The nature of the material released from the cell walls of *Bacillus megaterium* on reaction with bacteriophage

In recent years there have been a number of reports on the existence of bacteriophage enzymes which play an important role in the attachment to and infection of susceptible bacteria[1-3]. During these processes, compounds are released from the site of attachment on the cell-wall surface[1-3]. With cell walls of the Gram-negative organism *Escherichia coli*, WEIDEL AND PRIMOSIGH[4] found that the purified phage enzyme released substances containing alanine, α,ε-diaminopimelic acid, glutamic acid, glucosamine and muramic acid. The production of phage-induced lytic enzymes has also received recent attention[5-7] and MURPHY[8] has reported that an enzyme purified from bacteriophage lysates of *B. megaterium* liberated amino sugar substances from the walls of this organism. Whether the lysins and phage-tail enzymes are identical is not yet known. We were interested in determining the nature of the products released during attachment and inactivation of the phage by isolated walls of *B. megaterium*.

^{14}C-labelled walls were prepared by growing *B. megaterium*, strain KM, on a modified synthetic medium[9] containing [^{14}C]sucrose (1 mC/10 mg) as the sole carbon source. The only modification to the synthetic medium C of ROBERTS *et al.*[9] was the addition of $MnCl_2$ to give a final concentration of 0.001 %. The medium containing [^{14}C]sucrose was inoculated with a washed inoculum of *B. megaterium* grown for 16 h at 30° on the synthetic medium containing non-radioactive sucrose. The walls from the ^{14}C-grown cells were isolated by disintegration as previously described[10] and the wall fraction was washed on the centrifuge to remove cytoplasmic components and then resuspended in 0.1 % Bacto peptone. A suspension of bacteriophage C was added to give a high multiplicity of phages to walls. Previous experiments showed that bacteriophage adsorption occurred to the extent of about 90 % within 5–10 min in the 0.1 % peptone medium.

At time intervals varying from 5 min to 16 h after the addition of the phage suspension to the walls, aliquots were taken and inactivated by heating (1 min at 100°), the walls deposited by centrifugation at 20,000 rev./min for 20 min and the supernatant fractions were then examined for the nature of the ^{14}C-labelled compounds released.

Using two-dimensional paper chromatography (phenol saturated with water; *n*-butanol–propionic acid–water, 2:1:1.4, v/v/v) and radioautography, it was possible to establish the specific liberation of a component (or components) from the walls. The chromatographic behaviour of the material, which migrated as a band in the phenol direction, did not correspond to any of the amino acid, or amino sugar constituents of the original wall. The amount of this material increased with time of contact between the bacteriophages and walls. This material was absent from the walls suspended in the peptone medium alone, thus excluding the possibility that the results observed were due to an autolytic process. Furthermore, the materal was also absent in a control incubation mixture of walls and phage lysate from which the bacteriophages had been removed by prior adsorption with non-radioactive cell walls (this control was examined after 16 h incubation of the ^{14}C-labelled walls with the adsorbed lysate).

The component liberated by bacteriophage action on the walls for 16 h was eluted

from the paper chromatograms with water and submitted to hydrolysis with 6 *N* HCl for 16 h at 107°. After two-dimensional paper chromatography (pyridine–water, 4:1, v/v; *n*-butanol–acetic acid–water, 6:1:2, v/v/v) and radioautography, 7 well separated spots appeared on the films and 6 of these could be identified by co-chromatography as being: alanine, α,ε-diaminopimelic acid, glutamic acid, aspartic acid, glucosamine and muramic acid. In addition to the above constituents, the walls of *B. megaterium* contained ribitol phosphate, the polymer of which has recently been found in the walls of bacteria by BADDILEY and coworkers[11]. Compound 7 on the radioautograph of the hydrolysed material was identified as the anhydroribitol compound formed on acid hydrolysis of the teichoic acids[11]. The molecular proportions of the amino acid, amino sugar and ribitol constituents of the original cell wall and the material released during phage attachment, were determined by elution from chromatograms and counting the ^{14}C-labelled compounds (the structure proposed by STRANGE[12] was used for the calculation of the proportion of muramic acid). The results are presented in Table I.

TABLE I

THE MOLECULAR PROPORTIONS OF THE CONSTITUENTS OF CELL WALLS AND MATERIAL RELEASED FROM PHAGE-TREATED WALLS OF *Bacillus megaterium*

	Relative molecular proportions	
	Cell walls	*Component released from phage-treated walls*
Glucosamine	9 (9.4)*	3 (2.9)
Muramic acid	2 (2.1)	2 (1.6)
Alanine	8 (8.3)	8 (7.7)
Glutamic acid	5 (5.2)	5 (4.6)
Aspartic acid	1	1
α,ε-Diaminopimelic acid	5 (5.05)	4 (3.7)
Ribitol	1–0.5 (0.8)	0.5 (0.5)

* The precise values obtained are given in brackets.

These results indicate that during the attachment of the bacteriophage to the cell walls a specific compound is liberated. This compound is a peptide-amino sugar complex which differs from the original cell wall in that it contains a considerably lower molecular proportion of glucosamine. It was of interest to note that the products released by phage action on the wall did not include any free amino acids or free amino sugars.

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