CONSTANT PEPTIDOGLYCAN DENSITY IN THE SACCULUS OF *ESCHERICHIA COLI* B/r GROWING AT DIFFERENT RATES

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1. Introduction

The determination and maintenance of bacterial cell shape are problems still far from being understood (reviewed [1,2]). Several models have been advanced during the last decade, for mechanisms governing the observed changes in the dimensions of rod-shaped Gram-negative bacteria [3,4] under different growth conditions ([5–13]; O. Pierucci; M. J. Case and A. G. Marr; B. Westling-Haggstrom, B. Engberg and S. Normark; personal communications). It has always been tacitly assumed that the molecule(s) that is responsible for maintaining cell dimensions is present in a constant density (amount per unit surface-area). The crucial importance of this implicit assumption for the applicability of most growth models has led us to determine the density of several major components of the cell wall.

Here we present results on measurements of peptidoglycan (PG), neutral carbohydrate and protein content in cells and in sacculi of *Escherichia coli* B/r strain H266, growing with different dimensions in a variety of media that support growth rates over most of the possible range ($0.4 \leqslant \mu \leqslant 2.9$ doublings/h).

2. Materials and methods

2.1. *Growth*

E. coli B/r (strain H266; [14]) was cultivated in AB minimal salts solution [15] supplemented with either alanine, succinate, alanine and proline, glycerol, glucose (with or without a mixture of 15 amino acids) or glucose and casein hydrolysate (Sigma; [11]). For the highest growth rate ($\mu = 2.9\ h^{-1}$) Luria Broth (LB; [16]) was used. The cultures were vigorously aerated at 37°C in a New-Brunswick gyratory water-bath shaker during at least 10 doubling times, and maintained below 0.5 A_{450} (Gilford microsample spectrophotometer) by periodic dilutions. Two identical samples, 30–40 ml each, were taken into ice-cooled centrifuge tubes containing sodium-azide (final conc. 20 mM), sedimented (5 min at 18 000 rev./min) and the cell pellets were washed 3 times with 12 ml AB-buffered salts solution. The whole procedure was performed in the cold and lasted less than 1 h, at the end of which pellets were stored at −70°C.

2.2. *Chemical determinations*

One sample was suspended in 2 ml 4% sodium dodecyl sulfate (SDS) and heated (30 min) at 100°C. The SDS-insoluble material (mostly peptidoglycan sacculi; [17,18]) was sedimented at 130 000 × *g* for 90 min and hydrolysed (6 N HCl containing 0.2 M dimethyl sulfoxide, 105°C, 18 h). Amino acid analysis (Beckman 121) was performed on the hydrolysate with 100 nmol norleucine included as an internal standard. The oxidation with dimethyl sulfoxide was done to avoid any overlapping of traces of methionine on the diaminopimelic acid peak [19]. Total cell surface-area per sample was calculated from the dimensions of the bacterium measured under identical conditions [11–13], using the equation $\bar{A} = 2.554 \cdot 2^{49.3\,\mu/60}$ (eq. (7) in [13]), where μ is the growth rate of the culture and $\bar{A}$ the average

surface-area per cell. (Cell concentration was determined by viability counting, with a counting chamber or with a Coulter Counter model Z_B.) The second sample was utilized for the determinations of protein [20] and neutral sugars by the phenol–sulfuric acid method using D-mannose as standard [21]. Total amino acid analysis of intact cell hydrolysates (6 N HCl, 105°C, 18 h) were done as above.

3. Results

The strain studied, *E. coli* B/r, H266, is known to increase its cell size by > 1 order of magnitude over the range of growth rates studied ([11–13], fig.1a). This enlargement results from an increase in both dimensions, which results in reduced surface/mass ratio. Results of the chemical determinations performed on samples of cells grown at the rates indicated are summarized in table 1. The average peptidoglycan density, expressed as the number of diaminopimelic acid molecules per square nanometer of sacculus surface area, did not change substantially with changes in μ (fig.1b), while that of neutral sugars (expressed analogously) increased over the entire range studied (fig.1c).

From amino acid analyses of the isolated sacculi preparations, 9 non-peptidoglycan amino acids (most likely belonging to the covalently-bound lipoprotein) were added-up, and the total was divided by the total amount of peptidoglycan. The resultant ratio is an approximation for relative content of lipoprotein per peptidoglycan unit, which seems not to alter systematically with μ (fig.2).

4. Discussion

The amount of neutral sugars in *E. coli* is a crude measure of the lipopolysaccharide (LPS) surrounding the cell [22] although some of it may derive also from storage macromolecules such as glycogen [23]. The linear proportionality between its density and growth rate (fig.1c) supports the view that the neutral-sugar macromolecules are not essential structures for normal metabolism and multiplication nor for cell shape determination or maintenance. This should not

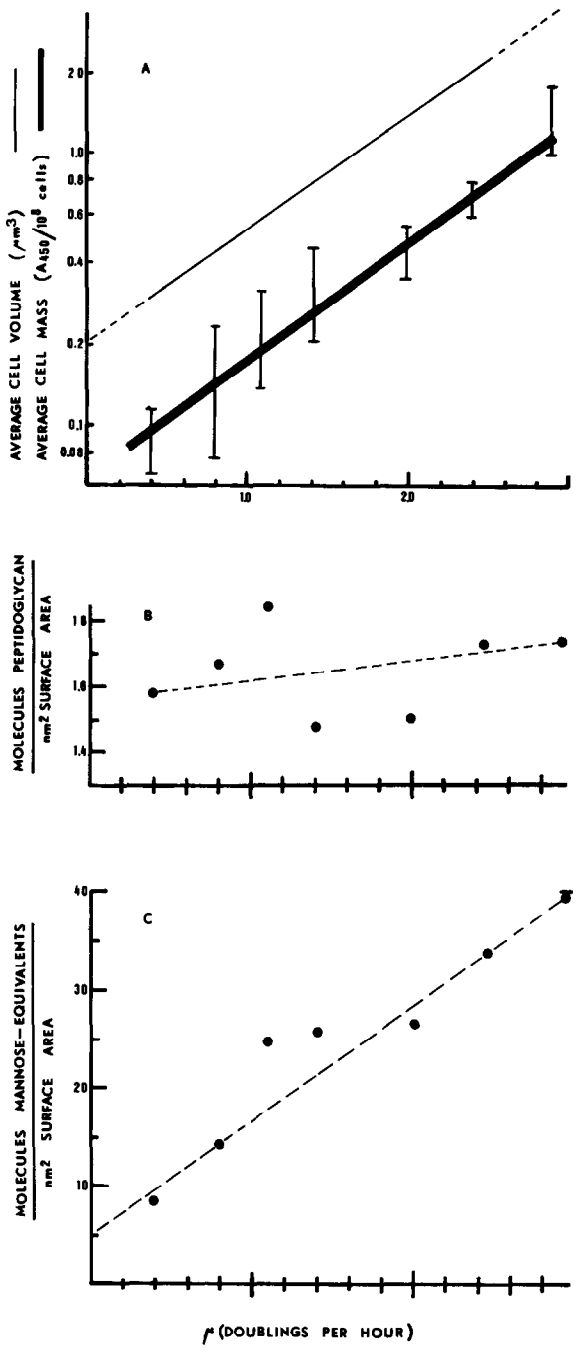

Fig.1. Average cell size (A) and surface densities of peptidoglycan (B) and of neutral carbohydrate (C) as functions of growth rate μ. Average cell volume $\bar{V}$ was calculated from cell dimensions using the equation $\bar{V} = 0.208 \cdot 2^{83.3\,\mu/60}$ (see [13]). Average cell mass was taken as the parallel line that fits best the measured values (inside the flags).

Table 1
Analyses of cells and of sacculi isolated from *E. coli* B/r cells (strain H266) growing at various rates

Growth rate (μ) (h^{-1})	Cell mass (A_{450})	Cell number (10^9)	Peptido-glycan (nmol)[a]	Mannose equiv. (μmol)	Relative lipo-protein[a]	Protein (mg)
2.93	12.50	1.04	42.58	0.922	1.36	1.37
2.45	12.33	1.67	49.76	0.961	1.89	1.18
2.00	13.15	2.74	55.11	0.961	2.49	1.45
1.40	15.30	5.67	79.17	1.367	3.54	1.50
1.10	16.83	8.41	123.63	1.667	4.66	1.80
0.80	14.25	9.50	106.58	0.922	2.87	1.75
0.40	11.63	11.63	97.40	0.517	4.23	1.55

[a] In sacculi

All absolute values are on a per-sample (30–40 ml culture) basis

be surprising since certain mutants almost completely lacking LPS or glycogen still display a normal rod-shape [22,23]. An interesting question that remains unanswered is whether the higher carbohydrate density found in faster-growing cells represents for example more LPS chains per units surface or longer chains.

The almost constant density of peptidoglycan in the sacculus of *E. coli* B/r irrespective of the growth medium or of cell size (fig.1b) indicates that this macromolecule may have a fixed structure which plays a role in the determination or maintenance of the rod-shape of the bacterium. The small increase in PG density (with μ) observed (~10% over the whole range) could mainly be explained by the large increase, over the same range of μ values, of the proportion of constricted cells (6% in 0.4 h^{-1}, 40% in 2.9 h^{-1} [4]);

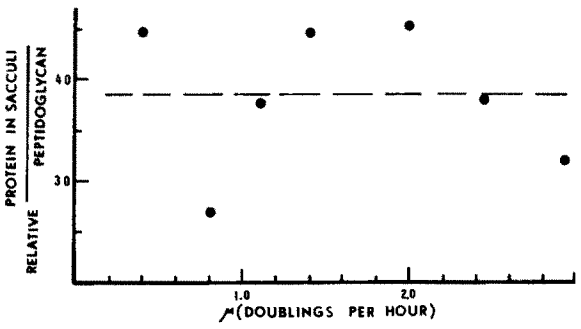

Fig.2. Protein content of sacculi relative to peptidoglycan as a function of growth rate μ.

an average constricted cell contains also two partially-completed septa, the contribution of which to total surface area was not considered here.

An average number of 1.65 repeating units of PG/nm^2 surface area was calculated (fig.1b). This number, together with the known length of a repeating disaccharide unit (1.00 nm, [18,24]), indicates that the average distance between two adjacent PG chains is 0.606 nm. This distance is longer by ~30% than the one estimated from X-ray diffraction studies (0.45 nm, [18,24]). Since the material prepared for X-ray crystallography is dried out PG foils, its structure may be different from that in the intact cell envelope. Some evidence has recently been presented which indicates that the PG lattice in the cell may be regarded as a paracrystalline foil consisting of amorphous regions and crystalline mosaic blocks [24]. A similar conclusion, that the PG density is not sufficient to cover all the cell surface as a crystalline chitin-like structure, has earlier been postulated [18,25]. However, it is not easy to conceive how such a layer, consisting of ~30% amorphous regions, will still retain the biological and physical properties characteristic for PG.

Almost all of the protein in sacculi preparations is lipoprotein (LP) bound to peptidoglycan [18], and therefore the data recorded here indicates that LP content of the sacculi is proportional to that of PG. The role of LP in cell shape maintenance and determination is not yet clear. Mutants completely lacking LP have been shown to grow and divide normally

[26]. It should be interesting to find out whether in such mutants, a change in μ does not affect the amount of PG per surface area.

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