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Non-Specific Uptake and Specific Binding of Sulphate by *Aspergillus nidulans*

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Yamamoto & Segel (1966) noted that the sulphate transport curves (plots of sulphate uptake versus time) for *Penicillium chrysogenum* did not go through the origin. They suggested that the intercept on the ordinate axis (sulphate uptake) resulted either from adsorption of sulphate on the mycelial surface or from penetration, by diffusion, into a free space (cf. 'apparent free space'; Briggs *et al.*, 1961). Under conditions of no net transport of sulphate, the uptake was non-saturatable, energy-independent, temperature-independent and unaffected by pH in the range 4–10.

Aspergillus nidulans wild type and its sulphite-requiring mutants (Arst, 1968) showed a similar sulphate uptake. In the transport-negative mutant sB-3 uptake of sulphate into the free space was non-saturatable and non-specific, and was independent of the energy and sulphur status of the mycelium and the time of exposure to external sulphate. Equilibrium with the external medium was achieved rapidly (15 s–1 min). Under conditions of no net transport, non-specific initial uptake also occurs in transport-positive mutants. The uptake accounts for 55–80% of the total volume of the pressed-dry mycelium, of which less than 20% is interstitial. The 'apparent free space' volume varies with osmotic changes (cf. Briggs, 1970). For example, the initial overshoot and delay in the establishment of steady sulphate uptake, reported by Scott & Spencer (1965) in transport studies, were observed only when harvested mycelia had been equilibrated with deionized water before being transferred to the buffered medium containing Na₂³⁵SO₄.

At an external sulphate concentration of 50 μM the 'apparent free space' of *Asp. nidulans* sB-3 accumulated 0.1 μmol of SO₄²⁻/g dry wt., but the intercept on the ordinate axis could increase to 0.7 μmol/g dry wt. in transport-positive mycelia after a period of sulphur starvation. In general, higher values for the intercept on the ordinate axis were associated with increased sulphate transport rates, but inhibition of protein synthesis during sulphur starvation prevented these increases. The elevation of the intercept on the ordinate axis was not affected by prior incubation of

the mycelium with 2,4-dinitrophenol, but pre-pulsing with 5 mM-K₂CrO₄ restricted the value to that of the transport mutant. These and other results indicate that the increase in the intercept on the ordinate axis, above the 'apparent free space' value, measures specific sulphate-binding to a protein, the synthesis of which is repressed during sulphur sufficiency. A specific sulphate-binding protein is known to form part of the transport system in bacteria (Pardee, 1966).

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The Effect of Growth Conditions on the Chemical Composition and Endotoxicity of Walls of *Aerobacter aerogenes* N.C.T.C. 418

By A. D. PEARSON and D. C. ELLWOOD (*Microbiological Research Establishment, Porton, nr. Salisbury, Wilts., U.K.*)

The core structure of the lipopolysaccharide of walls of Gram-negative bacteria contains two characteristic components, i.e. 3-deoxy-2-octulonic acid and heptose. Walls of *Aerobacter aerogenes* grown in a chemostat under different growth conditions were examined for these two components. It was found that the 3-deoxy-2-octulonic acid and heptose contents and their ratio to one another varied quite markedly, suggesting that the composition of the lipopolysaccharide was phenotypically variable (Ellwood & Tempest, 1967; Tempest & Ellwood, 1969).

3-Deoxy-2-octulonic acid is the sugar that links the lipid A (the lipid A moiety gives the lipopolysaccharide its endotoxic properties; Lüderitz *et al.*, 1966) to the core polysaccharide, and some correlation between the 3-deoxy-2-octulonic acid content of walls and their toxicity could be expected.

Wall preparations of *A. aerogenes* used in the previous chemical study have now been examined further with respect to their toxicity in mice. The LD₅₀ was determined by standard procedures, and it was found that organisms grown glycerol-limited at a dilution rate of 0.8 h⁻¹ had walls that gave an LD₅₀ in mice of 3 mg/20 g body wt. (walls of batch-grown *Escherichia coli* give LD₅₀ in mice of 2–3 mg/20 g). All other wall preparations prepared from organisms grown at different growth rates or different growth

limitations were markedly less toxic. In fact, the walls of Mg^{2+} -limited organisms grown at a low rate (dilution rate 0.1 h^{-1}) appeared to be non-toxic.

The 3-deoxy-2-octulonic acid contents of walls of fast-growing (dilution rate 0.8 h^{-1}) glycerol-limited organisms and walls of slow-growing (dilution rate 0.1 h^{-1}) Mg^{2+} -limited organisms were similar, but the toxicities of these walls were very different. This suggests that no correlation between the 3-deoxy-2-octulonic acid content of walls and their toxicity exists.

A characteristic component of the lipid A portion of the lipopolysaccharide of the Enterobacteriaceae is 3-hydroxymyristic acid. Thus again it would be expected that the 3-hydroxymyristic acid content of wall preparations would correlate with the toxicity result. The 3-hydroxymyristic acid content of these wall preparations was determined by methanolysis of the walls and g.l.c. of the methyl esters of the fatty acids formed. The 3-hydroxymyristic acid (characterized by retention times and mass spectra of its methyl ester) content of these walls again varied markedly, but there was no apparent correlation with the toxicity results.

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The Effect of Growth Conditions on the Muropeptide Composition of Walls of *Bacillus subtilis* W23

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This communication reports some preliminary findings on the influence of growth conditions on the muropeptide composition of *Bacillus subtilis* W23 when grown in a chemostat.

B. subtilis W23 was grown in a chemostat with different nutrient-limiting growth. Samples of organisms were removed from cultures and immediately heated at 100°C (for 20 min) to inactivate the autolytic enzymes. After the heating the organisms were isolated (after being washed three times with phosphate buffer) and incubated with lysozyme. Results showed that the rate of lysis of the organisms was different with respect to the growth limitation applied. Mg^{2+} -limited organisms lysed at a lower rate than did organisms from phosphate- or nitrogen-limited cultures. The difference in lysozyme sensitivity between Mg^{2+} - and phosphate-limited organ-

isms could be due to the different anionic polymers (teichoic acid and teichuronic acid) being present in the walls. This is unlikely, however, since ammonia-limited organisms, which also have teichoic acid in their wall (Ellwood, 1970) lysed at a faster rate than did phosphate-limited organisms. In fact, no correlation could be found between the wall anionic polymer content and the rate of lysis by lysozyme, suggesting that this difference might be due to the structure of the muropeptide.

Walls were prepared from organisms grown in a chemostat under a variety of conditions, and the anionic polymers present in the wall were removed by trichloroacetic acid extraction to leave a residue composed of muropeptide together with some protein. This residue was then analysed for muropeptide components and was found to be composed of glucosamine, muramic acid (presumably as the *N*-acetyl derivatives), alanine, glutamic acid and diaminopimelic acid. In all the wall preparations examined the qualitative composition was the same. However, quantitative analysis showed marked differences in the ratios of muramic acid and glucosamine to glutamic acid, whereas the ratio of the peptide components one to another was roughly constant. The molar ratios of muramic acid and glucosamine relative to glutamic acid in the muropeptide of ammonia-limited organisms were about unity, close to the theoretical value assuming that the sugar backbone of the muropeptides is unsubstituted. With Mg^{2+} - and sulphate-limited organisms the muropeptide contained a higher proportion of glucosamine than muramic acid, whereas the muropeptide of K^{+} -limited, glucose-limited and phosphate-limited organisms contained a higher proportion of muramic acid than glucosamine.

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The Respiratory-Chain Dehydrogenases of *Azotobacter vinelandii*

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Phosphorylating respiratory membranes from the nitrogen-fixing aerobe *Azotobacter vinelandii* catalyse the rapid oxidation of NADH, L-malate [NAD(P)^{+} -independent] and NADPH (Pandit-Hovenkamp, 1967).

NADH (K_m 0.040 mM), L-malate (K_m 0.167 mM) and NADPH (sigmoidal saturation kinetics, $s_{0.5}$ 0.440 mM) were oxidized at rates of 1.25, 1.59 and $1.49\text{ }\mu\text{g-atom of O/min per mg of protein}$ respectively with corresponding P/O ratios of 0.88, 0.54 and 0.58.