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Variable Nature of the Bacterial Cell Surface

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Introduction

Bacterial cell surfaces bear sites involved in bacterial adhesion to and colonization on other surfaces, sites that are immunogenic and sites that act as receptors for DNA and bacteriophage. The chemical natures of bacterial cell surfaces are consequently of great importance in defining many of the interactions of bacterial cells with their environments, whether these interactions involve colonization in natural ecosystems or the aetiology of bacterial disease.

In 1951 Salton and Horne described a ballistic method for the disintegration of bacteria and isolation of a cellular fraction that retained the original shape of the bacterial cells but which was relatively free of cell membrane and cytoplasmic components. Since that time much information has accumulated on the chemistry, biosynthesis, topography and function of bacterial cell walls and their components (for recent reviews see Daneo-Moore and Shockman 1977; Duckworth 1977; Ghuysen 1977; Sargent 1978; Sletyr 1978; Tipper and Wright 1979; Tomaz 1979; Tonn and Garder 1979; Wright and Tipper 1979; Rogers *et al.* 1980; Wicken and Knox 1980; Beveridge 1981; Rogers 1981; Shockman *et al.* 1981; Shockman and Wicken 1981; Ward 1981; Shockman and Barrett 1983; Wicken 1984). Early chemical analyses of bacterial cell walls revealed a degree of constancy of composition that could be readily allied, for instance, to the concept of group- and type-specific surface antigens exploited in reliable serological classification of bacteria. At the same time the clearly recognizable covalent association of the various macromolecular components of the isolated cell wall of Gram-positive bacteria in particular suggested an architecture that resembled a 'brick-wall'. Neither of these concepts is wholly true. Indeed, given the known adaptability of many bacterial species to different environments and the benefit of hind-sight, it might be considered surprising that such a holistic concept of the bacterial cell wall and the nature of the bacterial cell surface could have survived into the 1980s. In this review discussion is restricted to Gram-positive bacteria but the issues raised are applicable to Gram-negative bacteria as well.

The Cell Surface of Gram-positive Bacteria

By cell surface is meant the face of the cell as seen from the perspective of the external environment or, in chemical terms, the particular spatial arrangements of macromolecules and reactive sites that are able to interact directly with components of the environment. Cell surface is not synonomous with cell wall. The cell wall of Gram-positive bacteria is the water-insoluble fraction obtained from mechanically disrupted bacteria. It is composed of a mesh-work of peptidoglycan with one or more carbohydrate-like or true polysaccharide polymers and sometimes protein, all of which are linked covalently to the peptidoglycan; the detailed chemistry is generally genus- and often species-specific. The cell wall is,

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however, an operational fraction in two senses. Firstly, in the process of its preparation any water-soluble elements, not covalently linked to peptidoglycan, will have been lost and, secondly, any wall preparation is derived from a population of cells at a particular time in their life cycle. We now know that many genera turn over the components of this insoluble fraction during the life cycle and the cell wall in such cases is a dynamic rather than a static entity (Daneo-Moore and Shockman 1977; Shockman and Barrett 1983). Both of these considerations should spell caution in regarding cell wall chemistry as a total reflection of the nature and degree of constancy of the bacterial cell surface.

In recent years has come the recognition of the existence of other cell wall components in Gram-positive bacteria that are loosely associated with, rather than being covalently linked to, the rigid mesh-work of peptidoglycan. These *cell-wall-associated* components are readily removed by comparatively mild procedures including those employed in purifying isolated cell wall fractions such as extraction with buffers and detergent solutions. The components include a variety of macromolecular species such as the amphiphiles, of which lipoteichoic acids are the best known examples, proteins, polypeptides and polysaccharides (for reviews see Wicken and Knox 1980; Wicken 1984). The amphiphiles may also retain an association with the cell membrane, while others, such as proteins and polysaccharides, may be variously and loosely associated with the peptidoglycan in depth or at its surface as a capsule. Most, if not all, of these cell-wall-associated components are also found in varying quantities in the extracellular fluid, either due to active excretion or cell-wall turnover. Spatially, therefore, cell-wall-associated polymers can be thought of as being: (*a*) intramural, or within the peptidoglycan mesh-work; (*b*) extramural, or beyond the peptidoglycan mesh-work while still retaining a physical association with its surface; (*c*) extracellular and no longer associated with the producing cell.

These spatial divisions are not rigid but represent regions in a continuum and individual types of cell-wall-associated polymers may be distributed across the continuum both spatially and also temporally if they are in transit. The extramural region is, by this definition, the true surface and 'face' of the bacterial cell. It will be composed of cell-wall-associated components as well as exposed portions of the rigid cell wall and filamentous penetrations such as flagella and fimbriae. In thickness it can vary from virtually a monolayer through the readily demonstrable capsules formed by some species in laboratory culture to the extensive glycocalyces described by Costerton *et al.* (1981*a*, 1981*b*) for individual organisms and embedded microcolonies in natural ecosystems. The extramural region is also highly hydrated and readily penetrable. It can be regarded as a gradient of polymers ranging from a high degree of order proximal to the rigid portion of the cell wall to a concentrated solution distally as this region merges with the truly extracellular environment. The bacterial surface thus has depth and interaction with the environment can occur at various levels within it. This is well illustrated by such imprecise descriptions of *Streptococcus mutans* polysaccharide antigens as being located 'in or on the cell wall' (Hamada and Slade 1980) or 'on or near the cell surface' (Iacono *et al.* 1976).

Cell Surface Variability in Gram-positive Bacteria

Having defined the nature of the surface of the Gram-positive bacterial cell, the question of constancy of its composition or expression still remains. Clearly this is an important question for what we are asking is does the surface of the organism grown in batch culture in rich media in the laboratory resemble the surface of the same organism growing in a natural ecosystem? Our studies have been concerned with representative strains of two genera of oral Gram-positive bacteria which are associated with dental plaque, namely the streptococci and lactobacilli and we have used the technique of continuous culture in the chemostat both to vary some of the parameters of growth, such as pH, generation time, carbohydrate energy source and cation balance as well as to try and mimic some of the growth conditions these organisms experience in their natural oral ecosystem—

plaque. The mean generation time of plaque organisms has been estimated to be of the order 7–12 h, with limiting carbohydrate being the main determinant (Hamilton *et al.* 1979). In laboratory batch culture with excess carbohydrate the same bacteria have generation time of less than 1 h.

Effect of Growth Conditions on the Surface Properties of Whole Organisms

Adhesion to saliva-coated hydroxyapatite (SHA) beads (as a model of the tooth surface), relative hydrophobicity, response to lytic enzymes and immunogenicity of surface polymers are properties of whole organisms that have been studied in our laboratories in relation to variation in growth conditions in batch and continuous culture.

Table 1. Relative adhesion of *S. sanguis* G9B and *S. mutans* Ingbritt to saliva-coated hydroxyapatite beads for organisms in batch and chemostat cultures

In batch cultures organisms were grown on 2% (w/v) glucose with and without pH control. In chemostat cultures the carbohydrate source was the limiting nutrient (0·2%) and the dilution rate set to maintain mean generation times of 1·4 or 7 h at the indicated controlled pH value. Results for each organism are based on relative amounts of inhibition by unlabelled cells from various growth conditions on the adhesion of radiolabelled batch cells, grown at pH 6·0 with glucose, to saliva-coated hydroxyapatite beads. Direct comparisons between species and hence the adhesion ratios of *S. sanguis/S. mutans* are based on the isotherms for the two organisms grown at pH 6·0 in batch cultures. Values estimated from data in Rosan *et al.* (1982); Campbell *et al.* (1983)

Growth conditions	Relative adhesion		Adhesion ratio
	S. sanguis	*S. mutans*	
	Batch culture		
Glucose, pH 6·0	*100*	*24*	4·2
Glucose, no pH control	80	10	8·0
	Chemostat culture		
Fructose, pH 6·0, 7 h	81	10	8·1
Glucose, pH 6·0, 1·4 h	89	23	3·9
Glucose, pH 6·0, 7 h	42	17	2·5
Fructose, pH 6·0, 1·4 h	74	32	2·3
Glucose, pH 7·5, 7 h	26	24	1·1

Streptococcus sanguis is commonly regarded as more adhesive than *S. mutans*. This view is supported by the SHA-model with batch cultures, particularly those with controlled pH (Table 1) but it is also evident that growth conditions can be manipulated in the chemostat to result in near equality of adhesive ability of the two species. In Table 2 the effects of growth conditions on the adhesion and surface hydrophobicity of *S. sanguis* G9B are examined in some detail. Here pH, carbohydrate source and cation balance of the medium have marked and independent effects on both surface properties.

Similarly the immunogenicity of the cell wall polysaccharides of *Lactobacillus fermentum* NCTC6991 (Knox *et al.* 1979*a*) and, to a lesser extent, *S. mutans* Ingbritt (Knox *et al.* 1979*b*; Grossi *et al.* 1983) when whole cells are injected into rabbits has been shown to be dependent on generation time and pH of growth. Lysis of *S. mutans* Ingbritt with an *N*-acetylmuramidase was more rapid in chemostat-grown cells with fructose as limiting carbohydrate than with glucose. That this difference was probably due to differences in access of the peptidoglycan substrate to the enzyme in whole cells was indicated by uniform rates of lysis of isolated and purified cell walls irrespective of the growth conditions (Knox *et al.* 1979*b*; Hardy *et al.* 1981).

Table 2. Effect of growth conditions on adhesion and surface hydrophobicity of *S. sanguis* G9B[A]

pH	Growth conditions[B] Carbohydrate	Major cation	Inhibition of adhesion[C] (%)	Hydrophobicity[D] (%)
		Chemostat culture		
7·5	Glucose	Na^+	36	83
		K^+	35	58
	Fructose	Na^+	69	48
		K^+	66	24
6·5	Glucose	Na^+	51	56
		K^+	32	44
	Fructose	Na^+	59	40
		K^+	69	66
5·5	Glucose	Na^+	49	73
		K^+	35	67
	Fructose	Na^+	53	67
		K^+	73	75
		Batch culture		
6·0	Glucose	Na^+, K^+	58–62[E]	47
—	Glucose	Na^+, K^+	48	97

[A] Data from Knox and Wicken (1985).
[B] Organisms were grown in chemostat culture at a dilution rate = 0·1 h^{-1} (generation time = 7 h) with limiting carbohydrate and at various controlled pH values. The complex medium normally used (Jacques *et al.* 1979*b*) was modified to contain Na^+/K^+ mole ratios of 8·6 : 1 or 0·9 : 1. In batch culture with glucose as carbohydrate source and with and without pH control, the Na^+/K^+ was not adjusted and had the intermediate value of 1·9 : 1.
[C] Inhibition of adhesion of radio-labelled pH 6·0 batch-grown cells to saliva-coated hydroxyapatite beads (Campbell *et al.* 1983).
[D] Percentage adhesion of organisms to hydrocarbon in a two-phase hexadecane–aqueous buffer system (Rosenberg 1984).
[E] Range for four different experiments.

Cell Wall versus Cell-wall-associated Components

While changes in growth conditions, for example phosphate limitation in continuous culture, can result in marked phenotypic changes to the cell wall of certain organisms (Tempest and Wouters 1981; Ellwood *et al.* 1982) our studies on representative strains of oral streptococci and lactobacilli indicate a high degree of phenotypic stability for the cell wall components at different generation times and pH of growth, although variations were found in specific instances. Some examples from our studies include:

1. The peptidoglycan and covalently associated polysaccharide of *S. mutans* Ingbritt remained constant over a wide range of generation times and pH of growth (Knox *et al.* 1979*b*).
2. The cell wall galactosyl–glycerol teichoic acid and polysaccharide of *S. mutans* BHT were both invariant in terms of amount and structure over a wide range of growth conditions (Wicken *et al.* 1982*a*).
3. The glucosyl–ribitol teichoic acid of *Lactobacillus plantarum* did not vary in amount with change in growth conditions but the degree of its secondary glucosyl substitution was responsive to growth conditions (Wicken *et al.* 1982*a*).
4. The hexosamine-containing group C antigen of *Lactobacillus casei* subsp. *rhamnosus* NCTC 10302 remained constant in amount and structure (Wicken *et al.* 1983).
5. Some variation was recorded, however, for rhamnose-containing cell wall polysaccharides in terms of amount in *L. casei* NCTC 6375 and *S. mutans* 6715

(Wicken *et al.* 1983; K. W. Knox and A. J. Wicken, unpublished observations), and differences in amount and composition in *S. mutans* B13 (Linzer *et al.* 1984).

In contrast major differences can occur in the amounts of cell-wall-associated components in response to changes in growth conditions and these differences occur in both the cellular and the extracellular situation. Examples of these are:

1. *L. casei* subsp. *rhamnosus* NCTC 10302 produces a rhamnose-containing capsular polysaccharide; under the usual batch culture conditions with glucose in excess, polysaccharide production corresponded to 24% of cell dry weight while in continuous culture with growth-limiting glycose polysaccharide production ranged from 57% for fast-growing cells (1·2 h mean generation time) up to 126% for slow-growing cells (14 h mean generation time). The trend was similar in continuous culture with limiting fructose although the results were quantitatively lower (Wicken *et al.* 1983).
2. The production of lipoteichoic acid is markedly affected by changes in all parameters of growth studied, i.e. growth rate, pH of growth, limiting carbohydrate source and Na^+/K^+ ratios in the medium employed and these variations are found both in the cellular and extracellular situation (Jacques *et al.* 1979*a*, 1979*b*; Hardy *et al.* 1981; Wicken *et al.* 1982*b*). Table 3 illustrates the wide variation in relative amounts of cellular lipoteichoic acid for several organisms grown at pH 6·0 with limiting glucose or fructose at different generation times. Similar variability can be observed in the extracellular situation and through variation of other growth conditions such as pH and cation balance.

Table 3. Relative amounts of cellular lipoteichoic acid for various chemostat cultures grown at pH 6·0 and with either limiting glucose or fructose

Data from Jacques *et al.* (1979*a*), Hardy *et al.* (1981), Wicken *et al.* (1982*b*), Knox and Wicken (1985) and expressed as the ratio of cellular lipoteichoic acid produced in cultures grown with limiting fructose to that produced in cultures with limiting glucose

Dilution rate (h^{-1})	*S. mutans* Ingbritt	*S. mutans* AHT	*L. casei* NCTC 6375	*L. fermentum* NCTC 6991
0·05	2·8	5·6	0·6	—
0·10	3·0	3·5	1·6	1·1
0·30	2·2	2·5	1·6	1·1
0·50	9·0	2·0	3·8	1·1

3. *S. mutans* strains produce appreciable quantities of extracellular proteins some of which are also located extramurally as cell-wall-associated antigens. The total amount of extracellular protein as well as relative proportions of individual proteins is profoundly affected by generation time, pH of growth, limiting carbohydrate source and Na^+/K^+ ratio (Hardy *et al.* 1981; Knox *et al.* 1982; Forester *et al.* 1983; Knox and Wicken 1985).

Conclusions

Gram-positive bacterial cell surfaces can be markedly phenotypically variable in response to changes in growth conditions and the variation is associated more with the synthesis of extramural cell-wall-associated components rather than with the relatively invariant components of the cell wall's rigid peptidoglycan–carbohydrate complex. The latter will, of course, include many chemical features characteristic of the genus or species but the quantitative expression or exposure of these at the cell surface will be influenced by the total composition of the extramural region. It is therefore important in any study involving bacterial cell surface interactions that as many as possible of the factors governing the growth conditions should not only be controlled but if possible also be comparable with

the situation *in vivo*. In our experience *S. mutans*, for example, grown at pH 6·0 or 7·0 with limiting carbohydrate and a generation time of 10 h is reproducibly very different in terms of its surface properties and chemistry from the same organism grown in excess carbohydrate in rich media with no pH control. We do not suggest that uncontrolled batch culture of microorganisms should be eliminated. However, in studies involving bacterial cell surfaces, uncontrolled batch culture has severe limitations both in difficulty of relating to *in vivo* growth conditions and in reproducibility of surface chemistry and surface properties.

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