	0.10 ± 0.01	0.22 ± 0.01
Set 3					
SC4	8325-4(pI524)	Mc^s	14	0.07 ± 0.006	0.19 ± 0.01
SC4*mec*C5	8325-4(pI524) *mec*	Mc^r He	14	0.13 ± 0.01	0.44 ± 0.02
Set 4					
DU4916	*mec*	Mc^r Ho	9–11	0.03 ± 0.01	0.05 ± 0.01
DU4916-K7	*mec*	Mc^r He	9–11	0.06 ± 0.004	0.42 ± 0.12
DU4916S		Mc^s	9–11	0.04 ± 0.01	0.48 ± 0.03

[a] He, heterogeneous; $Mc^{(s)}$, methicillin resistance was reduced but not to the level of a completely susceptible strain, such as BB255; Ho, homogeneous.
[b] Expressed as the first-order rate constant (h^{-1}).

ble and resistant strains produced by chemical and transposon mutageneses and transduction.

MATERIALS AND METHODS

Organisms and growth conditions. Four sets of genetically related organisms were studied. These included strains produced by chemical mutagenesis and isogenic strains created by transposon Tn*551* mutagenesis and transduction (Table 1). The first set was used to focus on the involvement of *femA* in autolytic activity. The second set included ANS46 (heterogeneous), which contains a genetically well-defined *mec* determinant, and ANS62 (susceptible), which was derived from ANS46 by acriflavine treatment (22). The third set included SC4*mec*C5 (heterogeneous), which was derived by transduction with methicillin-resistant strain C5 (heterogeneous) as a donor and SC4 [8325-4(p1524)] (6, 14) as a recipient. The fourth set included DU4916 (homogeneous Mc^r), DU4916-K7 (heterogeneous Mc^r), and DU4916S (susceptible) (9–11), the latter two being derived from DU4916 by 5-aminoacridine–HCl treatment. Sets two and three were graciously provided by P. R. Stewart of the Australian National University, Canberra, New South Wales, Australia.

All autolysis and crude cell wall (CCW) isolation experiments were done with cells grown in a liquid medium (PYK medium) consisting of 5.0 g of Bacto Peptone (Difco, Detroit, Mich.), 5.0 g of yeast extract (Difco), and 3.0 of K_2HPO_4 per liter at pH 7.2. Cultures were grown at 30°C with shaking (200 rpm) in a rotary shaking incubator. Daptomycin-induced lysis experiments were carried out with cation-supplemented (50 mg of Ca^{2+} and 25 mg of Mg^{2+} per liter) Mueller-Hinton broth (Difco) at pH 7.2 and 30°C. Southern hybridization studies were done with cells grown at 37°C in L broth (1) medium. A 2% (vol/vol) inoculum was used to initiate growth in all experiments.

DNA manipulations. Southern hybridization was performed essentially as described by Maniatis et al. (21). Chromosomal DNA digested with *Eco*RV was probed with a 10.5-kb *Pst*I chromosomal fragment known to include *femA* and the adjacent regions.

CCW isolation. CCW isolation procedures have been described in detail elsewhere (42). Organisms from a 1-liter PYK culture grown to an A_{580} of 1.0 were harvested by centrifugation (13,800 × *g*, 4°C, 10 min), washed by resuspension in 200 ml of cold distilled water, and reharvested. The cells were resuspended in 50 ml of cold distilled water, and the suspension was placed in a 100-ml Bead Beater (Biospec, Bartlesville, Okla.) blending unit with 50 ml of cold 0.1-mm glass beads. The cells were broken in five consecutive 2-min intervals with the apparatus packed in bags of ice; the machine was allowed to rest for 2 min between intervals. The glass beads were removed with a sintered glass filter under vacuum, and the filtrate was retained. The CCWs were harvested from the filtrate by centrifugation as described above, washed twice in 100 ml of cold distilled water by resuspension, and centrifuged before being lyophilized.

Triton X-100-stimulated autolysis. Triton X-100-stimulated autolysis was measured essentially as described by Gustafson and Wilkinson (11). Washed exponential-phase cells were resuspended in 0.05 M Tris-HCl (pH 7.5) buffer (unstimulated) or 0.05 M Tris-HCl (pH 7.5) buffer containing 0.05% (wt/vol) Triton X-100 (stimulated) in spectrophotometer cuvettes and incubated stationary at 30°C. The rates of

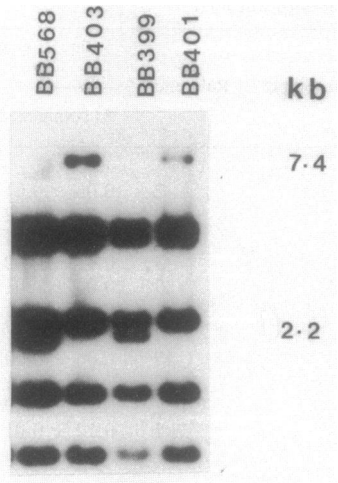

FIG. 1. Hybridization of the cloned *Pst*I fragment known to include *femA* with *Eco*RV restriction nuclease digests of homogeneous methicillin-resistant *S. aureus* BB568 and BB399 and their *femA* derivatives BB403 and BB401, respectively.

autolysis were calculated as first-order rate constants (log A_{580} at time zero/logA_{580} at time t = kt/2.303) by simple least-squares regression of logA_{580} at time zero/logA_{580} at time t versus t.

Daptomycin-induced autolysis. Daptomycin-induced autolysis was measured as described by Gustafson and Wilkinson (11).

CCW autolysis. CCWs were resuspended in 0.01 M sodium acetate buffer (pH 5.0) or 0.05 M potassium phosphate buffer (pH 7.2) in spectrophotometer cuvettes to an A_{580} of 0.6 to 0.7, with a brief sonic treatment. Attempts were made to stimulate CCW autolysis by adding RNase (0.5 mg/ml) or lysozyme (0.5 mg/ml) (41) to CCWs in sodium acetate buffer or by adding trypsin (5 μg/ml) (31) to CCWs in potassium phosphate buffer. CCWs were incubated at 30°C.

RESULTS

Transposon Tn*551* insertional inactivation of *femA* in BB568 (COL) and BB399 (DU4916). Southern hybridizations with a *femA* probe were performed on *Eco*RV digests of chromosomal DNAs from strains BB568, BB403, BB399, and BB401 (Fig. 1). In BB568 and BB399, *femA* is located on a 2.2-kb fragment. This fragment disappears upon insertion of the 5.2-kb transposon Tn*551* and is replaced by a 7.4-kb fragment in strains BB403 and BB401. These results suggest that the insertion has occurred in the same region of the chromosome in these two strains.

Triton X-100-stimulated whole-cell autolysis. The first-order rate constants for unstimulated and Triton X-100-stimulated whole-cell autolysis were measured in all the strains used in this study (Table 1). The results obtained with set 1 are also shown in graphic form in Fig. 2.

The heterogeneous methicillin-resistant transductant SC4 *mec*C5 showed a faster rate of Triton X-100-stimulated autolysis than did its methicillin-susceptible parental strain, SC4. Strain BB270, a heterogeneous methicillin-resistant transductant, showed a faster rate of unstimulated autolysis and daptomycin-induced autolysis (Fig. 3) than did the transduction recipient BB255 and a slightly faster rate of Triton X-100-stimulated autolysis than did BB255. Heterogeneous resistant strain ANS46 showed a faster rate of autolysis than did a susceptible derivative created by acriflavine treatment.

Strain BB308, which is BB270 with a Tn*551* insertion in *femA*, showed a slower rate of Triton X-100-stimulated autolysis than did BB270. A *femA*$^+$ revertant of BB308, strain BB314, regained higher autolytic activity. A Tn*551* insertion in the *femA* gene of methicillin-susceptible parental

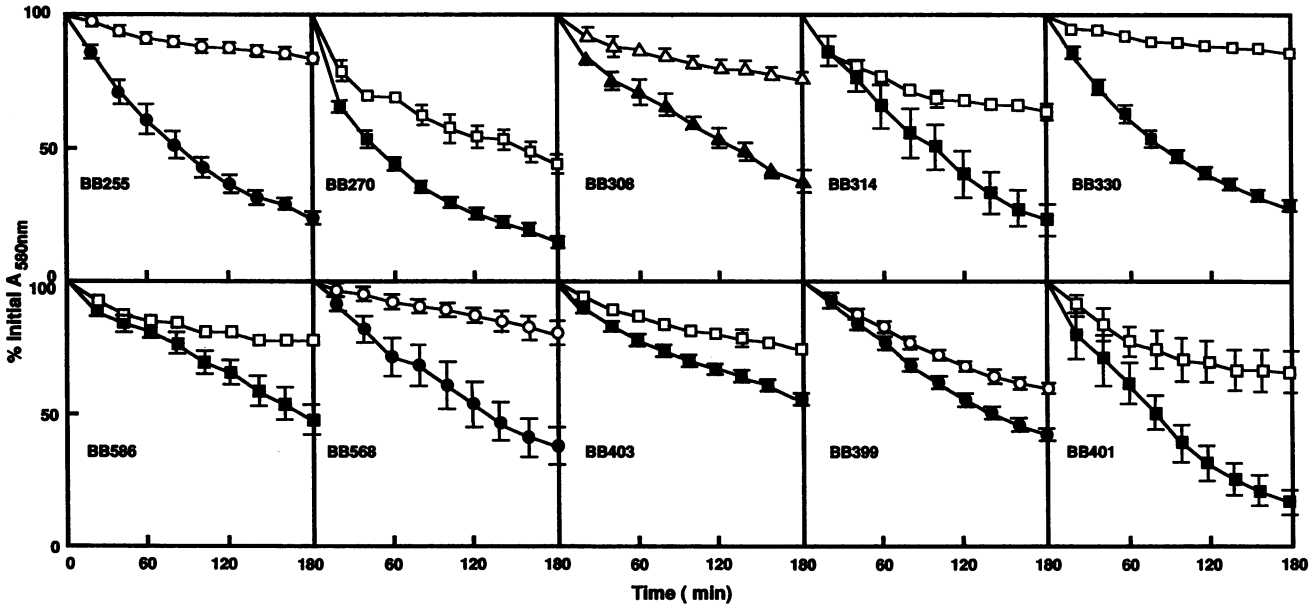

FIG. 2. Unstimulated and Triton X-100-stimulated autolysis of methicillin-resistant and -susceptible *S. aureus* (set 1). Open symbols represent cells in 0.05 M Tris-HCl buffer (pH 7.5), and closed symbols represent cells in 0.05 M Tris-HCl buffer (pH 7.5), and closed symbols represent cells in 0.05 M Tris-HCl buffer (pH 7.5) containing 0.05% (vol/vol) Triton X-100. Each point represents the mean of three experiments, and the standard deviations of the points are represented by error bars.

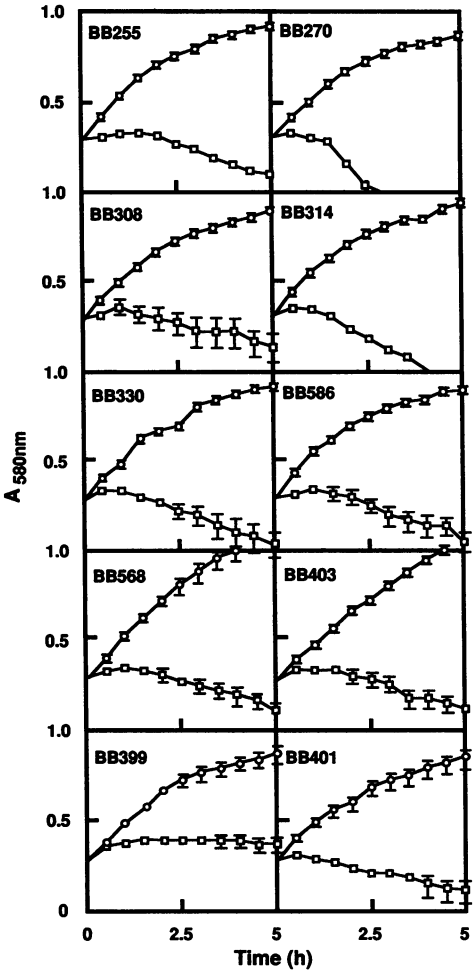

FIG. 3. Daptomycin-induced autolysis of methicillin-resistant and -susceptible *S. aureus* (set 1). Daptomycin (20 μg/ml) was added to growing cultures at an A_{580} of 0.2 to 0.3. Symbols: ○, control; □, daptomycin. Each point represents the mean of three experiments, and the standard deviations of the points are represented by error bars.

strain BB255, yielding strain BB330, resulted in a small decrease in the rate of autolysis. Taken together, these results suggest that functional *mec* and *femA* determinants are necessary for a faster rate of autolysis. However, when strain BB308 was transformed with plasmid pBBB31 carrying the cloned *femA* determinant, the resulting strain, BB586, had an autolytic activity similar to that of strain BB308. Strain BB586 was chloramphenicol and methicillin resistant, suggesting that *femA* was being expressed. Plasmid pBBB31 contains *femA* and a truncated version of *femB* (open reading frame 419) (2). Complementation of BB308 by pBBB31 resulted in methicillin resistance expression that was slightly lower than that in the parent, BB270, suggesting that the expression of open reading frame 419 was needed to attain the full level of methicillin resistance expression (2). Likewise, *femB* may be necessary for restoring the full, parental level of autolysis. Maidhof et al. (20) found that pBBB31 (strain BB586) was unable to restore completely parental (strain BB270) levels of cell wall turnover and whole-cell autolysis to strain BB308.

The influence of *femA* on the autolysis of homogeneous Mc^r strains was also studied. The *femA* mutant (BB403) of BB568 (COL) showed lower autolytic activity than did its parent, in accordance with the findings described above. However, *femA* inactivation in BB399 (DU4916) resulted in an increase in autolytic activity. Apparently, the genetic background of the strain affects the influences of *mec* and *femA* on autolytic activities.

When the DU4916 (fourth) set of strains was examined, we confirmed our previously reported finding with these organisms (11). DU4916 showed very low autolytic activity, whereas DU4916-K7 and DU4916S showed high Triton X-100-stimulated autolytic activities (Table 1).

It should be noted that BB399 (DU4916 maintained in the laboratory of B. Berger-Bachi for about a decade) showed a faster rate of Triton X-100-stimulated autolysis than did DU4916 (DU4916 maintained in the laboratory of B. J. Wilkinson). The reason for this is not known. However, the daptomycin-induced and CCW autolysis rates were very similar.

Overall, unstimulated rates of autolysis were slow and either were not much different between strains, e.g., ANS46 and ANS62, or tended to parallel Triton X-100-stimulated autolysis rates, e.g., SC4 and SC4*mec*C5.

Daptomycin-induced autolysis. Daptomycin-induced autolysis was used to determine whether the findings noted above would also be observed with another method of stimulating autolysis. The results with the heterogeneous *femA* series (set 1) are shown in Fig. 3. The fastest rate of lysis was seen with BB270, which contains *femA* and *mec*. BB308 (*mec*) showed a slow rate of autolysis, whereas the *femA*$^+$ revertant of BB308 (BB314) showed a faster rate of autolysis.

In the other sets of strains studied, daptomycin-induced autolysis tended to parallel Triton X-100-stimulated autolysis, although the results were not as dramatic (data not shown). As was noted previously for strain DU4916 (11) (data not shown) and strain BB399 (Fig. 3), daptomycin inhibited growth, but lysis did not follow.

CCW autolysis. CCW autolysis in the presence and absence of lysozyme, RNase, and trypsin was measured for strains DU4916, DU4916-K7, and DU4916S; ANS46 and ANS62; BB255, BB270, and BB308; BB568 and BB403; and BB399 and BB401. CCW autolysis was faster in 0.05 M sodium acetate buffer (pH 5) than in 0.05 M potassium phosphate buffer (pH 7.2). RNase and lysozyme stimulated autolysis, whereas trypsin had little effect. There were no striking differences in CCW autolysis for strains DU4916, DU4916-K7, and DU4916S; BB255, BB270, and BB308; and BB568 and BB403. These observations suggest that in these strains, differences in autolysis seen in intact cells were not reflected in CCWs. However, strain BB401 CCWs autolysed much more rapidly than did those of strain BB399, mirroring the differences seen in the autolysis of whole cells (Fig. 4).

DISCUSSION

Three distinct heterogeneous methicillin-resistant strains showed faster rates of autolysis than did their methicillin-susceptible parental transduction recipients or derived methicillin-susceptible strains. This result implies that methicillin resistance is associated with a faster rate of autolysis in these heterogeneous strains. This finding is also compatible with the report by Seligman (30) of an unusual increased autolytic activity in methicillin-resistant strains. Also, Wale et al. (40) observed that a methicillin-resistant strain of *S.*

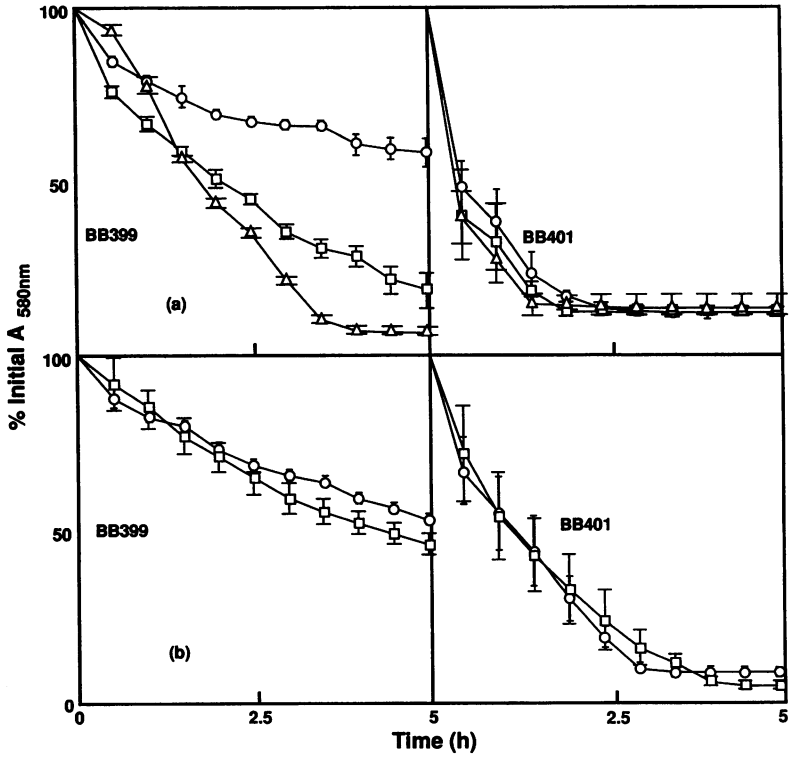

FIG. 4. Autolysis of CCWs of homogeneous methicillin-resistant *S. aureus* BB399 and *femA* mutant strain BB401. (a) Isolated CCWs suspended in 0.1 M sodium acetate buffer (pH 5.0) with no additions (○) or with RNase (0.5 mg/ml) (□) or lysozyme (0.5 mg/ml) (△). (b) Isolated CCWs suspended in 0.05 M potassium phosphate buffer (pH 7.2) with no additions (○) or with trypsin (5 μg/ml). Each point represents the mean of three experiments, and the standard deviations of the points is represented by error bars.

aureus showed a faster rate of daptomycin-induced autolysis than did an unrelated methicillin-susceptible strain.

The expression of methicillin resistance is affected by auxiliary genes other than the *mec* determinant and located elsewhere on the chromosome (2, 24). One such gene is *femA* (2). From our studies, it appears that *femA* interacts with *mec* to influence the rate of autolysis of a strain. *femA* mutants of heterogeneous resistant transductant BB270 and homogeneous resistant strain COL had slower rates of autolysis than did the *femA*$^+$ parental strains. de Jonge et al. (7) studied the autolysis characteristics of Tn*551* insertion mutants with insertions outside the *mec* determinant of homogeneous strain COL, including one, RUSA III-3, in which the insertion may have been in *femA*. The mutants, which had greatly decreased methicillin resistance and showed heterogeneous resistance expression, also showed reduced autolysis and cell wall turnover rates. *femA* codes for a 48-kDa protein, and no significant homologies were found with other known proteins, except for that coded for by a related gene, *femB* (2). The precise role of the FemA protein in the cell is unknown. However, Maidhof et al. (20) showed that *femA* mutants of methicillin-resistant and -susceptible strains had a reduced peptidoglycan glycine content. Compared with *femA*$^+$ strains, these mutants showed slower rates of lysostaphin digestion of peptidoglycan, whole-cell autolysis, and cell wall turnover and increased susceptibility to β-lactam antibiotics.

It appears that the *mec* determinant in some way increases the autolytic activity of heterogeneous resistant strains. This increased autolytic activity cannot be expressed in the absence of a functional *femA* gene. The results of Maidhof et al. (20) suggest that cells with a reduced peptidoglycan glycine content cannot express methicillin resistance well. It is possible that peptidoglycan with a lower glycine content is a poor participant in peptidoglycan biosynthesis in methicillin-resistant strains in the presence of β-lactam antibiotics.

How might the *mec* region of the chromosome influence the autolytic activity of a strain? The *mec* region, in addition to encoding the gene for PBP 2a, may also encode resistance to tetracycline, mercurial agents, and cadmium ions (15). The expression of some of these determinants may well involve the production of membrane proteins which may affect the autolytic activity of a cell. Alternatively, it is not inconceivable that PBP 2a could also have some autolytic activity. Dollinger et al. (8) reported that the second peptidoglycan hydrolase of *Enterococcus hirae* (*Streptococcus faecium*) covalently binds penicillin. However, de Jonge et al. (7) found that a mutant of a methicillin-resistant strain with a Tn*551* insertion in *mec* retained a relatively fast rate of autolysis, apparently arguing against PBP 2a having autolytic activity.

The increased autolytic activities of cells bearing intact *mec* and *femA* genes were not reflected in faster rates of CCW autolysis. It appears that autolysins are regulated differently in intact cells than in isolated CCWs. CCWs from strain DU4916 and with poorly cross-linked peptidoglycan show a fast rate of autolysis that is not expressed by intact cells (25). Similar findings were obtained with strains BB270 and BB568 grown in the presence of methicillin (data not shown). In an intact cell, the wall is subjected to hydrostatic pressure that is not present in isolated CCW suspensions. According to Koch's surface stress theory of wall growth, the stress from the hydrostatic pressure on bonds in peptidoglycan makes the bonds less stable, lowers the free

energies of hydrolysis and activation, and accelerates splitting by autolytic enzymes (16).

Phenotypic expression of methicillin is increased or decreased by a variety of chemical and physical factors (29). In view of the possible correlation of methicillin resistance and autolysis, it is interesting to examine the effects of resistance-altering factors on autolysis. Growth with NaCl and in the presence of β-lactams increases resistance expression (29) and increases the potential for autolysis (19, 25). Growth at high temperatures, at pH 5.2, and in the presence of chelating agents decreases resistance expression. The effects of these factors on autolysis do not appear to have been studied systematically.

de Jonge et al. (7) suggested that the fast rate of wall turnover seen in methicillin-resistant strains serves to excise anomalous segments of wall inserted during the different degrees of acylation of PBPs that occur upon exposure to β-lactams (44). However, methicillin-resistant strains can apparently tolerate large amounts of peptidoglycan that is abnormal in being poorly cross-linked (25, 45). Rather than excising anomalous segments of wall, the function of the increased rate of autolysis might be to aid in cell division. Although methicillin-resistant strains grow in the presence of β-lactam antibiotics, the wall that is synthesized is chemically abnormal (25, 45) and septa are grossly enlarged (43); these abnormalities may interfere with cell division and separation.

The results obtained with DU4916 and its heterogeneous and susceptible derivatives in our previous study (11) and the current study are exactly opposite those obtained with the other strains studied. Strain DU4916 shows a variety of low lytic activities, including unstimulated lysis and lysis stimulated by Triton X-100, growth with NaCl, daptomycin, vancomycin, and D-cycloserine (this study; 11, 25). The reasons for the low lytic activity phenotype of this strain are not known. Indications are that the "defect" involves the autolysin substrate or autolysin regulation, rather than an absence of autolysins. Autolytic activity is expressed by CCWs and is extractable by freezing-thawing or LiCl treatments, and strains derived from DU4916 have higher autolytic activities (11, 25). It is possible that there is something about the chemistry and structure of the DU4916 cell wall that confers a low susceptibility to autolysins to this strain relative to strains DU4916-K7, DU4916S, and BB401. The *femA* mutant of the other homogeneous strain studied, COL (BB568), showed a lower autolytic activity. However, we do not know whether the rate of autolysis of COL is fast or slow compared with those of heterogeneous and susceptible derivatives created by means other than Tn*551* mutagenesis. Recently, Chambers et al. (4a) reported that a spontaneous heterogeneous mutant of COL (COL-het) was more lytic than the homogeneous parent, COL.

It is also not clear, given our findings with the other sets of strains studied, how DU4916 can express homogeneous and high-level methicillin resistance in this low autolytic activity background. It is interesting that an early paper by Lacey (18) on this organism was entitled "*Staphylococcus aureus* DU4916—an atypical methicillin-resistant isolate?" All methicillin-resistant strains carry *mec*, but its expression is modulated by various auxiliary genes (37). Presumably, the genetic background of DU4916 is different from those of the other strains examined.

In summary, there is an association between the heterogeneous expression of methicillin resistance and increased autolysis involving at least the *mec* and *femA* determinants. Further work is necessary to establish whether autolysis plays a direct role in methicillin resistance and to describe the mechanistic details of increased autolytic activity.

ACKNOWLEDGMENTS

This work was supported by Swiss National Science Foundation grant 32-26279.89 to Brigitte Berger-Bächi.

We are grateful to Eli Lilly & Co. for supplying daptomycin.

REFERENCES

1. **Berger-Bächi, B.** 1983. Insertional inactivation of staphylococcal methicillin resistance by Tn*551*. J. Bacteriol. **154:**479–487.
2. **Berger-Bächi, B., L. Barberis-Maino, A. Strässle, and F. H. Kayser.** 1989. *femA*, a host-mediated factor essential for methicillin resistance in *Staphylococcus aureus*: molecular cloning and characterization. Mol. Gen. Genet. **219:**263–269.
3. **Berger-Bächi, B., A. Strässle, and F. H. Kayser.** 1986. Characterization of an isogenic set of methicillin-resistant and susceptible mutants of *Staphylococcus aureus*. Eur. J. Clin. Microbiol. **5:**697–701.
4. **Chain, E., and E. S. Duthie.** 1945. Bacteriocidal and bacteriolytic activity of penicillin on the staphylococcus. Lancet **i:**652–657.

4a. **Chambers, H. F., C. J. Hackbarth, and C. Miick.** 1991. Program Abstr. 31st Intersci. Conf. Antimicrob. Agents Chemother., abstr. 488.

5. **Chambers, H. F., B. J. Hartman, and A. Tomasz.** 1985. Increased amounts of a novel penicillin-binding protein in a strain of methicillin-resistant *Staphylococcus aureus* exposed to nafcillin. J. Clin. Invest. **76:**325–331.
6. **Cohen, S., and H. M. Sweeney.** 1970. Transduction of methicillin resistance in *Staphylococcus aureus* dependent on an unusual specificity of the recipient strain. J. Bacteriol. **104:**1158–1167.
7. **de Jonge, B. L. M., H. de Lencastre, and A. Tomasz.** 1991. Suppression of autolysis and cell wall turnover in heterogeneous Tn*551* mutants of a methicillin-resistant *Staphylococcus aureus* strain. J. Bacteriol. **173:**1105–1110.
8. **Dollinger, D. L., L. Daneo-Moore, and G. D. Shockman.** 1989. The second peptidoglycan hydrolase of *Streptococcus faecium* ATCC 9790 covalently binds penicillin. J. Bacteriol. **171:**4355–4361.
9. **Dornbusch, K., and H. O. Hallander.** 1973. Transduction of penicillinase production and methicillin resistance-enterotoxin B production in strains of *Staphylococcus aureus*. J. Gen. Microbiol. **76:**1–11.
10. **Dornbusch, K., H. O. Hallander, and F. Lofquist.** 1969. Extrachromosomal control of methicillin resistance and toxin production in *Staphylococcus aureus*. J. Bacteriol. **98:**351–358.
11. **Gustafson, J. E., and B. J. Wilkinson.** 1989. Lower autolytic activity in a homogeneous methicillin-resistant *Staphylococcus aureus* strain compared to derived heterogeneous-resistant and susceptible strains. FEMS Microbiol. Lett. **59:**107–112.
12. **Hartman, B. J., and A. Tomasz.** 1984. Low-affinity penicillin-binding protein associated with beta-lactam resistance in *Staphylococcus aureus*. J. Bacteriol. **158:**513–516.
13. **Hartman, B. J., and A. Tomasz.** 1986. Expression of methicillin resistance in heterogeneous strains of *Staphylococcus aureus*. Antimicrob. Agents Chemother. **29:**85–92.
14. **Henenine, N., and P. R. Stewart.** 1986. Physiological determination of methicillin resistance in *Staphylococcus aureus*: comparison of clinical and genetically derived isolates. J. Antimicrob. Chemother. **17:**705–715.
15. **Inglis, B., P. R. Mathews, and P. R. Stewart.** 1990. Induced deletions within a cluster of resistance genes in the *mec* region of the chromosome of *Staphylococcus aureus*. J. Gen. Microbiol. **136:**2231–2239.
16. **Koch, A. L.** 1985. How bacteria grow and divide in spite of internal hydrostatic pressure. Can. J. Microbiol. **31:**1071–1083.
17. **Kornblum, J., B. J. Hartman, R. P. Novick, and A. Tomasz.** 1986. Conversion of a homogeneously methicillin resistant strain of *Staphylococcus aureus* to heterogeneous resistance by Tn*551*-mediated insertional inactivation. Eur. J. Clin. Microbiol. **5:**714–718.

18. **Lacey, R. W.** 1974. *Staphylococcus aureus* DU4916—an atypical methicillin-resistant isolate? J. Gen. Microbiol. **84:**1–10.
19. **Madiraju, M. V. V. S., D. P. Brunner, and B. J. Wilkinson.** Effects of temperature, NaCl, and methicillin on penicillin-binding proteins, growth, peptidoglycan synthesis, and autolysis in methicillin-resistant *Staphylococcus aureus*. Antimicrob. Agents Chemother. **31:**1727–1733.
20. **Maidhof, H., B. Reinicke, P. Blümel, B. Berger-Bächi, and H. Labischinski.** 1991. *femA*, which encodes a factor essential for expression of methicillin resistance, affects glycine content of peptidoglycan in methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* strains. J. Bacteriol. **173:**3507–3513.
21. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
22. **Mathews, P. R., K. C. Reed, and P. R. Stewart.** 1987. The cloning of chromosomal DNA associated with methicillin and other resistances in *Staphylococcus aureus*. J. Gen. Microbiol. **133:**1919–1929.
23. **Matsuhashi, M., M. D. Song, F. Ishino, M. Wachi, M. Doi, M. Inoue, K. Ubukata, N. Yamashita, and M. Konno.** 1986. Molecular cloning of the gene of a penicillin-binding protein supposed to cause high resistance to β-lactam antibiotics in *Staphylococcus aureus*. J. Bacteriol. **167:**975–980.
24. **Murakami, K., and A. Tomasz.** 1989. Involvement of multiple genetic determinants in high-level methicillin resistance in *Staphylococcus aureus*. J. Bacteriol. **171:**874–879.
25. **Qoronfleh, M. W., and B. J. Wilkinson.** 1986. Effects of growth of methicillin-resistant and -susceptible *Staphylococcus aureus* in the presence of beta-lactams on peptidoglycan structure and susceptibility to lytic enzymes. Antimicrob. Agents Chemother. **29:**250–257.
26. **Reynolds, P. E.** 1984. Resistance of the antibiotic target site. Br. Med. Bull. **40:**3–10.
27. **Reynolds, P. E., and D. F. J. Brown.** 1985. Penicillin-binding proteins of beta-lactam-resistant strains of *Staphylococcus aureus*: effect of growth conditions. FEBS Lett. **192:**28–32.
28. **Rogers, H. F.** 1967. Killing of staphylococci by penicillins. Nature (London) **213:**31–33.
29. **Sabath, L. D.** 1977. Chemical and physical factors influencing methicillin resistance of *Staphylococcus aureus* and *Staphylococcus epidermidis*. J. Antimicrob. Chemother. **3**(Suppl. C)**:**47–51.
30. **Seligman, S. J.** 1971. Autolytic activity in methicillin-resistant *Staphylococcus aureus*, p. 218–222. Antimicrob. Agents Chemother. 1970.
31. **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.
32. **Smith, P. F., and B. J. Wilkinson.** 1981. Differential methicillin susceptibilities of peptidoglycan syntheses in methicillin-resistant *Staphylococcus aureus*. J. Bacteriol. **148:**610–617.
33. **Song, M. D., M. Wachi, M. Doi, F. Ishino, and M. Matsuhashi.** 1987. Evolution of an inducible penicillin-target protein in methicillin-resistant *Staphylococcus aureus* by gene fusion. FEBS Lett. **221:**167–171.
34. **Sutherland, R., and G. N. Rollison.** 1964. Characteristics of methicillin-resistant staphylococci. J. Bacteriol. **87:**887–889.
35. **Tesch, W., C. Ryffel, A. Strässle, F. H. Kayser, and B. Berger-Bächi.** 1990. Evidence of a novel staphylococcal *mec*-encoded element (*mecR*) controlling expression of penicillin-binding protein 2′. Antimicrob. Agents Chemother. **34:**1703–1706.
36. **Tomasz, A.** 1987. Resistance and tolerance to β-lactam antibiotics in pneumococci and in *Staphylococcus aureus*, p. 616–628. *In* P. Actor, L. Daneo-Moore, M. L. Higgins, M. R. J. Salton, and G. D. Shockman (ed.), Antibiotic inhibition of bacterial cell surface assembly and function. American Society for Microbiology, Washington, D.C.
37. **Tomasz, A., S. Nachman, and H. Leaf.** 1991. Stable classes of phenotypic expression in methicillin-resistant clinical isolates of staphylococci. Antimicrob. Agents Chemother. **35:**124–129.
38. **Tomasz, A., and S. Waks.** 1975. Mechanism of action of penicillin: triggering of the pneumococcal autolytic enzyme by inhibitors of cell wall synthesis. Proc. Natl. Acad. Sci. USA **72:**4162–4166.
39. **Ubukata, K., N. Yamashita, and M. Konno.** 1985. Occurrence of a beta-lactam-inducible penicillin-binding protein in methicillin-resistant staphylococci. Antimicrob. Agents Chemother. **30:**577–583.
40. **Wale, L. J., A. P. Shelton, and D. Greenwood.** 1989. Scanning electronmicroscopy of *Staphylococcus aureus* and *Enterococcus faecalis* exposed to daptomycin. J. Med. Microbiol. **30:**45–49.
41. **Wecke, J., M. Lahav, I. Ginsberg, and P. Giesbrecht.** 1982. Cell wall degradation of *Staphylococcus aureus* by lysozyme. Arch. Microbiol. **131:**116–123.
42. **Wilkinson, B. J., K. D. Dorian, and L. D. Sabath.** 1978. Cell wall composition and associated properties of methicillin-resistant *Staphylococcus aureus* strains. J. Bacteriol. **136:**976–982.
43. **Wilkinson, B. J., and M. J. Nadakavukaren.** 1983. Methicillin-resistant septal peptidoglycan synthesis in a methicillin-resistant *Staphylococcus aureus* strain. Antimicrob. Agents Chemother. **23:**610–613.
44. **Williamson, R., and A. Tomasz.** 1985. Inhibition of cell wall synthesis and acylation of the penicillin-binding proteins during prolonged exposure of growing *Streptococcus pneumoniae* to benzylpenicillin. Eur. J. Biochem. **151:**475–483.
45. **Wyke, A. W., J. B. Ward, and M. V. Hayes.** 1982. Synthesis of peptidoglycan in vivo in methicillin-resistant *Staphylococcus aureus*. Eur. J. Biochem. **127:**553–558.