THE ACTION OF DILUTE ALKALI ON SOME BACTERIAL CELL WALLS

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Ikawa (1961) found that brief exposure to dilute sodium hydroxide at 25° removed substances from the isolated cell walls of several species of bacteria. However, extraction was not complete and the effect of more prolonged exposure was not described. The isolation of wall polymers with alkali provides a possible alternative to the more usual procedures using a low pH. Linkages that are acid labile may be preserved in alkaline conditions (Knox and Morgan, 1954; Morgan, 1960). We have found that 0.1N sodium hydroxide at 35° in the absence of oxygen removes rapidly and completely the teichoic acids present in walls of Bacillus subtilis W23, Bacillus subtilis 168 and Bacillus licheniformis N.C.T.C. 6346. In the last-mentioned organism, the teichuronic acid polymer present in the walls (Janczura, Perkins and Rogers, 1961) is resistant to alkaline extraction, whereas in acid both teichoic acid and teichuronic acid are made soluble. The mucopeptide component of these walls remains insoluble during alkaline extraction.

B. licheniformis cell walls were extracted (4 mg/ml) with 0.1N sodium hydroxide at 35° under nitrogen and the results were compared to those obtained by treatment of walls (4 mg/ml) with 0.1N hydrochloric acid at the same temperature (Fig. 1). Dilute acid removed phosphorus and glucuronic acid as expected, since the teichoic acid and teichuronic acid are dissolved by 5°/o (w/v) trichloroacetic acid (Janczura et al., 1961; Hughes, 1965). The extraction by 0.1N hydrochloric acid was slow, and in the case of

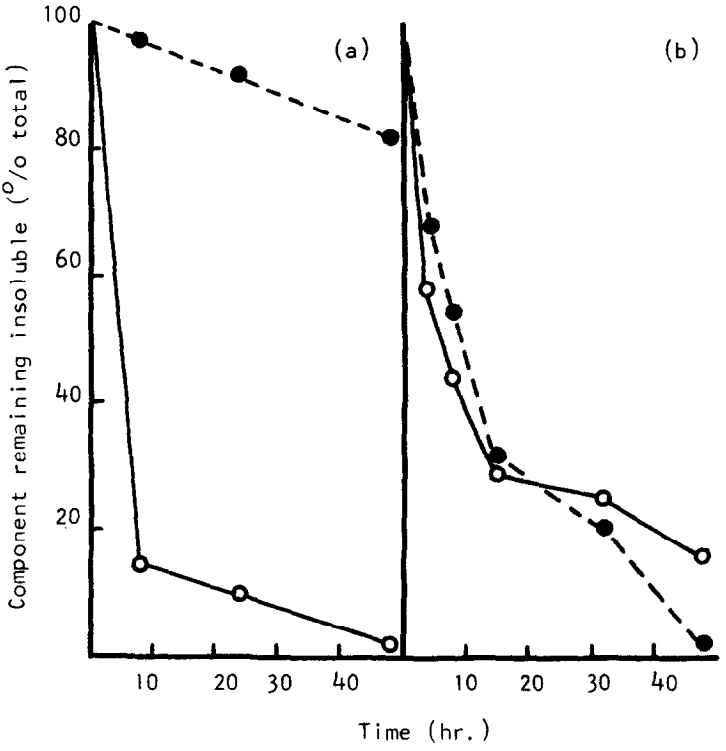

Fig. 1. Extraction of components from the cell wall of B. licheniformis. Walls (4 mg/ml) were suspended either in 0.1N sodium hydroxide under N_2 or in 0.1N hydrochloric acid and kept at 35°. At intervals, samples containing 10 mg of walls were withdrawn and centrifuged. The insoluble and soluble fractions were analysed. —o—, phosphorus; —●—, uronic acid. (a) 0.1N NaOH; (b) 0.1N HCl.

phosphorus was not complete even after 48 hr. In contrast, phosphorus was removed faster and almost completely (at least 95°/o) from the walls by dilute alkali. Thus over 85°/o of the wall phosphorus was soluble within 8 hr of exposure to 0.1N sodium hydroxide, while dilute acid in the same time removed only about 55°/o of the total. A striking difference was found in the behaviour of teichuronic acid (Fig. 1). Only minor amounts of glucuronic acid were made soluble by alkaline treatment, even after exposure for 48 hr.

Samples of the alkali-soluble fractions were examined, after acid hydrolysis, by paper chromatography in butan-1-ol-pyridine-water (6:4:3). Alanine was present, presumably derived from the ester-linked alanine of

the teichoic acid. No glutamic acid or αε-diaminopimelic acid was detected, showing that the mucopeptide component was absent.

The teichoic acid of B. subtilis W23 is a glucosylated ribitol-phosphate polymer (Chin, Burger and Glaser, 1966). Approximately 97% of the phosphorus content of these walls was removed after treatment with 0.1N sodium hydroxide at 35° for 48 hr, and most of this amount was removed in the first 4 hr. Material made soluble (2.0 ml, equivalent to 10 μmoles of total phosphorus) was treated overnight at 35° with Escherichia coli phosphatase (0.5 ml, 10 mg/ml) in 0.1M TRIS-hydrochloric acid buffer, pH 8.4 (2.5 ml) containing 0.1M magnesium sulphate. The phosphatase digest was examined by paper chromatography in butan-1-ol-pyridine-water (6:4:3, by vol.) or propan-1-ol-ammonia-water (6:3:1, by vol.). Material containing organically-bound phosphorus remained at the origin after chromatography, and only trace amounts of glucosylribitol or ribitol were detected. These last products would be produced by phosphatase treatment of the phosphomonoester derivatives that are formed from the teichoic acid after vigorous alkaline hydrolysis (Armstrong, Baddiley and Buchanan, 1960).

The teichoic acids present in B. subtilis 168 cell walls (Young, 1967) behaved similarly (Fig. 2), with acid or alkali, to those in B. licheniformis and B. subtilis W23. Phosphorus was rapidly and almost completely removed from the walls by 0.1N sodium hydroxide, and more slowly and incompletely by 0.1N hydrochloric acid. Fractions made soluble (Fig. 2) either by acid or by alkali contained, after strong acid hydrolysis, hexosamine material (Elson and Morgan, 1933), and chromatography showed the presence of galactosamine. The rate of release of galactosamine was only slightly faster in alkali than in acid, and extraction of galactosamine by acid was complete when only about 55% of the wall phosphorus was made soluble.

Material prepared by treatment of B. subtilis 168 cell walls with 0.1N hydrochloric acid was applied to a column of Sephadex G-50 (Fig. 3a). Fraction S1 (Fig. 3a) contained phosphorus, glycerol, glucose and D-alanine in the

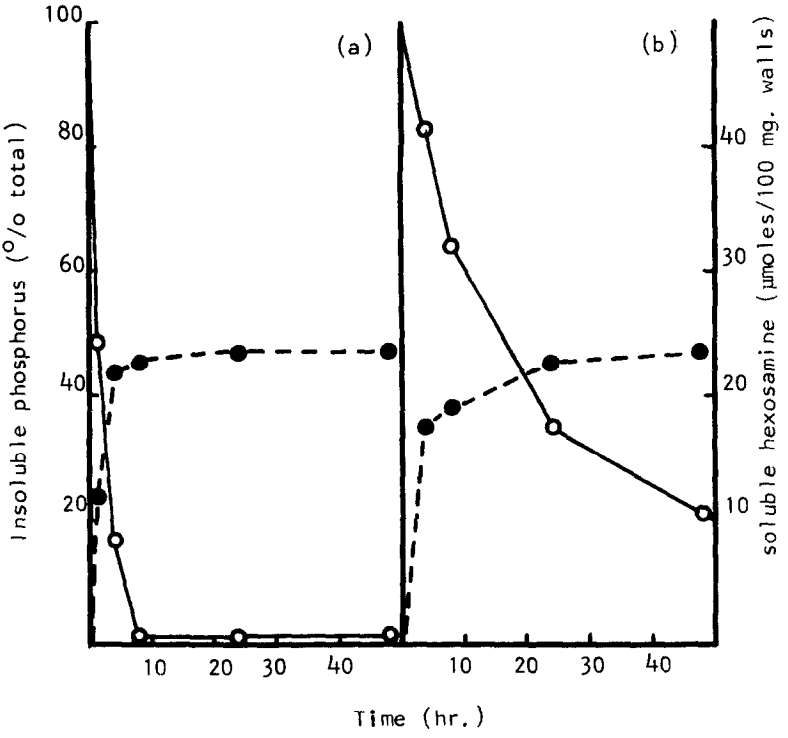

Fig. 2. B. subtilis 168 cell walls were exposed at 35° either to dilute acid or dilute alkali as described in Fig. 1. The insoluble and soluble fractions were analysed. —o—, phosphorus; —●—, hexosamine material after total acid hydrolysis. (a) 0.1N NaOH; (b) 0.1N HCl.

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molecular proportions 1.00 : 1.08 : 0.96 : 0.09, and accounted for 76°/o of the total phosphorus. The remainder of the phosphorus was eluted later from the column together with over 90°/o of the total galactosamine. Compound S2 (Fig. 3a) was reducing, and after treatment with 1°/o (w/v) sodium borohydride followed by acid hydrolysis, the galactosamine content had decreased to 6°/o of the starting value. Therefore, each residue of galactosamine or N-acetyl-galactosamine present in compound S2 (Fig. 3a) carried a free reducing group.

Chromatography on Sephadex G-50 (Fig. 3b) of the alkali-soluble fraction of walls (Fig. 2) gave a single peak (S1) of high molecular size and containing virtually all of the phosphorus, glucose and galactosamine

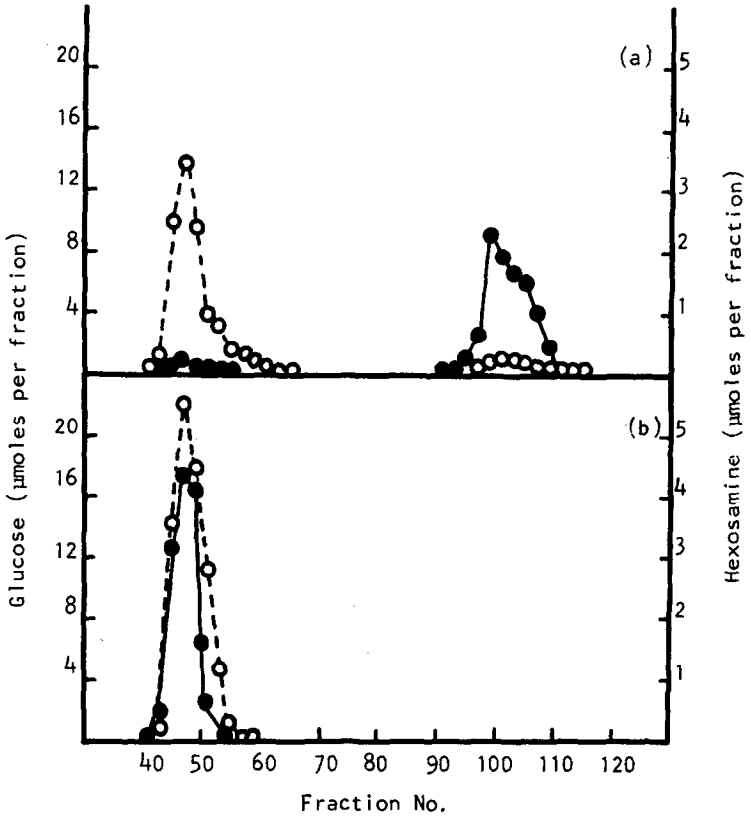

Fig. 3. Chromatography on Sephadex G-50 of soluble extracts obtained from B. subtilis 168 cell walls. Material was applied to the column (2 x 130 cm) in water (4 ml) and the column was equilibrated and eluted with 0.1M pyridine-acetate buffer, pH 5.1. Fractions (2.5 ml) were collected and analysed for glucose (—o—) by the anthrone reagent and, after acid hydrolysis, for hexosamine material (—●—). (a) Walls (110 mg) were extracted for 20 hr at 35° with 0.1N hydrochloric acid and the soluble fraction, after concentration, was applied to the column. (b) Walls (145 mg) were treated with 0.1N sodium hydroxide for 16 hr at 35° and the neutralised and concentrated soluble fraction was applied to the column.

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of the wall. Fraction S1 (Fig. 3b) was non-reducing and all of the galactosamine was recovered after treatment with sodium borohydride. Thus the C-1 positions of the galactosamine units that were free in the acid extracts were now blocked. After hydrolysis of S1 (Fig. 3b) with 0.1N-hydrochloric acid overnight at 35°, over 85°/o of the galactosamine residues became susceptible to sodium borohydride.

These experiments suggest that the N-acetylgalactosamine units are covalently linked to a part of the glucosylated glycerophosphate polymer present in B. subtilis 168 walls. These covalent linkages are stable to dilute alkali but are hydrolysed during extraction of the walls with dilute acids. At the same time, reducing groups of N-acetylgalactosamine residues are exposed. For example, N-acetylgalactosamine-1-phosphate is labile to acid (Leloir and Cardini, 1956), but alkali would not be expected to hydrolyse this compound.

Cell walls contain free reducing groups (Salton, 1964), and it is known that alkaline degradation of polysaccharides proceeds from free reducing terminals (Ballou, 1954; Whistler and BeMiller, 1958). In order to control this effect, the alkaline extractions of cell walls have been carried out after treatment of the walls (5 mg/ml) with 1°/o (w/v) sodium borohydride at 35° for 5 hr. No phosphorus was removed from walls of B. subtilis W23 or B. licheniformis N.C.T.C. 6346 and no uronic acid was removed from B. licheniformis walls during this treatment. An equal volume of 0.2N sodium hydroxide containing 2°/o (w/v) sodium borohydride was added and extraction continued at 35°. The results then obtained were identical to those described earlier (Fig. 1).

In conclusion, dilute alkali may be useful in removing polymers from bacterial cell walls. In many cases the covalent linkages broken during alkaline extraction may be different in type and number from those that are hydrolysed during extraction with dilute acids, or with formamide.

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