RHEOLOGICAL INTERACTIONS OF CYSTIC FIBROSIS TRACHEAL MUCIN AND PSEUDOMONAS AERUGINOSA EXTRACELLULAR ALGINATE

Y.M. Smedley, C. Marriott, N. Hodges, S.L. James, Department of Pharmacy, Brighton Polytechnic, Moulsecoomb, Brighton BN2 4GJ

Patients with cystic fibrosis (CF) suffer from progressive lung disease and are particularly susceptible to infection with mucoid strains of Ps.aeruginosa. Colonization with these strains leads to a rapid deterioration in pulmonary function and clearance of thick sticky respiratory mucus becomes a serious problem. Mucoid cells of Ps.aeruginosa secrete a polyanionic exopolysaccharide which resembles alginic acid and forms gels in the presence of calcium and other divalent cations. Since it has been reported that calcium levels are elevated in the CF lung (Kilbourn 1984), it is of importance to determine the extent to which Pseudomonas alginate contributes to the rheological properties of CF mucus, and whether various cations are of significance in any interaction. Tracheal mucin from chronic bronchitics (CB) was also studied since this represents a control with a similar level of infection.

Mucin was purified from CF and CB sputum by gel filtration and concentrated by ultrafiltration to 8% w/w. Clinical isolates of Ps.aeruginosa from CF sputum were grown in static liquid medium (casamino acids 1%, potassium gluconate 2%) for 144 hours at 37°C. The bacteria were removed by centrifugation and the alginate extracted by caesium chloride isopycnic density centrifugation and concentrated to 3% w/w. Solutions of calcium chloride (100 mM) and sodium chloride (200 mM) were prepared in HEPES buffer (0.05 M, pH 7.4) and mixtures of these salt solutions (4% v/v) with mucin (80% v/v) plus alginate (16% v/v) were equilibrated for 18 h at 4°C. The rheological properties were measured using an oscillating sphere microrheometer over the range 0.2 - 20.0 Hz. The values at 10 Hz, which approximates to the rate of ciliary beating, were used to calculate the percentage increase in storage and loss moduli (G' and G" respectively) of mucin/salts solutions on addition of the alginate. These percentage increases together with the standard errors (SE) are tabulated below.

Treatment	% Increase in Storage and Loss Moduli							
	CF G'	SE	CF G"	SE	CB G'	SE	CB G"	SE
Buffer	91.7	10.6	81.8	10.2	70.2	14.0	84.7	13.9
4 mM $CaCl_2$	141.6	11.5	163.3	12.4	75.5	13.9	50.9	15.2
8 mM NaCl	26.4	5.8	32.9	8.3	59.2	24.2	96.0	17.1

Addition of alginate caused significant increases in both moduli which were of a similar magnitude for CF and CB mucin. In the presence of Ca^{2+} the effect of alginate on the elasticity of CF mucin was significantly enhanced, whereas for CB mucin there was no significant enhancement. Elasticity can be related to the number of permanent interactions beween molecules and enhancement may be due to divalent cations causing cross-links. For CF mucin, addition of a monovalent cation, Na^+, significantly reduced the interaction between mucin and alginate. The Na^+ ions may bind to the same site on the molecules as the divalent cations, but would not be able to cross-link the molecules. The monovalent cations would also neutralise the negative charge on the molecules, reducing the electrostatic repulsion between them leading to compaction and a reduction in the probability of entanglements; this is shown as a reduction in viscoelasticity.

The results clearly demonstrate that CF and CB mucins, although ostensibly similar as products of chronic obstructive airways disease, may exhibit fundamentally different characteristics with the former being susceptible to cation-induced changes in its interaction with Ps.aeruginosa alginate. The results also show that the presence of alginate may contribute sigificantly to the thickness of CF bronchial secretions and to the clinical problem of mucus clearance.

Kilbourn, J.P. (1984) Current microbiol. 11: 19-22