Polypelectrolyte Nature of Bacterial Teichoic Acids


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Several physicochemical properties of the teichoic acid of Bacillus subtilis 168 have been determined. The teichoic acid partial specific volume was found to be 0.57 ml/g. The apparent weight-average molecular weight of the polymer was 24,800. Sedimentation was strongly dependent on solvent. The sedimentation coefficient of the teichoic acid was found to have a value of $s_{20,w}^o = 1.90S$. In dilute buffers and distilled water, the teichoic acid possessed a rigid rod or extended conformation. Salts induced a loss of secondary structure in the polymer, resulting in a random coil configuration. Salt-induced structural changes in the teichoic acid were determined by viscosities, ultraviolet difference spectra, and inhibition of precipitation with concanavalin A. Divalent cations such as Mg$^{2+}$ had little effect on the teichoic acid structure. The salt-induced structural changes were reversible, as evidenced by return of the original properties upon dialysis of the teichoic acid against water. Sodium chloride inhibited the adsorption of bacteriophage $\phi$25 to B. subtilis cell walls. Teichoic acid conformation may have a significant influence on the physiology of bacteria.

Baddiley (7) has defined teichoic acids as "all wall and membrane polymers that possess phosphodiester groups, polyols, and/or sugar residues, and usually, but not always, $\beta$-alanine ester residues." Teichoic acids, containing either glycerol or ribitol as the polyol, occur in the cell walls of most gram-positive bacteria (5). Glycerol teichoic acids are found associated with lipid in the membranes of many gram-positive bacteria (39, 43).

The structure and function of bacterial teichoic acids is still not well understood. A great amount of information has accumulated concerning composition and sequence (5, 7); however, little is known about the physicochemical properties of the polymers. Functionally, teichoic acids appear to play several roles. Kohoutová (20) has shown that teichoic acids serve as competence factor receptor sites in Diplococcus pneumoniae. Young (47) and Glaser et al. (17) have shown that teichoic acids serve as bacteriophage receptor sites in Bacillus subtilis. Hughes et al. (18) proposed that teichoic acids, by virtue of their divalent cation binding ability, function to sequester necessary ions for cation-dependent membrane enzymes. However, more recent evidence from experiments with teichoic acid-deficient staphylococci demonstrates that teichoic acid does not significantly bind divalent cations (27). Teichoic acid-free mutants of bacilli frequently possess bizarre morphologies (40). Thus, teichoic acids may have a role in cell separation. In this respect, it is interesting to note that the autolytic system of Diplococcus pneumoniae is largely inactive when choline is absent from its teichoic acid (38), and these cells are non-competent as well (37).

We have initiated studies on the purification (16), interaction properties (15; T. Z. Kan, R. J. Doyle, and D. C. Birdsell, Carbohydrate Res., in press), location in the cell wall, and site of biosynthesis of the B. subtilis teichoic acid. In this report, we show that teichoic acid structure is markedly sensitive to ionic environment. The data suggest that in water and dilute buffers the teichoic acid exists in an extended conformation, but that in solutions of high ionic strength a random coil configuration is present. (This work was presented in part at the Annual Meeting of the American Society for Microbiology, Philadelphia, Pa., 1972.)

MATERIALS AND METHODS

Bacterial cultures and growth. B. subtilis 168 was used for all the experiments described here. The cells were grown in Spizizen minimal medium (32) supplemented with 0.08% casein hydrolysate (Nutritional Biochemicals Corp., Chagrin Falls, Ohio), 0.005% L-tryptophan, and 0.5% glucose as the carbon source.
source. The cells were harvested as described previously (15).

**Bacteriophage.** A lysate of bacteriophage φ25 was prepared as described earlier (10).

**Cell walls and teichoic acid.** Cell walls from *B. subtilis* strain 168 were then autolyzed the prepared at 4°C acid isolated from the amino acid was washed twice with acetone-ethanol and once with cold ether, dissolved in water, and lyophilized.

**Preparation of Con A and [¹⁴C]Con A.** Concanavalin A (Con A) was purified by the method of Agrawal and Goldstein (1) and stored as a salt-free powder at –20°C. The [¹⁴C]Con A was prepared by a modification of the method described by Agrawal et al. (3), in which acetic anhydride is used to acetylate free amino groups. At 15-min intervals, three 15-μlter volumes of acetic [¹⁴C]anhydride (New England Nuclear Corp., Boston, Mass.) were added to 500 ml of Con A dissolved in half-saturated sodium acetate. The solution was kept cold in an ice bath. One hour after the last addition of acetic anhydride, the solution was placed in a dialysis bag and dialyzed against cold 0.04 M tris(hydroxymethyl)aminomethane hydrochloride (pH 7.4) until the dialysate was free from radioactivity. The [¹⁴C]Con A was then frozen until ready for use.

**Scintillation counting.** All samples for radioactivity determinations (0.5 ml) were dissolved in 12 ml of Multisol (Isolab, Inc., Akron, Ohio) and counted in a Packard Tri-Carb automatic scintillation spectrometer. Internal standards were run ([¹⁴C]Con A) to correct for quenching.

**Chemical analyses.** Glucose was determined by the method of Mokrash with the anthrone reagent (26). Phosphorous was determined by the ashing technique of Ames (4). Protein was quantitated by the standard Lowry (21) method with Con A as a standard. Con A solutions were standardized by ultraviolet absorbance measurements, assuming that 1 mg of Con A per ml gives an absorbance of 1.14 at 280 nm (2).

**Sedimentation and molecular weight studies.** Sedimentation equilibrium studies were performed by the method of Yphantis (48). A Spinco model E ultracentrifuge equipped with Rayleigh interference optics and automatic temperature control was used in all sedimentation runs. Teichoic acid (0.03%) was dissolved in 0.3 M NaCl and dialyzed against the same solvent. The dialysate was used as the reference solvent. An AnD rotor was employed by using the standard, double-sector, aluminum-filled Epon cell with a 12-mm light path. The rotor speed was 33,450 rpm. Equilibrium times were determined by trial and error. The runs were terminated when there was no further increase in fringe displacement with time. All runs were at 20°C. A Gaertner two-dimensional comparator was employed to measure fringes. The results were plotted in the usual manner (13, 48): log c versus r², where c is the net fringe displacement (corrected for solvent) and r is the position in the cell. The apparent weight-average molecular weight of the teichoic acid was calculated by the following equation (13):

\[
M_w = \frac{2RT}{(1 - \bar{v}_p)w^2} \times \frac{2.303}{d(r^2)} \frac{\text{log c}}{\text{water}}
\]

where R is the universal gas constant (8.314 ergs per degree per mol), T is the absolute temperature, \(\bar{v}\) is the partial specific volume, \(\rho\) is the density of solvent, and \(w\) is angular velocity of the rotor in radians per second.

Sedimentation velocity runs were performed by using schlieren optics. All runs were at 20°C with a speed of 59,760 rpm. Observed sedimentation coefficients, s₂₀,w, were corrected to the conditions of 20°C and water by the following equation (13):

\[
s_{20,w} = s_{20,0} \times \frac{\eta_{\text{solvent}}}{\eta_{\text{water}}} \times \frac{(1 - \bar{v}_p)\text{water}}{(1 - \bar{v}_p)\text{solvent}}
\]

where \(s_{20,w}\) is the corrected sedimentation coefficient, \(\eta\) is the viscosity, and \(\rho\) is the density of water or solvent at 20°C. Temperature corrections were unnecessary since all runs were at 20°C. The densities and viscosities of solvent were determined experimentally.

**Partial specific volumes.** The apparent partial specific volume, \(\bar{v}\), of the teichoic acid was determined pycnometrically (13) with 1- and 2-ml pycnometers (Arthur H. Thomas, Philadelphia, Pa.). The solvent was water. Teichoic acid concentrations of 1 and 2% were used. All determinations were made at 22°C.

**Viscosities.** Teichoic acid viscosities were determined at 25°C in Cannon-Ubbelohde dilution viscometers (Cannon Instrument Co., State College, Pa.). Solvent flow times were between 180 and 240 s, thus eliminating the requirement for Ubbelohde correction. Intrinsic viscosities were determined according to the following equation:

\[
[\eta] = \lim_{c \to 0} \eta_{sp,c}
\]

where [\(\eta\)] is the intrinsic viscosity in dl per gram, \(\eta_{sp,c}\) is the specific viscosity, \(c\) is teichoic acid concentration in grams per dl. Plots of \(\eta_{sp,c}\) (reduced viscosity) versus \(c\) were constructed, and [\(\eta\)] was obtained by extrapolation to zero concentration.

**Difference spectra.** Ultraviolet spectra were obtained by use of a Cary 15 spectrophotometer equipped with a 0- to 0.1-absorbance slide-wire. Difference spectra were determined by the standard tandem arrangement (46). Spectra were recorded at room temperature (22 ± 2°C).

**Isionic point.** The teichoic acid is isionic pH, which approximates the isoelectric pH (39), was determined according to a modification of the method of Janus et al. (42). Teichoic acid (5 mg/ml) in deionized, deionized water was dialyzed overnight against water containing suspended Amberlite MB-3 mixed-bed exchange resin. A constant stream of nitrogen purged
the dialysis system. After dialysis, the pH of the teichoic acid solution was obtained by use of a Corning model 7 pH meter equipped with a combination electrode. The dialysis bags were first treated with boiling 1% ethylenediaminetetraacetic acid and then stored under nitrogen in the presence of the resin.

RESULTS

Viscosity studies. Teichoic acids should exhibit typical polyelectrolyte properties in aqueous solutions. In distilled water, a plot of reduced viscosity versus teichoic acid concentration yields an upward curvature at low teichoic acid concentrations (Fig. 1). The intrinsic viscosity, \( \eta_0 \), obtained by extrapolation, was found to be 0.48 dl/g for the teichoic acid in water. In the presence of 10^{-3} M Mg^{2+}, an intrinsic viscosity of 0.44 dl/g was determined. However, at low teichoic acid concentrations, a downward curvature was observed for the teichoic acid in 10^{-3} M Mg^{2+}. The intrinsic viscosity of the same teichoic acid preparation in 1.0 M sodium chloride was found to be 0.13 dl/g, and the reduced viscosity concentration curve was linear. Thus, sodium chloride markedly affects the teichoic acid structure in solution.

If the teichoic acid undergoes a gradual structural change in the presence of sodium chloride, plots of reduced viscosity versus sodium chloride concentration should be smooth. The change of teichoic acid-reduced viscosity with sodium ion concentration is shown in Fig. 2. It is apparent that the transition was complete above 0.25 M sodium chloride. Similar changes are observed when intrinsic viscosities are obtained as a function of sodium chloride concentration (inset, Fig. 2). The change in the intrinsic viscosity of the teichoic acid was linear between sodium chloride concentrations of approximately 10^{-3} M and 0.2 M. Thus, below 10^{-3} M and above 0.2 M sodium chloride, the intrinsic viscosity of the teichoic acid did not significantly change. The intrinsic viscosity in deionized water was the same as that in 10^{-3} M sodium chloride. In addition to sodium chloride, other salts, such as potassium chloride, lithium chloride, and guanidine hydrochloride, also induce similar changes in the viscosity of the B. subtilis teichoic acid.

Partial specific volume of B. subtilis 168 teichoic acid. To calculate molecular weights and to correct observed sedimentation coefficients, it was necessary to determine the partial specific volume for the teichoic acid. Based on seven separate pycnometric determinations, the B. subtilis 168 teichoic acid partial specific volume was found to be 0.57 ± 0.03 ml/g. To our knowledge this is the first report for the determination of a teichoic acid partial specific volume.

Sedimentation studies. Attempts to obtain sedimentation velocity profiles for the teichoic acid dissolved in distilled water were not successful. The teichoic acid remained at the top of the cell during centrifugation. However, when the teichoic acid was dissolved in dilute Tris-
hydrochloride buffer or in 1.0 M sodium chloride, sedimentation readily occurred. A typical sedimentation profile is shown in Fig. 3. There is little information in the literature concerning the sedimentation behavior of purified teichoic acids. Some macromolecules have a strong concentration dependence on sedimentation. The concentration dependence for the B. subtilis teichoic acid sedimentation coefficient is shown in Fig. 4. In Tris-hydrochloride (pH 7.4) the concentration dependence is marked, whereas the effect is less pronounced in 1.0 M sodium chloride. Sedimentation coefficients, corrected to the conditions of 20°C and water, were found to be $s_{20,w}^2 = 1.90 \pm 0.15S$. By constructing a series of $s_{20,w}^2$ versus concentration curves using the points shown in Fig. 4, it was possible to compute the standard deviation. It is important to mention that the pH values of teichoic acid solutions in either distilled water or sodium chloride were approximately 5.6.

However, the isoionic pH was found to be 4.9. When the teichoic acid was dissolved in Tris-hydrochloride buffers, no changes in the buffer pH values were detected.

The molecular weight of the B. subtilis 168 teichoic acid was found to be 24,800. The results were computed from the slope of log c (fringe displacement) versus $r^2$ (Fig. 5). The slope of the curve indicates that the teichoic acid preparation was homogeneous, as deviations from linearity show heterogeneity (48). Equilibrium was reached within 24 h. It is possible to relate molecular weights to viscosities and determine the shape of a polyelectrolyte (36, 42). This is done by use of the empirical Staudinger relationship (36, 42):

$$|\eta| = K \cdot MW^a$$

where $K$ is a constant indicating solute-solvent interaction properties, and $a$ is the shape factor. When $a > 1$, an extended or rigid rod conformation exists; when $0.5 < a < 0.8$, a random coil conformation is present (36, 42). The Staudinger relationship is valid providing that lower-molecular-weight fractions are derived from a common high-molecular-weight parent.
Bello et al. (9) found that degraded or chemically modified gelatins obeyed the Staudinger equation when compared to their parent gelatins. Thus, application of this method to the teichoic acid necessitated having two teichoic acid fractions of different molecular weights and viscosities, but of the same origin. This was accomplished by degrading the teichoic acid prepared by affinity chromatography \((MW = 24,800\) and \(\eta = 0.48\)) by sonic treatment in distilled water. The degraded teichoic acid had an average molecular weight of 18,500 (as determined by sedimentation equilibrium) and an intrinsic viscosity of 0.35 dl/g in water. In 1.0 M sodium chloride, its intrinsic viscosity was 0.11 dl/g. From these data and the viscosities obtained from Fig. 1, it was possible to use the Staudinger relationship to determine the shape of the teichoic acid in water and in 0.1 M sodium chloride. The data are shown in Fig. 6, where \(\log [\eta]\) is plotted against \(\log MW\). The shape factor, \(a\), is then obtained from the slope of the respective curves. In water, \(a\) was found to be 1.08, indicating that the teichoic acid possessed a rigid rod conformation. In 1.0 M sodium chloride, however, \(a\) was determined to be 0.54, a value consistent with a random coil conformation.

Salt-induced difference spectrum. The inset in Fig. 7 shows the ultraviolet absorption spectrum of the teichoic acid. Analyses have shown that the teichoic acid is free from protein and nucleic acid (16). The teichoic acid exhibits high absorption only below 250 nm. When the teichoic acid is mixed with sodium chloride, a difference spectrum is generated characterized by inflection points at 234 and 226 nm (Fig. 7). The difference spectrum must originate as a result of salt-induced changes in the teichoic acid. Similar difference spectra were obtained by using hydroxylamine-treated teichoic acids (29) and trichloroacetic acid-extracted teichoic acids. In addition, dialysis to remove the salt eliminates the difference spectrum. Thus, the structural transitions induced by salts are reversible.

**Sodium chloride-induced inhibition of bacteriophage adsorption.** Most of the \(B.\ subtilis\) bacteriophages adsorb specifically to the glucosylated teichoic acid (47). Adsorption of bacteriophage \(\phi 25\) depends primarily on glucosylation of teichoic acid in wild-type \(B.\ subtilis\) (47). If salts alter the structure of the teichoic acid, then phage \(\phi 25\) adsorption to cell walls should be reduced in the presence of salts. The data in Fig. 8 show that phage \(\phi 25\) binding to cell walls is highly sensitive to the presence of sodium chloride. A progressive decrease in binding is observed up to a 1.5 M sodium chlo-

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**Fig. 5.** Determination of the \(B.\ subtilis\) 168 teichoic acid molecular weight by equilibrium ultracentrifugation. Details of the calculations are given in the text.

**Fig. 6.** Staudinger viscosity-molecular weight plots for teichoic acids in water and 1.0 M sodium chloride. The higher molecular weight represents the teichoic acid prepared by affinity chromatography of \(B.\ subtilis\) 168 cell wall autolysates. The smaller-molecular-weight teichoic acid was obtained by sonication (Raytheon sonic oscillator, model DF101; maximal voltage, 1.0 A; 10 min; temperature 2 C) of the high-molecular-weight sample.
ride concentration. Control experiments (absence of cell walls) demonstrated that sodium chloride has no effect on the ability of the bacteriophage to initiate infection. It is interesting that complete inhibition of phage adsorption was not observed, even with a 2.0 M sodium ion concentration.

**Effect of ions on teichoic acid-Con A interactions.** Salts readily inhibit the precipitation between *B. subtilis* 168 cell wall digests and Con A (15). The precipitin profile between purified teichoic acid and Con A is shown in Fig. 9. In dilute Tris-hydrochloride buffer, a typical precipitin curve was observed, with a broad equivalence zone between 0.125 to 0.75 mg of teichoic acid and a reduction in the amount of Con A precipitated above 0.75 mg of teichoic acid. When the precipitin curve was obtained in the presence of $10^{-3}$ M Mg$^{2+}$, the equivalence zone was larger (curve A, Fig. 9). Under the same conditions but in the presence of 1.0 M sodium chloride, no precipitation between Con A and the teichoic acid occurred (curve C, Fig. 9). The salt inhibition of teichoic acid precipitation must reflect a change in the teichoic acid structure since Con A is unaffected by high concentrations of salts (15, 31).

Dissolution of preformed Con A-teichoic acid complexes is less sensitive to salts (Table 1). For example, 1.0 M sodium chloride or 1.0 M potassium chloride would not dissolve preformed Con A-teichoic acid complexes. The protein denaturant guanidine-hydrochloride completely dissolved the complex. Also, the Con A inhibitor (31) methyl-$\alpha$-D-mannopyranoside rendered the complex soluble, whereas $\alpha$-galactose, a sugar which does not bind to Con A, had no effect on the complex.

**DISCUSSION**

The results of these studies show that salts induce structural transitions in the teichoic acid of *B. subtilis* 168. Doyle and Birdsell (15), Kan et al. (in press), Reeder and Ekstedt (28), and Archibald and Coapes (6) have shown that ions...
tend to inhibit the precipitin reaction between Con A and teichoic acids from several bacterial species. Recently, Knox and Wicken (19) showed that ions inhibited the reaction between teichoic acids and anti-teichoic acid antibodies. Doyle and Birdsell (15) speculated that sodium chloride induced a random coil conformation in the teichoic acid of B. subtilis 168. The present study, using several criteria, establishes that teichoic acids undergo conformational changes in salt solutions.

The observation that increasing concentrations of sodium ion produced a gradual decrease in viscosity (Fig. 2) indicates that abrupt conformational changes did not occur in the teichoic acid. Thus, the teichoic acid probably exists as a freely permeable polymer in water (14). The shape factor, \( a = 1.08 \), derived from the slope of the Staudinger viscosity-molecular weight plot (Fig. 6) further supports the contention that, in distilled water, the teichoic acid exists in an extended or rigid conformation (24, 42). On the other hand, in 1.0 M sodium chloride, the slope of 0.54 suggests that the teichoic acid is in a random coil conformation (24, 42). Moreover, the upward curvature of the reduced viscosity concentration plot (Fig. 1) of teichoic acid in water also suggests a rigid conformation for the polymer (12, 24). The upward curvature of the viscosity concentration curve at low teichoic acid concentrations indicates that the degree of ionization (secondary phosphate groups) increases. A concomitant increase in viscosity would then occur. At the higher ionic strengths (1.0 M sodium chloride, Fig. 1), ionization is effectively suppressed, leading to a linear viscosity concentration profile. Some polyelectrolytes become helical in the presence of binding ligands (34). The present studies do not rule out a possible helical conformation in teichoic acids. Rotatory dispersion and circular dichroism measurements will be necessary before an estimate of helical contributions can be made. The intrinsic viscosity of the teichoic acid in the presence of \( \text{Mg}^{2+} \) ions suggests that divalent cations, at relatively low concentrations, do not significantly change the teichoic acid structure (Fig. 1). Similar viscometric results have been described for well-characterized polyelectrolytes. For example, carboxymethylcellulose has a low viscosity and random coil conformation in 0.2 M sodium chloride, but in 0.005 M sodium chloride the polymer has a rigid rod conformation and a relatively high viscosity (12).

The finding that the teichoic acid sedimentation coefficient increased with decreasing concentration is not unexpected (Fig. 4). The fact that sedimentation was more dependent on concentration in dilute Tris-hydrochloride than in 1.0 M sodium chloride suggests that in the salt solution the teichoic acid is more compact.

Table 1. Solubilization of preformed concanavalin A-teichoic acid complexes by salts and saccharides

<table>
<thead>
<tr>
<th>Salt</th>
<th>Soluble Con A (( \mu )g)</th>
</tr>
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<tbody>
<tr>
<td>Tris-hydrochloride buffer</td>
<td>49</td>
</tr>
<tr>
<td>(0.05M)</td>
<td></td>
</tr>
<tr>
<td>NaCl (1.0 M)</td>
<td>645</td>
</tr>
<tr>
<td>NaCl (0.1 M)</td>
<td>390</td>
</tr>
<tr>
<td>NaCl (0.01 M)</td>
<td>95</td>
</tr>
<tr>
<td>KCl (1.0 M)</td>
<td>580</td>
</tr>
<tr>
<td>GuCl (2.0 M)*</td>
<td>839</td>
</tr>
<tr>
<td>Methyl-D-mannopyranoside (0.1 M)</td>
<td>827</td>
</tr>
<tr>
<td>d-Galactose (0.1 M)</td>
<td>55</td>
</tr>
</tbody>
</table>

* [14C]concanavalin A (1,000 \( \mu \)g; specific activity, 3,750 counts per min per mg) and teichoic acid (1,000 \( \mu \)g) were mixed in 2.0 ml of 0.05 M Tris-hydrochloride buffer (pH 7.4). After an incubation of 2 h at room temperature, the complexes were centrifuged and washed once with 5 ml of Tris-hydrochloride buffer. To the precipitates was added 4.0 ml of salt or sugar solution in Tris-hydrochloride, and the resulting suspensions or solutions were allowed to incubate an additional 2 h. After incubation, the samples were centrifuged, and the amount of soluble concanavalin A was determined by scintillation counting of the supernatant fraction. The original precipitates contained 850 ± 30 \( \mu \)g of concanavalin A.

* GuCl, Guanidine hydrochloride.
This view is consistent with the viscosity data. Varga (41) found that a decrease in the ionic strength of the solvent resulted in a decreased sedimentation coefficient for hyaluronic acid. Strominger and Ghuysen (35) reported a $s_{20,w}^2$ value of 1.66S for the “native” teichoic acid of Staphylococcus aureus, although solvent conditions and a dependence on concentration were not described. Sanderson et al. (30) also reported a sedimentation coefficient of 1.13S for the teichoic acid of S. aureus Copenhagen without defining the conditions for its determination. Our data show that it is necessary to determine the concentration dependence for the sedimentation of teichoic acids regardless of the solvent system (Fig. 4).

The value determined in these studies for the teichoic acid molecular weight is higher than any reported previously (7). Strominger and Ghuysen (35), however, reported a value of 23,000 for the teichoic acid of S. aureus. This value may be erroneous, since it was apparently derived from uncorrected sedimentation and diffusion coefficients. It is possible to compute an approximate chain length of 53 units from the teichoic acid molecular weight (24,800) and its phosphorus content of 2.13 μmol/mg (16). This is more than double the chain length for any teichoic acid reported previously (7). The longer chain length may be reflected in the method of teichoic acid preparation. Our method, based on affinity chromatography of cell wall autolysates, is probably less hydrolytic than the acid or organic solvent extraction procedures commonly used to purify teichoic acids (5, 7).

Frequently, structural transitions in polymers may be detected by changes in their ultraviolet absorbance characteristics. This is particularly true for proteins which contain chromophores absorbing in the ultraviolet region of the spectrum. The observation that sodium chloride induced an ultraviolet difference spectrum in the teichoic acid (Fig. 7) supports the concept that ions cause structural changes in the polymer. The fact that hydroxylamine-treated and trichloroacetic acid-extracted teichoic acids gave similar difference spectra with sodium chloride suggests that ester-linked D-alanine or a contaminant was not responsible for the spectral changes. It is likely that the difference spectrum reflects changes in the conformation of chromophores that absorb below 240 nm. In this regard, Wood (45) has shown that a large difference spectrum between 210 and 240 nm is generated in soluble collagen when the molecule undergoes a transition of helix to random coil.

Two possible explanations exist for the finding that ions inhibited bacteriophage φ25 adsorption to B. subtilis 168 cell walls. The bacteriophage may bind to the receptor sites on the cell walls via a direct ionic interaction. Thus, ions would competitively inhibit the adsorption. Another and more plausible explanation is that ions change the structure of the glucosylated teichoic acid receptor site. This interpretation would be consistent with the viscosity and spectral changes induced by ions in pure teichoic acid preparations. Marquis (22) found that high-ionic-strength media induced an expansion in the cell walls of B. megaterium. The changes observed by Marquis, however, appear to be related to wall peptidoglycan, since the walls were most compact at pH 4, a hydrogen ion concentration close to the isoelectric pH of peptidoglycan. If sodium chloride induces a structural change in the bacteriophage receptor site, complete inhibition should be observed. We consistently found that complete inhibition of φ25 adsorption to the walls did not occur (Fig. 8). It is possible that higher concentrations of ions are required to induce structural changes in the teichoic acid of isolated walls than for teichoic acid molecules in aqueous solution or that there are other secondary phage receptors in the cell wall which may be partially enmeshed by sodium chloride.

Teichoic acid-Con A complex formation can be used to study the structure of teichoic acids. For example, the inhibition of gel precipitin bands between Con A and alpha-glucosylated teichoic acids is markedly sensitive to ionic strength (15). Fully glucosylated teichoic acids react more strongly with Con A than partially glucosylated teichoic acids (6). It is interesting that Mg$^{2+}$ did not greatly alter the Con A-teichoic acid precipitin curve (Fig. 9) even though divalent cations bind well to teichoic acids (18). It is important to note also that, even with relatively high teichoic acid concentrations, the precipitin reaction with Con A did not occur in 1.0 M sodium chloride (Fig. 9). This finding agrees with other studies (6, 15, 28; T. Z. Kan et al., in press). However, Mauck and Glaser (25) reported that Con A would precipitate with the glucosylated teichoic acid of B. subtilis Marburg in 1.0 M sodium chloride. An explanation for this discrepancy is not obvious. It is surprising that 1.0 M sodium or potassium chloride would not completely solubilize Con A-teichoic acid complexes (Table 1). So and Goldstein (31) and Doyle and Birdsell (15) have shown that sodium chloride concentrations greater than 2.0 M have no effect on Con A-neutral polysaccharide complexes. It is possible that, with the
formation of insoluble Con A-teichoic acid complexes at low ionic strength, a matrix is formed which excludes ions. Thus, the complex, once formed, would be less sensitive to salts than the initial interaction between the protein and teichoic acid. Guanidine-hydrochloride probably solubilized the complexes by unfolding Con A.

The change in bacterial teichoic acid structure induced by salts may have a significant influence on the physiology of the cells. For example, Barnhart and Herriot (8) found that 0.3 M sodium chloride inhibited the binding of exogenous deoxyribonucleic acid by *Haemophilus influenzae*. Our studies show that in 0.3 M sodium chloride the teichoic acid structure is changed (Fig. 2). The bactericidal effect of \( \beta \)-lysin on *B. subtilis* is reduced markedly by solutions of ionic strength greater than 0.2 (24). Matheson et al. (23) suggested that the interaction between intact cells or protoplasts and \( \beta \)-lysin involved electrostatic forces. Reusch and Neuhaus (29) found that sodium and potassium ions inhibited the incorporation of \( \alpha \)-alanine into the glycerol teichoic acid of *Lactobacillus casei*. This inhibition may be related to the conformation of the teichoic acid. Brown et al. (11) have shown that autolysins bind strongly to teichoic acids of *B. subtilis*. Wise et al. (44) observed that the teichoicase of *B. subtilis* was inhibited by high salt concentrations. Unfortunately, the above studies only permit a partial catalog of potential functions for teichoic acid. Before the function(s) of bacterial teichoic acids can be understood, it will be necessary to examine the physicochemical properties of the teichoic acids in solution. The present data show that the teichoic acid of *B. subtilis* 168 exhibits a “native” or extended conformation in solutions of low ionic strength. In solutions of intermediate and high ionic strength, the teichoic acid exists as a random coil.

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LITERATURE CITED


