JOURNAL OF BACTERIOLOGY, June 1967, p. 2022-2023

Vol. 93, No. 6
Printed in U.S.A.

Ion-binding Properties of the Cell Wall of *Staphylococcus aureus*

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Received for publication 10 February 1967

We have investigated the binding capacity of ions by the cell wall of *Staphylococcus aureus*, with relation to its polyionic structure. The cell wall, prepared by Salton's scheme (M. R. J. Salton, *The bacterial cell wall*, p. 58, Elsevier Publishing Co., New York, 1964) from *S. aureus* strain 22 ISI (Istituto Sieroterapico Italiano), was further treated with 0.1 M ethylenediaminetetraacetic acid (*p*H 7.5; EDTA). Electron microscopic tests as well as chemical measurements were used to check its purity. Experiments were carried out with 50 mg of lyophilized cell wall in 10 ml of cation solution, the concentration of which ranged between 2.5 and 100 meq per liter depending on the experiment, with an average of 50 meq per liter. The ions were added as chlorides. Preliminary tests showed that the highest binding capacity occurred after an incubation period of 20 hr. After incubation for 20 hr at 25 C in reciprocating shaker-baths, the cell wall material was collected by centrifugation at 7,000 $\times$ *g* for 20 min, and was washed three times with an equal volume of previously boiled, deionized water. The liquid from the third washing was cation-free. The samples were dried at 110 C, weighed, and then ashed at 500 to 600 C for 6 hr; they were then suspended again in 6 N HCl, dried, and finally taken up in a measured quantity of 0.1 N HCl. Na^+ and K^+ were measured by flame photometry with a Beckman model DU spectrophotometer; Mg^{++} and Ca^{++} were measured by titration with EDTA, with Eriochrome Black T (A. Holasek and A. Flaschka, Z. Physiol. Chem. **288**:144, 1951) and murexide (J. R. Dunstone, Med. J. Australia **2**:571, 1957), respectively, as indicators. Hydrogen ion-binding capacity was studied by suspending 50 mg of lyophilized cell wall in 10 ml of HCl at a concentration of 0.01 to 0.000001 N. After 6 hr of incubation at 25 C with shaking in a nitrogen environment, the hydrogen ion concentration was measured with a Beckman Expandomatic *p*H meter.

S. aureus cell wall bound approximately 14 μeq of K^+ and Na^+ and approximately 85 μeq of Mg^{++} and Ca^{++} per g of cell wall (dry weight). The binding capacity, though different for monovalent and bivalent ions, was the same for ions of the same valence; this indicates that ions of the same valence may be bound through the same mechanism. The binding capacity, studied in relation to the concentration of ions in the incubating medium, rapidly reached its maximum with concentrations above 10 meq per liter. Influence of anions on the binding capacity of the cell wall was studied with various salts of K^+, Na^+, Ca^{++}, and Mg^{++} in 0.01 M tris(hydroxymethyl)aminomethane (Tris) buffer at *p*H 7.4. The anion to which the cation is bound was of no importance. The ion-binding capacity varied considerably with the *p*H of the medium. High *p*H levels increased the amount of ions bound, this phenomenon being more pronounced with Mg^{++} and Ca^{++}. Cell wall preparations at *p*H 2.0 bound approximately 245 μeq of H^+ per g of cell wall (dry weight), indicating that a corresponding quantity of dissociable acidic groups was present. The results are shown in Table 1.

To check the formation of complexes, potenti-

TABLE 1. *Ion-binding capacity of the cell wall of Staphylococcus aureus strain 22 ISI*[a]

*p*H of the medium	Amt (μeq) of ion bound per g of cell wall (dry wt)				
	H^+	K^+	Na^+	Ca^{++}	Mg^{++}
2.10[b]	245	—	—	—	—
2.30[c]	—	5.6	5.6	31.2	32.0
3.16[b]	132	—	—	—	—
4.28[b]	13	—	—	—	—
4.50[d]	—	7.4	7.5	62.7	63.1
7.20[e]	—	14.3	14.7	85.9	86.2
9.10[e]	—	16.8	16.9	129.0	130.0

[a] Results are the mean values of 10 experiments.
[b] HCl solution.
[c] Glycine hydrochloride buffer, 0.05 M.
[d] Citric acid-sodium citrate buffer, 0.05 M.
[e] Tris chloride buffer, 0.05 M.

ometric-titration curves of the cell wall in the presence or the absence of cations were determined. A 200-mg amount of cell wall was suspended in 50 ml of neutral, deionized water and titrated with carbonate-free 0.1 N KOH in both the absence and the presence of cation (0.005 M). The final volume was 50 ml; 0.5 ml of 0.1 N KOH was added every 30 min. After every addition of KOH, the *p*H was measured with a Beckman Expandomatic *p*H meter. Nitrogen was bubbled through the liquid to obtain good mixing and to keep the atmosphere above the solution inert.

Figure 1 shows that, when the cell wall suspension was titrated in the presence of Ca^{++} and Mg^{++}, the titration curve shows a definite decrease of *p*H, whereas, with Na^{+} and K^{+}, the decrease is noticeably smaller. There was sufficient evidence to indicate that a bivalent cation is bound by two dissociable acidic groups, thus forming complexes. Na^{+} and K^{+} also caused a decrease in *p*H which, however, was much smaller than that observed for Ca^{++} and Mg^{++}. It has to be postulated that for Na^{+} and K^{+} there is also an ionic-type bond with the acidic groups of the cell wall. These data suggest that the cell wall behaves like a weak ion-exchange resin. Studies on the cell wall exchange capacity are in progress in our laboratory.

This investigation was carried out with the help of the "Consiglio Nazionale delle Ricerche."

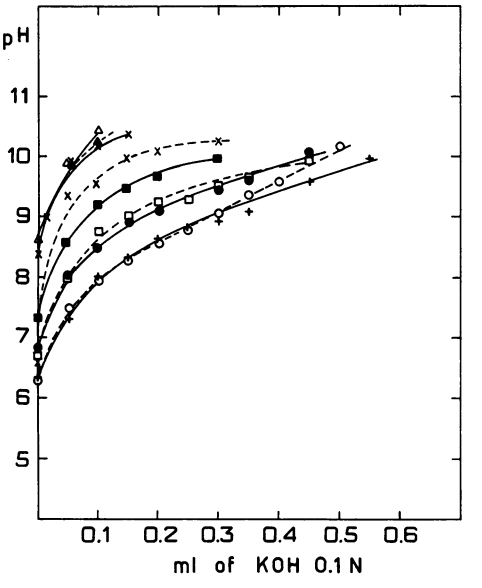

FIG. 1. *Potentiometric titrations of cations in the presence and absence of cell wall. Symbols:* ■, *200 mg of cell wall in water;* ▲, *0.005 M NaCl;* × (*solid line*), *0.005 M KCl;* △, *0.005 M* $CaCl_2$; × (*broken line*), *0.005 M* $MgCl_2$; □, *200 mg of cell wall in water plus 0.005 M NaCl;* ●, *200 mg of cell wall in water plus 0.005 M KCl;* ○, *200 mg of cell wall in water plus 0.005 M* $CaCl_2$; +, *200 mg of cell wall in water plus 0.005 M* $MgCl_2$.