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Ghuysen J-M. Use of bacteriolytic enzymes in determination of wall structure and their role in cell metabolism. *Bacteriol. Rev.* 32:425-64, 1968.
[Service de Bacteriologie, Université de Liège, Belgium]

The bacterial wall peptidoglycan is a network structure. Glycan strands of alternate 3,1-4 linked N-acetylglucosamine and N-acetylmuramic acid pyranoside residues are substituted through the D-lactyl group of N-acetylmuramic acid by L-Ala-γ-D-Glu-L-Xaa$_3$-D-Ala peptide units where L-Xaa$_3$ is most often a diamino acid, occasionally a neutral amino acid. Peptide units substituting adjacent glycan strands are linked together by means of bridges that involve the carboxyl group of the terminal D-Ala of one peptide and either the ω-amino group or the diamino acid L-Xaa$_3$ or the α-carboxyl group of D-Glu of another peptide. Depending on the composition and location of the bridges, the wall peptidoglycans fall into four main chemotypes. [The *SCI*® indicates that this paper has been cited in over 435 publications.]

The Rigid Matrix of Bacterial Cell Walls

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In the early 1950s, the bacterial cell wall had emerged as a new field of research. Milton R.J. Salton had succeeded in isolating the walls of several types of bacteria, the first analyses of which had revealed the occurrence of compounds that had never before been encountered in nature. Claes Weibull had observed that, upon treatment with lysozyme, resting cells of *Bacillus megaterium* were transformed into wall-less, fragile protoplasts, and Joshua Lederberg had seen that cells of *Escherichia coli* growing in a hypertonic medium were changed into fragile spherical cells when penicillin was added to the culture. Though the underlying mechanisms were different, lysozyme and penicillin brought about a loss of integrity of the wall whose essential function was to keep the bacteria alive under usual environmental conditions.

I became aware of these studies when, after receiving my PhD in physical chemistry at the University of Liège, I came to Maurice Welsch's laboratory at the medical school, a place known for its contributions to the concept of antibiosis. Welsch had obtained from the culture filtrate of *Streptomyces albus* G, a crude preparation called actinomycetin, which had bacteriolytic activities. He was convinced that the lytic agent was of enzymatic nature, but his attempts to isolate it had failed. He probably thought that I might have better luck.

I met Salton for the first time at the third International Congress of Biochemistry in Brussels, in 1955, and the following year I worked for a few months in his laboratory at the University of Manchester, England. We found that, in marked contrast to lysozyme, the wall-degrading activity of two fractions that I had laboriously isolated from actinomycetin was attributable to distinct peptidases. The data were illuminating. Possessing the right assortment of hydrolases should make it possible to establish the structure of the bacterial cell walls by controlled and progressive degradations.

I took about 12 years to carry the project to real achievement. Original hydrolases, each of them hydrolysing specific linkages, were isolated and used to dismantle, in an ordered manner, the walls of various types of bacteria into increasingly smaller fragments. The fragments were isolated, their structure was established, and the "puzzle" was reconstituted, giving rise to the concept of the wall peptidoglycan, a term that, following a roundtable discussion that took place in Detroit, I used for the first time in one of my "biochemistry" papers published in 1966.

I believe that the popularity of the article was due to the unified view it presented. Despite endless variations in the primary structure, all the bacterial wall peptidoglycans are built on the same general pattern. Simplicity had emerged from a seemingly inextricable complexity.

One of the bacteriolytic enzymes described in the article was in fact a DD-carboxypeptidase. This enzyme catalysed an important shift in my research and that of my associates as we became interested in a detailed study of this penicillin-resistant metallo (Zn) DD-peptidase[1,2] of the serine DD-peptidases, penicillin-binding proteins, and β-lactamases.[3-6]

1. **Dideberg O, Charlier P, Dive G, Joris B, Frère J-M & Ghuysen J-M.** Structure at 2.5 Å resolution of a Zn^{++}-containing D-alanyl-D-alanine-cleaving carboxypeptidase. *Nature* 299:469-70, 1982. (Cited 30 times.)
2. **Kelly J A, Dideberg O, Charlier P, Wéry J P, Libert M, Moews P C, Knox J R, Duez C, Fraipont C, Joris B, Dusart J, Frère J-M & Ghuysen J-M.** On the origin of bacterial resistance to penicillin. Comparison of a β-lactamase and a penicillin target. *Science* 231:1429-37, 1986. (Cited 40 times.)
3. **Duez C, Piron-Fraipont C, Joris B, Dusart J, Urdea M S, Martial J A, Frère J-M & Ghuysen J-M.** Primary structure of the *Streptomyces* R61 extracellular DD-peptidase. 1. Cloning into *Streptomyces lividans* and nucleotide sequence of the gene. *Eur. J. Biochem.* 162:509-18, 1987.
4. **Dideberg O, Charlier P, Wéry J P, Dehottay P, Dusart J, Erpicum T, Frère J-M & Ghuysen J-M.** The crystal structure of the β-lactamase of *Streptomyces albus* G at 0.3 nm resolution. *Biochemical J.* 245:911-3, 1987.
5. **Joris B, Ghuysen J-M, Dive G, Renard A, Dideberg O, Charlier P, Frère J-M, Kelly J A, Boyington J C, Moews P C & Knox J R.** The active-site-serine penicillin-recognizing enzymes as members of the *Streptomyces* R61 DD-peptidase family. *Biochemical J.* 250:313-24, 1988.
6. **Ghuysen J-M.** Bacterial active-site serine penicillin-interactive proteins and domains: mechanism, structure, and evolution. *Rev. Infec. Dis.* 10:726-32, 1988.

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