Inhibition by spermine of the action of a bacterial cell-wall lytic enzyme

The cell-wall fraction of a Gram-negative marine bacterium[1] is subject to autodegradation under suitable ionic conditions. This degradation is mediated by an enzyme and is inhibited by the polyamine, spermine, at a concentration of 10^{-3} *M*.

The organism was grown with aeration for 18 h at 30° in sea water containing Difco peptone (1 %, w/v). Cells were harvested, suspended in cold distilled water, and broken by shaking in a Mickle disintegrator for about 18 min with cooling in an ice bath after 9 min. All manipulations in the preparation of the cell walls were performed in an ambient temperature of 4°. The cell-wall fraction was purified by centrifugation in distilled water alternately at 12,200 × *g* for 30 min and 1000 × *g* for 10 min. In general, 4 such cycles were sufficient to yield a cell-wall fraction of acceptable purity. Cell-wall fractions were autolysed at 35° in 0.05 *M* or 0.067 *M* phosphate buffer, pH 8. The extent of the breakdown was followed by measuring changes in absorbancy of the suspensions at 700 mμ. A preliminary qualitative analysis of soluble breakdown products was made after incubating the cell-wall fraction for 35 min in 0.05 *M* buffer and separating the soluble materials from the residual wall by centrifugation at 14,500 × *g* for 45 min. The supernatant solution (lysate) was dialysed for 2 days at 4° against 3 changes of distilled water. The non-dialysable fraction was concentrated at about 30° and freeze-dried. The dialysates were pooled, concentrated at about 30°, inorganic phosphate was largely removed by crystallization in the cold and the supernatant solution was freeze-dried. Amino acids and amino sugars were identified in acid hydrolysates (6 *N* HCl for 16 h at 100°) by 2-dimensional paper chromatography in water–pyridine (80:20, v/v) followed by butan-1-ol–acetic acid–water (6:1:2, v/v/v) and reaction with ninhydrin. α,ε-Diaminopimelic acid was located specifically by paper chromatography in the solvent system of RHULAND *et al.*[2]. Sugars and polyols which were released by acid hydrolysis (2 *N* HCl for 2 h at 100°) were separated by paper chromatography in the butanol–acetic acid solvent system and in propan-1-ol–NH_3 (15 *N*)–water (6:3:1, v/v/v). Such chromatograms were sprayed with aniline phthalate for sugars and with a periodate–Schiff's base reagent[3] for polyols. The heptose present in the cell wall of this organism[1] was detected by the H_2SO_4–cysteine reaction of DISCHE[4].

Fig. 1 shows lysis of the cell wall at pH 8 (properties of the system will be described in greater detail elsewhere) and it will be noted that under these conditions spermine completely inhibited the lytic process. (On the other hand, spermine did not affect the degradation by lysozyme of the cell wall of a species of *Micrococcus*).

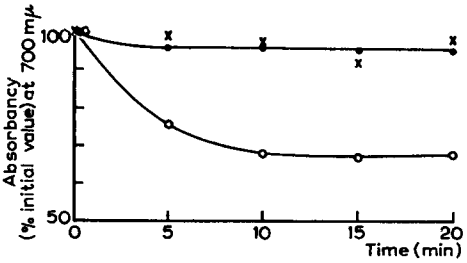

Fig. 1. Autolysis of the cell-wall fraction at 35° in 0.067 *M* phosphate buffer, pH 8. ○, untreated cell walls; ●, heated cell-walls (10 min at 100°), ×, cell walls + 10^{-3} *M* spermine.

Table I lists materials released by acid hydrolysis from the cell-wall lysate and the cell-wall residue (or whole cell-wall fraction). It is noteworthy that whereas the "typical" cell-wall constituents, hexosamine and muramic acid, were released,

TABLE I

COMPOUNDS DETECTED IN ACID HYDROLYSATES OF THE CELL WALL AND ITS AUTO-DEGRADATION PRODUCTS

Type of compound	*Cell wall (whole and residue)*	*Lysate*	
		Non-dialysable fraction	*Dialysate*
Amino acids	Diaminopimelic acid + normal protein constituents	Normal protein constituents	Alanine Aspartic acid Glutamic acid Glycine Valine or Methionine Leucine or isoleucine
Amino sugars	Hexosamine + muramic acid*	Hexosamine + muramic acid*	—
Sugars	Glucose + heptose	Trace of heptose?	—
Polyols	Glycerol + unidentified polyols	Unidentified polyols	Glycerol

* The proportion of muramic acid to hexosamine was judged by inspection of chromatograms to be greater in the lysate than in the cell walls.

diaminopimelic acid was not. It is also of interest that the dialysable fraction of the lysate consisted of peptides. "Fingerprints"[5] of this fraction separated about 30 peptides, suggesting that the breakdown of the crude cell wall is caused by an enzyme and not merely by spontaneous disintegration under appropriate physico-chemical conditions. The presence in the dialysable fraction of some hydrolysable compound(s) of glycerol probably signifies that the enzyme has degraded lipoprotein. A possible mechanism for the overall breakdown of the cell-wall material might involve enzymic removal of a lipoprotein "cement" followed by spontaneous release of other cell-wall constituents. The inhibition of such a process by spermine provides an interesting extension of MAGER's findings that this substance increases the stability of bacterial cells and spheroplasts against osmotic damage[6–8].

C.S.I.R.O., Marine Laboratory, Cronulla, Sydney (Australia) A. D. BROWN

[1] A. D. BROWN, *J. Gen. Microbiol.*, 23 (1960) in the press.
[2] L. E. RHULAND, E. WORK, R. F. DENMAN AND D. S. HOARE, *J. Am. Chem. Soc.*, 77 (1955) 4844.
[3] J. G. BUCHANAN, C. A. DEKKER AND A. C. LONG, *J. Chem. Soc.*, (1950) 3162.
[4] Z. DISCHE, *J. Biol. Chem.*, 204 (1953) 983.
[5] V. M. INGRAM, *Biochim. Biophys. Acta.*, 28 (1958) 539.
[6] J. MAGER, *Nature*, 176 (1955) 933.
[7] J. MAGER, *Biochim. Biophys. Acta*,. 36 (1959) 529.
[8] J. MAGER, *Nature*, 183 (1959) 1827.

Received June 27th, 1960