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Influence of broad spectrum protease inhibitors on *Staphylococcus epidermidis* biofilm formation in vitro

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Staphylococcus epidermidis is the leading cause of infections relating to implanted medical devices (Rohde et al 2005). In most natural environments, a biofilm consists of a multispecies bacterial community, however, in almost 80% of biomaterial-associated infections *S. epidermidis* is the main causative organism (Götz 2002). The traditional approach to prevent biofilm formation at material surfaces involves the use of established antimicrobial agents. Currently, all antimicrobial reagents are evaluated against bacteria in the planktonic state; however, numerous studies have demonstrated that bacteria existing in biofilm communities may be up to one hundred fold more resistant to antimicrobials when compared with their planktonic counterparts. This inherent resistance of bacterial biofilms to antimicrobial agents coupled with ever increasing emergence of bacterial resistance highlights the need to develop novel therapies directed against pathogens associated with biomaterial-related infections (Götz 2002). Recent studies indicate that proteolysis plays a pivotal role in the pathogenicity of many microorganisms. In *S. epidermidis*, proteolysis is a critical factor in the establishment and maintenance of biofilms (Rohde et al 2005). Early studies have shown that biofilm formation in the clinically significant *S. epidermidis* depended upon the expression of a 140-kDa extracellular Accumulation-associated protein Aap (Hussain et al 1997; Rohde et al 2005). The truncated Aap domain mediates intercellular adhesion in a polysaccharide-independent manner. In contrast, expression of full length Aap does not lead to a biofilm-positive phenotype. Therefore, to gain adhesive function, full-length Aap requires proteolytic processing through the action of Staphylococcal proteases (Rohde et al 2005). This project seeks to demonstrate the importance of protease involvement in biofilm formation by *S. epidermidis* and to identify the proteases involved in the post-translational modification of Aap during this process. The Calgary biofilm device (CBD) was used to determine the effect of the broad range protease inhibitor α -2-macroglobulin on *S. epidermidis* biofilm formation. Addition of α-2-macroglobulin to the growth medium led to a significant decrease in biofilm formation of *S. epidermidis* (Table 1). The reduction in biofilm viable count observed upon addition of the broad spectrum protease inhibitor, α-2 –macroglobulin suggests that proteases are involved in biofilm formation. The proteases are thought to be involved in the post-translational modification of Aap, the cell-wall bound protein, which is essential for the accumulation of *S. epidermidis* on polymer surfaces (Hussain et al 1997), most probably through the action of a serine or metalloprotease (Rohde et al 2005). The protease, as yet unidentified, represents a therapeutic target for the development of specific inhibitors as putative antibiofilm compounds.

Table 1 Influence of the broad range protease inhibitor alpha-2-macroglobulin on *S. epidermidis* biofilm formation as determined by the CBD

Concn of α-2-macroglobulin (nM)	Mean biofilm viable count (cfu/ml)	Mean planktonic viable count (cfu/ml)
462 nM	$7.62 \pm 1.41 \times 10^3$	$5.85 \pm 1.98 \times 10^{10}$
308 nM	$2.33 \pm 1.25 \times 10^3$	$4.76 \pm 1.29 \times 10^{10}$
154 nM	$5.67 \pm 0.65 \times 10^3$	$5.08 \pm 0.69 \times 10^{10}$
0 (Control)	$1.24 \pm 1.01 \times 10^6$	$8.00 \pm 0.55 \times 10^{10}$

Data are means ± s.d.

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Hussain, M. et al (1997) *Infect. Immun*. **65**: 519–524
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