SURFACE ELECTROKINETIC PROPERTIES OF TWO STRAINS OF *STAPHYLOCOCCUS AUREUS* DURING THEIR CELL GROWTH CYCLE

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SUMMARY: Two strains of *Staphylococcus aureus* were investigated: *S. aureus* H, a normal wild-type strain, and 52A5, a mutant strain whose cell wall contains no teichoic acid but is made up entirely of mucopeptide. *S. aureus* H cells in the lag or stationary phase of growth had an electrophoretic mobility of -1.10 μm/s/V/cm while those in the logarithmic phase had a mobility of -0.80 μm/s/V/cm in saline at pH 7.2, 0.6 mM $NaHCO_3$, 25°C (I = 0.145 g-ions/l). *S. aureus* 52A5 cells in the same solution had a mobility of -0.87 μm/s/V/cm in lag and stationary growth phases but a mobility of -1.30 μm/s/V/cm in the logarithmic growth phase. The *S. aureus* H cell surfaces at lag phase had pKs of 3.2 and 9.5; at logarithmic phase, 4.2 and 9.0; and at stationary phase, 3.0 and 9.5. The 52A5 cell surfaces at lag phase had pKs of 2.3 and 10.3; at logarithmic phase, 1.7 and 8.5; at stationary phase, 2.6 and 10.2.

Because of their size and of limitations due to particle conductance, bacteria have not been as readily studied by the technique of micro-particle electrophoresis as have mammalian cells. Hence much valuable information on bacterial cell surfaces has been neglected. A very substantial exception to this is the extensive work of James and co-workers (1-6) and that contained in a recent review (7). Plummer and James (3) reported that *Aerobacter aerogenes* electrophoretic mobility increased to a maximum value early in the logarithmic period of the growth cycle, while James and Brewer (1) demonstrated that pH-mobility curves of laboratory strains of *Staphylococcus aureus* passed through maxima in the range of pH 4-5. These latter authors attributed one of the determinants of the electrophoretic mobility of *S. aureus* to be cell-surface teichoic acid. In the present communication we have investigated the electrophoretic mobilities of two strains of *S. aureus*: *S. aureus* H, the parental laboratory strain with a normal cell wall, and 52A5, a mutant strain that has no teichoic acid in its cell wall, which is made up entirely of mucopeptide (8,9).

The curve of cell electrophoretic mobility vs the pH of the measuring solution, determined by the method of microelectrophoresis, is an important

characteristic of the particle surface. By this means and by measurements of electrophoretic mobility in the presence of surface-modifying agents, a great deal of information on the cell surface can accrue. The present study on the surface of microbial cells describes the electrokinetic parameters of two strains of *S. aureus* with completely different surface properties.

MATERIALS AND METHODS

Cells and culture. The properties, culture, and maintenance of *S. aureus* H and *S. aureus* 52A5 were as described previously (8,9).

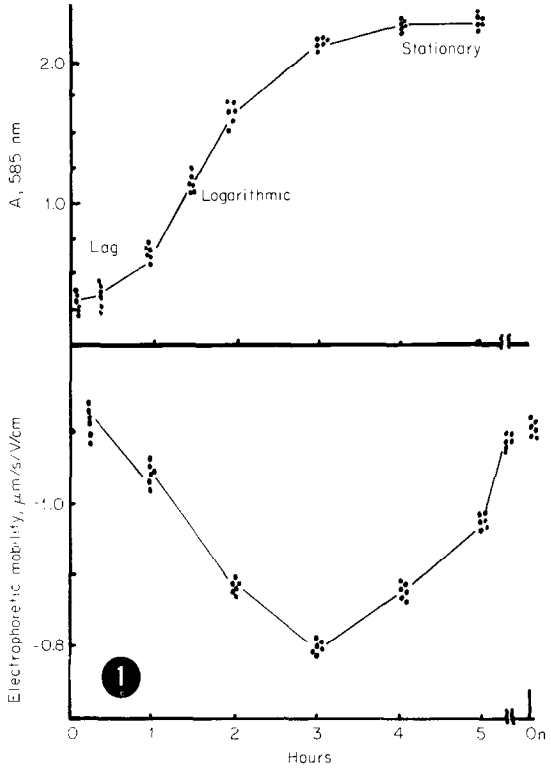
Experimental design. The strains of *S. aureus* were grown overnight to a cell density of about 2-3 x 10^9/ml; this is designated on the figures as ON and represents 0 hr. An aliquot of the overnight *S. aureus* culture was dispensed into fresh medium, and this culture was sampled at intervals between 0 hr and 5 hr. Aliquots of the culture were sampled for determination of growth by measuring absorbance at 585 nm and for determination of electrophoretic mobility.

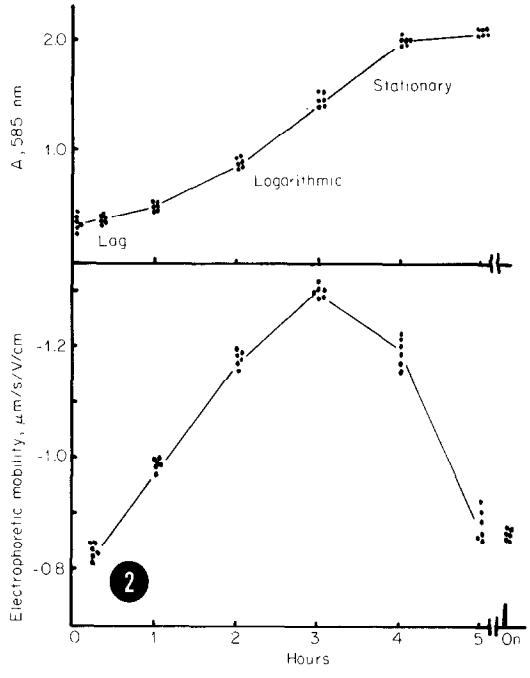
Solutions. All solutions were prepared in glass-distilled water. The solution used in these studies, termed saline, contained 0.145 NaCl, 0.6 mM $NaHCO_3$, pH 7.2 ± 0.1. This solution and isosmotic solutions of NaOH and HCl for adjusting solution pH were prepared as described by Heard and Seaman (10).

Electrophoretic mobilities. Measurements were made at 25°C (±0.1) in a horizontal cylindrical chamber of 5 ml volume equipped with reversible blacked platinum electrodes (11-14). The method essentially measures the movement of particles in an electric field in a defined medium. The surface is treated as an ampholyte much like a protein so multiple *apparent bulk pK* values result as mobility is measured as a function of pH; at a mobility of zero the *isopotential point* of the surface is measured, similar to the isoelectric point of a protein. Since in the present studies *apparent bulk pK* values were determined graphically, differences of greater than 0.2 units are significant. The chamber was viewed by transillumination in the apparatus obtained from Rank Brothers, Bottisham, England. The mobilities of the particles were calculated in μm/s/V/cm; each value was obtained by timing the movement of at least 20 bacteria, with reversal of polarity after each measurement. The alignment of the apparatus was checked by the method of Heard and Seaman (10). In order to calibrate and calculate instrument parameters human erythrocytes were used as standard particles. Determinations of the mobility of washed human erythrocytes were made in 0.0145 M NaCl, 4.5% sorbitol made 0.6 mM with respect to $NaHCO_3$. Normal blood for this purpose was obtained from healthy donors of the phenotype A Rh^+, taken into EDTA, immediately washed, and electrophoretic mobilities were determined. Heard and Seaman (10) reported a value of -2.78 ± 0.08 μm/s/V/cm for the electrophoretic mobility of human erythrocytes; in the present experiments we found a value of -2.78 ± 0.04 μm/s/V/cm in saline sorbitol. A minimum of six independent experiments were performed for each electrophoretic mobility determination.

RESULTS AND DISCUSSION

The data of Fig. 1 indicate that the lag phase *S. aureus* H cells had an electrophoretic mobility of -1.10 μm/s/V/cm while the lag phase *S. aureus*

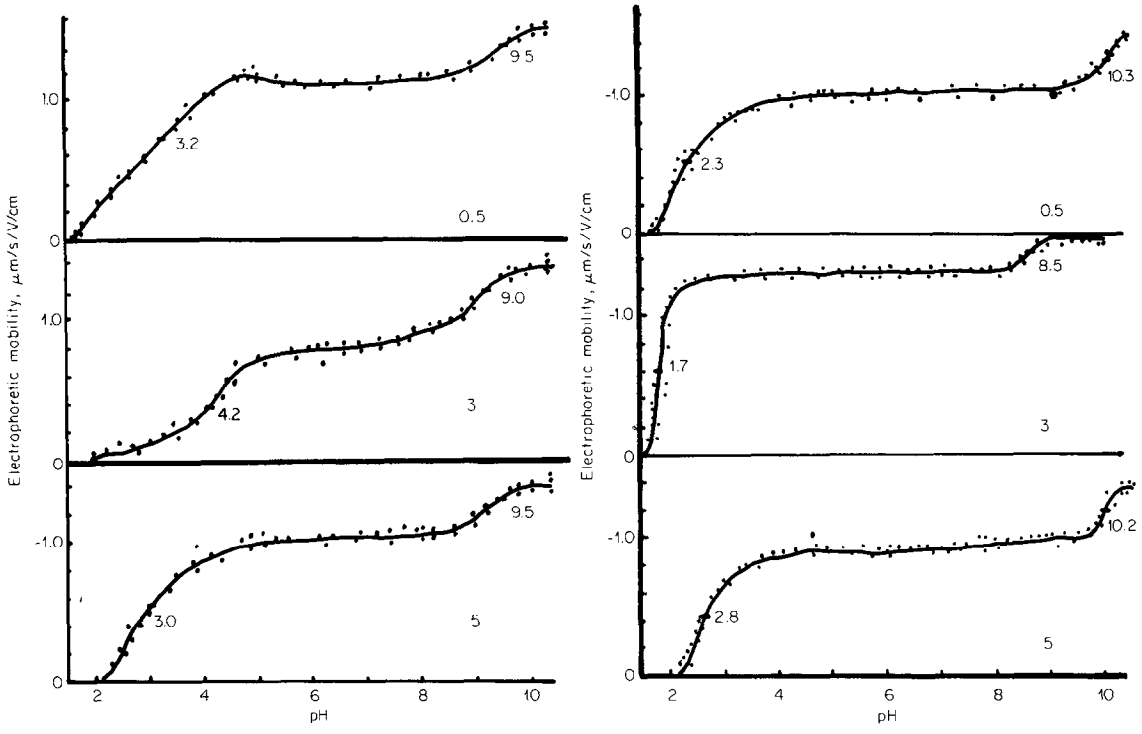




Figures 1 and 2. Electrophoretic mobility of *Staphylococcus aureus* as a function of growth cycle time. The upper panel represents the growth cycle and the lower panel, the electrophoretic mobility in saline at pH 7.2, 0.6 mM $NaHCO_3$, 25°C (I = 0.145 g-ions/l). Experiments were carried out as given in Materials and Methods. For the growth determinations each point represents mean values (3 determinations) for single independent experiments. For the electrophoretic mobilities each point represents mean value of at least 20 bacteria measured in a single independent experiment. ON refers to cells held overnight. Figure 1: *S. aureus* H. Figure 2: *S. aureus* 52A5.

52A5 cells had a mobility of -0.87 μm/s/V/cm (Fig. 2). The curves in Fig. 3 show that the *S. aureus* H cell surfaces in lag phase had pK values of 3.2 and 9.5, while the curves of Fig. 4 demonstrate that the lag phase *S. aureus* 52A5 cell surfaces had pK values of 2.3 and 10.3. These data indicate that in lag phase the *S. aureus* H surface is characterized by anionic groups, probably from both teichoic acid and amino acids, and also contains free amino groups. The 52A5 surface at lag phase is characterized by carboxyl groups of amino acids and free amino groups, possibly of amino acids (e.g., pK_2 of proline = 10.6).

The data for the logarithmic phase cells are striking. They show (1) a *major* difference from the respective lag phase surface characteristics, and (2) extreme differences between the *S. aureus* H and 52A5 surfaces. As shown



Figures 3 and 4. Electrophoretic mobility of *Staphylococcus aureus* as a function of pH and time of growth cycle. The numbers 0.5, 3, and 5 refer to cells grown for 0.5, 3, and 5 hr, as shown in Figure 1. Electrophoretic mobilities were determined in saline at the given pH, 25°C (I = 0.145 g-ions/l) as given in Materials and Methods. For the electrophoretic mobilities each point is a mean value of at least 20 bacteria measured in a single independent experiment. Approximate pK values of the surfaces are indicated. Figure 3: *S. aureus* H. Figure 4: *S. aureus* 52A5.

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in Fig. 1, at the 3-hr logarithmic point the *S. aureus* H cells have a mobility of -0.80 μm/s/V/cm whereas the 52A5 cells have a mobility of -1.30 μm/s/V/cm (Fig. 2). The pK values for the logarithmic *S. aureus* H cells were 4.2 and 9.0 (Fig. 3) while for the 52A5 cells the surface pK values were 1.7 and 8.5 (Fig. 4). This indicates that the *S. aureus* H cell surfaces are probably mainly characterized by teichoic acid and free amino groups whereas the 52A5 cell surfaces are composed of very low pK carboxyl groups of amino acids (e.g., pK_1 of aspartic acid = 1.88) and low pK free amino groups (e.g., pK_2 lysine = 8.93). These data, when compared with the lag phase data, point up the very important facts that (1) the *S. aureus* cell surface is turning over very rapidly, (2) at different times in the cell growth cycle different groups are ionogenically expressed at the cell surface, and (3) the external cell sur-

face properties are absolutely predicated on the materials available for their biogenesis--i.e., if teichoic acid is not programmed to be an integral part of the cell wall, it cannot contribute to the bacterium's electrokinetic properties.

Although the electrophoretic mobilities of the stationary phase cells of the two *S. aureus* strains investigated were rather similar to the lag phase values (Figs. 1 and 2), the pK values for the surfaces were somewhat different (Figs. 3 and 4). This is particularly true for the pH mobility curves (Fig. 3) for *S. aureus* H; the lag phase curve has a maximum at pH 4.8 and a pK_1 of 3.2 while the stationary phase cells have a broad maximum of pH 5.5 to 8.0 and a pK_1 of 3.0. This seems to indicate that the stationary cells have more amino acid carboxyl groups and less teichoic acid phosphorous groups at the external surface than do the lag phase cells, although the pK difference is not significant.

Our results indicate that the electrokinetic parameters of *S. aureus* differ dramatically over the growth cycle and that a cell wall mutant of *S. aureus* has drastically different electrokinetic parameters. The results should be of great importance to work on microbial surfaces and on membrane turnover, molecular insertion, and fluidity in general.

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