THE NATURE OF THE CELL WALLS OF SOME GRAM-POSITIVE AND GRAM-NEGATIVE BACTERIA

by

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Until fairly recently there has been little evidence as to the exact chemical composition of bacterial cell walls; but the results of earlier macro- and micro-chemical studies of what has been largely assumed to be cell walls have left few doubts about their probable complexity and variation from one organism to another[1]. Several procedures for the preparation of bacterial cell walls in a high state of purity have now been described[2,3,4]. Recent studies[2,3,5-8] have made it abundantly clear that the composition of the rigid cell walls of different bacteria varies considerably. Furthermore, a comparison of the results of the investigation of the cell-wall composition of the Gram-negative *Esch. coli*[2,3] with those of the Gram-positive *Strep. faecalis*[5,6] and *Staph. aureus*[7] suggested that there may be some well-defined differences between the compositions of the cell walls of Gram-negative and Gram-positive bacteria. With this in mind, studies of the composition of bacterial cell walls have been extended to several Gram-positive and Gram-negative bacteria.

Cell walls of *Strep. pyogenes*, *M. lysodeikticus*, *Sarcina lutea*, *B. subtilis*, *Esch. coli* and *Salmonella pullorum* have been prepared by mechanical disintegration of the cells, according to the procedure outlined in a previous communication[4]. Investigation of the composition of the cell walls of these bacteria has revealed several interesting differences between the Gram-positive and Gram-negative groups of bacteria. The lipid content of the cell walls of the two Gram-negative bacteria, *Esch. coli* and *Salmonella pullorum*, is considerably higher than that of any of the walls of the Gram-positive bacteria investigated (*e.g.* total lipid contents of 22.6% and 19.0% for *Esch. coli* and *Salmonella pullorum* respectively, compared with 2.6% for *B. subtilis*, 1.2% for *M. lysodeikticus* and for *Sarcina lutea*, 1.1%). The amino-acid components of the cell-wall preparations have been examined by paper-chromatography, and with the exception of *Strep. pyogenes* cell wall, which has retained its type-specific M-protein, the cell walls of the Gram-positive bacteria have a limited complement of amino acids, being devoid of both aromatic and sulphur-containing amino-acids. The cell walls of the two Gram-negative organisms investigated possess a much more complete range of amino acids, including aromatic, certain sulphur-containing amino acids, arginine and proline. On removal of the M-protein from the cell wall of *Strep. pyogenes* (by treatment with 1% trypsin, in PO_4 buffer at pH 8, for 2 hr at 37° C according to the method described by LANCEFIELD[9]), its amino-acid constitution conforms to the general features observed for the other Gram-positive wall preparations, and furthermore reveals a striking similarity to the amino-acid constitution of the cell wall of *Strep. faecalis*[5,6].

The inclusion of *Strep. pyogenes* cell wall in the present studies has been of interest from the immunological viewpoint. The cell wall of this organism appears to be characterized by antigenic heterogeneity. The retention of the M-protein by the cell wall, on mechanical disintegration of *Strep. pyogenes*, was not surprising in view of the observations reported by ZITTLE AND MUDD[10]. The presence of the M-protein was confirmed by the strong positive precipitin reaction obtained with extracts of *Strep. pyogenes* cell wall and M-protein antiserum. Treatment of the cell wall with trypsin removes the M-protein without apparently affecting the mechanical properties or the general appearance of the cell wall. It would appear that the group-specific polysaccharide (C substance) is at least partly involved as a structural unit of the cell wall. The enzyme preparation from *Streptomyces albus*, which MAXTED[11] had shown was capable of liberating the C substance from β-haemolytic Streptococci, was also found to be capable of lysing the turbid cell-wall suspensions of *Strep. pyogenes*. Extracts of *Strep. pyogenes* wall obtained with this enzyme preparation also gave positive precipitin tests, specific for group A antiserum. The cell walls of *Strep. pyogenes* could be dissolved by alkaline hypochlorite, and the material precipitated by adjusting to pH 4.5 with acetic acid and adding 2 vols. ice-cold ethanol was water-soluble and gave a strong specific precipitin test with group A antiserum. The group A Streptococcus polysaccharide investigated by SCHMIDT[12] contained glucosamine and rhamnose, and it was not surprising therefore that hexosamine and rhamnose were the only reducing substances detected in the cell wall of *Strep. pyogenes*.

Details and discussion of the results of the investigation of the composition of the cell walls will be submitted for publication in this journal.

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C-TERMINAL GROUPS IN CHYMOTRYPSINOGEN AND DFP-α-CHYMOTRYPSIN IN RELATION TO THE ACTIVATION PROCESS

by

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Although the action of carboxypeptidase has been generally related to the hydrolysis of simple peptides, evidence has come to fore that this peptidase is also capable of liberating amino acids from protein substrates[1,2], presumably from the carboxyl (C-terminal) end of polypeptide chains. This enzymatic reaction is of interest since it permits the controlled, stepwise degradation of proteins and the identification of C-terminal amino acids of the original and residual protein. However, the success of this method as an analytical tool depends on the removal of the last traces of active endopeptidases (chymotrypsin and trypsin) and free amino acids from the preparations of enzyme and substrate. In applying this method to chymotrypsinogen and DFP-α-chymotrypsin as substrates, purified crystalline preparations of these proteins were subjected to additional crystallizations until chymotrypsin and trypsin contents (as determined by hydrolysis of synthetic substrates) were reduced to less than 0.2%. Last traces of these proteinases were inactivated by di*iso*propylfluorophosphate (DFP)[3] which does not significantly inactivate carboxypeptidase, and free amino acids and peptides were removed by exhaustive dialysis against 0.001 *M* HCl. Carboxypeptidase, dissolved in lithium chloride, and substrates were incubated for varying lengths of time at 25° and pH 7.8 and the liberated amino acids were determined with a modified colorimetric ninhydrin method[4] after precipitation of proteins by trichloracetic acid.

With chymotrypsinogen as substrate (substrate/enzyme mole ratios of 17 and 50) not more than 0.2 to 0.3 moles of amino acids per mole of chymotrypsinogen (molecular weight 22,500) were maximally found in less than 30 minutes of hydrolysis. However, when crystalline DFP-α-chymotrypsin was used as substrate (substrate/enzyme mole ratio of 22 and 32), 2 moles of amino acids per mole of substrate were maximally liberated after 2 to 3 hours of incubation. Carboxypeptidase remained fully active even after 6 hours of incubation.

In order to identify the amino acids, one- and two dimensional paper chromatography was used with phenol—water, and *n*-butanol—acetic acid—water (200:30:75), respectively, as solvent systems. The enzymatic reaction was terminated either by the addition of trichloracetic acid or by acidification with HCl to pH 3.0. No supporting buffers were employed but lithium chloride (0.5%)

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