The Action of Dilute Alkali on Bacterial Cell Walls

By A. R. ARCHIBALD, HILARY E. COAPES and G. H. STAFFORD

Microbiological Chemistry Research Laboratories, University of Newcastle upon Tyne, NE1 7RU

(Received) 30 May 1969

Hughes & Tanner (1968) have reported that complete extraction of teichoic acids from the walls of certain bacteria may be achieved by incubation in 0.1 M-NaOH at 35° and that the mucopeptide components of these walls remains insoluble during this treatment. We have also found extraction with alkali to be of value since the possibility of acid hydrolysis of glycosidic substituents on the teichoic acid is thus avoided (Archibald, Baddiley & Heptinstall, 1969). However, we have found that on such treatment of walls of staphylococci the mucopeptide component dissolves completely. This finding contrasts with that of Hughes & Tanner (1968) and with our own observations on the walls of *Bacillus megaterium* 7581, *Lactobacillus plantarum* C106, *Lactobacillus plantarum* 17-5 and *Micrococcus lysodeikticus* N.C.T.C. 2665, in all of which the mucopeptide component is almost entirely insoluble in dilute alkali.

Samples of the above walls (5 mg./ml.) were suspended in 0.5 M-NaOH at room temperature and the change in E_{550} was noted with time. Walls of *Staphylococcus lactis* N.C.T.C. 2102, *Staphylococcus saprophyticus* I2 and *Staphylococcus aureus* H were similarly examined. It was found that walls of the staphylococci dissolved completely in 50 hr. whereas the extinction of the other wall suspensions remained essentially constant after an initial fall of 10–20%. In all cases, however, the phosphorus was released almost completely in 4 hr.

The proportions of amino acids and amino sugars present in the walls of *S. lactis* N.C.T.C. 2102 were essentially the same as those found after dissolution in alkali (Table 1), and the absence of any marked destruction of glucosamine and muramic acid indicates that the dissolution of these walls is not caused by extensive degradation of the polysaccharide backbone. In agreement with this conclusion it was found that prior treatment with NaBH_4 has no effect on the dissolution of these walls with alkali. Although the action of alkali on walls of staphylococci is not yet understood, it is noteworthy that the other walls studied differ markedly in their solubility. Of particular interest is the observation that the mucopeptide components of *S. aureus* H and *S. saprophyticus* I2 are resistant to dissolution by dilute aqueous dimethylhydrazine whereas the walls of *B. megaterium* and

M. lysodeikticus are very rapidly dissolved by this reagent (Anderson, Archibald, Baddiley, Curtis & Davey, 1969). This difference had been thought to be due to the more extensive cross-linking found in staphylococcal mucopeptide, but the contrasting action of alkali on these different walls makes the conclusion less certain. The present results show, not only that alkali and dimethylhydrazine differ markedly in their mode of action on walls, but suggest that the integrity of these cell walls may primarily be due to different structural features.

In larger-scale experiments teichoic acids were obtained by a 4 hr. extraction with alkali of walls of *S. lactis* N.C.T.C. 2102 (Archibald, Baddiley, Button, Heptinstall & Stafford, 1968) and *L. plantarum* C106 (Adams, Archibald, Baddiley, Coapes & Davison, 1969). In each case the extract contained mucopeptide components and the teichoic acids were purified to establish whether their extraction might be primarily due to a preferential solubilization of that fraction of the mucopeptide to which the teichoic acids were attached.

After 4 hr. at room temperature almost 100% of the phosphorus was extracted from walls of *L. plantarum* C106 (250 mg. in 20 ml. of 0.5 M-NaOH). The supernatant solution was removed by centrifugation and neutralized with 0.5 M-HCl. Samples of the extract and the wall residue were hydrolysed at 100° in 6 M-HCl for 16 hr. and analysed for amino acids (Table 1). A further sample of the extract (3 μ moles of P) was dialysed against 1 l. of water for 5 days at room temperature and the non-diffusible fraction was analysed as before. Less than 5% of the phosphorus was removed although such dialysis resulted in the loss of a proportion of the mucopeptide components.

Alkali extract (15 μ moles of P) from *L. plantarum* C106 was subjected to electrophoresis as a band on paper in sodium barbiturate-HCl buffer, pH 8.6, for 1 hr. at 17.9 v/cm. A single phosphorus-containing spot was detected (Hanes-Isherwood spray) moving towards the anode at a distance of 15 cm. from the origin. The phosphorus-containing material was eluted, and a sample was hydrolysed (6 M-HCl for 16 hr. at 100°) and analysed for amino acids (Table 1).

Alkali extract from *S. lactis* N.C.T.C. 2102 (20 μ moles of P) was subjected to electrophoresis in

Table 1. *Composition of cell-wall samples of S. lactis N.C.T.C. 2102 and L. plantarum C106*

Intact walls of *S. lactis* and *L. plantarum*, the soluble fractions obtained by extraction of these with 0.5M-NaOH for 4 hr., the insoluble residues so obtained ('alkali-extracted cell walls') and the purified teichoic acids ('samples subjected to electrophoresis') were analysed for amino compounds and phosphate. The solution resulting from dissolution of *S. lactis* walls by incubation in 0.5M-NaOH for 50 hr. was also analysed.

Component	Composition (μ moles/g. of sample)								
	Cell walls		Alkali-solubilized cell walls (50 hr.)	Alkali-extracted cell walls (4 hr.)		Alkali extract of cell walls (4 hr.)		Sample subjected to electrophoresis	
	2102	C106	2102	2102	C106	2102	C106	2102	C106
Muramic acid	382	368	367	687	638	—	—	—	—
Glutamic acid	505	546	460	532	945	93	87	14	27
Serine	413	—	413	310	—	122	—	—	—
Glycine	1685	55	1685	1475	46	292	63	99	7
Alanine	979	864	918	1470	1296	144	400	—	15
Lysine	444	—	428	706	—	146	—	—	—
Glucosamine	903	455	920	692	935	455	60	743	46
Diaminopimelic acid	—	445	—	—	907	—	17	—	2
Phosphorus	1319	846	1319	117	111	2440	1533	3100	1809

pyridinium acetate buffer, pH 5.3, for 1.5 hr. at 17.9 v/cm. Again a single phosphorus-containing spot was detected, at a distance of 12.6 cm. from the origin. Elution and acid hydrolysis of this showed that only extremely small quantities of amino acids were associated with the phosphorus (Table 1).

Treatment of the alkali extract from *L. plantarum* C106 with alkaline phosphatase yielded 6% inorganic phosphorus, indicating the presence of terminal phosphomonoester groups that are not due to hydrolysis of the interunit phosphodiester linkages of the teichoic acid since further treatment of the extract with 0.5M-NaOH for 4 hr. did not markedly increase the number of phosphomonoester groups present.

These results show that negligible hydrolysis of the interunit phosphodiester linkages takes place in 0.5M-NaOH at room temperature. This suggests that the phosphomonoester residue found in the extracted teichoic acid may originate from a terminal phosphodiester linkage to the wall (cf. Hay, Archibald & Baddiley 1965).

With *S. lactis* N.C.T.C. 2102, too, essentially all of the mucopeptide components were removed from the teichoic acid on dialysis and electrophoresis, although in this case no phosphomonoester groups were detected.

These studies are in agreement with those of Hughes & Tanner (1968) in showing that teichoic acid extracted by dilute alkali is polymeric and that even in cases where extensive dissolution of the mucopeptide occurs it may be obtained free from mucopeptide components. Although such extraction removes alanine ester groups it avoids the possibility of acid hydrolysis and so is a valuable alternative to previous extraction procedures.

The differential activities of NaOH and dimethylhydrazine on various walls are not at present understood.

We thank the Science Research Council for a research studentship (G.H.S.) and Professor J. Baddiley, F.R.S., for his interest.

- Adams, J. B., Archibald, A. R., Baddiley, J., Coapes, H. E. & Davison, A. L. (1969). *Biochem. J.*, **113**, 191.
 Anderson, J. C., Archibald, A. R., Baddiley, J., Curtis, M. M. & Davey, N. B. (1969). *Biochem. J.* **113**, 183.
 Archibald, A. R., Baddiley, J., Button, D., Heptinstall, S. & Stafford, G. H. (1968). *Nature, Lond.*, **219**, 855.
 Archibald, A. R., Baddiley, J. & Heptinstall, S. (1969). *Biochem. J.* **111**, 245.
 Hay, J. B., Archibald, A. R. & Baddiley, J. (1965). *Biochem. J.* **97**, 723.
 Hughes, R. C., Tanner, P. J. (1968). *Biochem. biophys. Res. Commun.* **33**, 22.