A COMMON ANTIGEN IN THE CELL WALLS OF THREE LYSOZYME-SENSITIVE BACTERIA

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Isolated cell walls from three lysozyme-sensitive bacterial species, *Bacillus megaterium*, *Micrococcus lysodeikticus* and *Sarcina lutea*, have been shown to contain a common antigen. This antigen has been demonstrated by agglutination and agar precipitation techniques.

Several workers have demonstrated the presence of specific antigens in the isolated cell walls of bacteria (Cummins, 1954; McCarty, 1952; Salton, 1952, 1953; Tomesik and Guex-Holzer, 1954; Vennes and Gerhardt, 1956). Such isolated cell walls from some Gram-positive bacteria are susceptible to lysis by lysozyme and it was felt that these walls might contain components which were both common substrates for lysozyme and also common antigens. The following work was carried out to investigate this possibility.

Methods and Materials

Crystallised egg white lysozyme was obtained from the Armour Pharmaceutical Co. Ltd., Eastbourne, England.

Organisms: Bacillus megaterium strain K.M. (non-sporing strain); *Micrococcus lysodeikticus* N.C.T.C. 2665; *Sarcina lutea*, N.C.T.C. 8340.

Cultures were grown in Difco nutrient broth in 2 litre flasks with forced aeration at 30° for 18 hr. The cells were harvested by centrifuging washed twice with distilled water and resuspended in M/15 phosphate buffer.

Cell wall preparation. Cell walls were prepared by the method of Salton and Horne (1951). Cell suspensions containing 10 mg./ml. dry wt. were mixed with Ballotini and shaken in a Mickle disintegrator for 10 min. The ruptured cell walls were collected by centrifuging, washed twice with distilled water, treated with trypsin for 30 min. and finally washed once in M sodium chloride then twice with distilled water. The washed cell walls were resuspended in M/15 phosphate buffer containing 0·001 per cent thiomersal as a preservative and were stored at 4°.

Lysozyme digests. Lysozyme digests of cells and of isolated cell walls were prepared by treating suspensions of cells (20 mg./ml.) or of walls (1 mg./ml.) in buffer with 100 μg. of lysozyme per ml. at 37° for 30 min. The resulting solutions were centrifuged to remove any residual particles and were stored at 4°: they were used within 72 hr. of preparation.

Antisera. Antisera against intact cells, isolated cell walls and lysozyme digests of cell walls from all three organisms were prepared in rabbits. The equivalent of 1 mg. dry wt. of cell walls was injected intravenously into rabbits every 2 days for a total of 22 days. Three weeks after the last injection 20 ml. of blood was removed from the ear vein of each rabbit, and the serum separated from the blood cells by centrifuging. 0·001 per

cent thiomersal was added as a preservative and the sera stored at 4° until required.

Agglutination titres. Agglutination titres of the antisera were measured against their homologous and heterologous cells and cell walls by mixing equal volumes of cell or wall suspensions (1 mg./ml.) and of diluted antisera. These mixtures were incubated in agglutination tubes at 37° for 24 hr. and examined for agglutination.

Agar diffusion precipitation reactions. Agar diffusion precipitation reactions between the antisera and the digests of intact cells and cell walls were made in a 1 per cent gel of washed agar in M/15 phosphate buffer at pH 7·2 containing 0·001 per cent thiomersal. 20 ml. volumes of the agar gel were poured into pyrex petri dishes and reservoirs cut in the agar with a 6 mm. cork borer. One reservoir was placed in the centre of the dish and was surrounded by five further reservoirs evenly spaced with their inner edges 10 mm. from the edge of the central one.

One antiserum was placed in the central hole in each plate and the digests in the surrounding reservoirs. The plates were sealed with plasticine and incubated at 37° in an airtight perspex box and examined at 24 hr. intervals for the development of precipitation bands.

Results

Using the injection schedule described above antibody titres reached a maximum level three weeks after the last injection but then fell off rapidly unless maintained by booster injections.

The results of agglutination tests are shown in Table I.

TABLE I

Antisera	Antigens					
	K.M.c.	K.M.w.	M.L.c.	M.L.w.	S.L.c.	S.L.w.
K.M.c.	512	128	16	16	8	8
K.M.w.	128	128	16	16	4	8
M.L.c.	8	8	256	64	16	16
M.L.w.	8	8	128	128	32	64
S.L.c.	2	4	8	8	128	16
S.L.w.	4	8	16	16	32	32

Figures are the reciprocals of agglutination titres.
K.M. = *Bacillus megaterium.* M.L. = *Micrococcus lysodeikticus.* S.L. = *Sarcina lutea.* c. = intact cells. w. = isolated cell walls.

These results show the presence of antigens specific to each organism but also an antigen or antigens common to all three species. The intact cells also show the presence of some surface antigens which are lost during the preparation of cell walls by the method described above.

Agar precipitation reactions give three or four bands of precipitation between each antiserum and its homologous digests. Two of these precipitation bands are still obtained when the antisera are tested against the heterologous digests. One of these common bands is however obtained when the reaction is carried out between the anti-digest sera and a solution of lysozyme and is thus due to the antigenic properties of the lysozyme. The remaining common band cannot however be accounted

for in this way and is also obtained when the digests are tested against antisera prepared against undigested cells or cell walls. These results indicate that there is at least one antigenic component which is common to the walls of all three species of bacteria.

Further work by the author (unpublished) has shown that this common component is present in the non-dialysable fraction of the cell wall lysozyme digests and is also still present when the walls are digested with the F Enzyme isolated from *Streptomyces albus* by Ghuysen (1957).

Discussion

It has been suggested that the immunological specificity of bacterial cells is carried in part by the mucopeptides in the cell walls (Rogers 1962). These mucopeptides occur in large amounts in the cell walls of Gram-positive bacteria particularly in those of lysozyme-sensitive organisms where they can account for up to 90 per cent of the dry weight of the walls. Such mucopeptides probably consist of polysacharide chains made up of *N*-acetyl glucosamine and *N*-acetyl muramic acid linked together at points by peptide chains of three or four amino-acids. Such molecules possess the physical properties required of antigens and variation in the composition and position of the peptide chains would be expected to give antigens of different specificity.

It seems probably from the above results that organisms which are highly sensitive to lysozyme have at least one common mucopeptide in their cell walls. Further work with other bacterial species is expected to confirm this.

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