Levan as a Virulence Determinant of *Actinomyces viscosus*

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ABSTRACT

Levan as a Virulence Determinant of *Actinomyces viscosus*

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Actinomyces viscosus is a significant microbial component of human dental plaque, and is capable of adhering to surfaces under a variety of nutritional conditions. This organism has been shown to produce dental caries in experimental animals and is a member of one of the few genera of oral microorganisms which has been frequently shown to destroy periodontal tissue in monocontaminated experimental animals. Many studies have shown a correlation between *A. viscosus* and periodontal disease, but essentially nothing is known about which components or products of *A. viscosus* are responsible for its pathogenic properties. Bacterial virulence can be related to the presence of a polysaccharide capsule, and some bacterial polysaccharides in dental plaque have been shown to participate in mechanisms of plaque formation. Other polysaccharides have been shown to be able to activate complement (C) by the alternate C pathway.

Levan was not present on the surface of glucose-grown *A. viscosus* cells, but after a brief sucrose incubation, these cells were completely encapsulated with a tenacious levan layer constituting 0.02% to 0.03% of the cellular dry weight. Sucrose-grown *A. viscosus* cells possessed a levan layer which was only moderately enhanced by a subsequent sucrose

incubation. Part of the levan on sucrose-grown cells was found to be a loose slime rather than a tenacious capsule. Purified extracellular levan from *A. viscosus* was shown to contain both $\beta(2\rightarrow1)$ and $\beta(2\rightarrow6)$ linkages, and when injected into monkeys, rats, and mice was weakly immunogenic or nonimmunogenic. Purified levan was shown to be a potent activator of the alternate C pathway in both human and guinea pig serum. Both levan-encapsuled and levan-deficient *A. viscosus* cells were capable of C activation, although the former proved more adept at C consumption. *Actinomyces viscosus* cells were aggregated by a short sucrose incubation, but not glucose or fructose. Since both levan and dextran also caused cellular aggregation, it is possible that sucrose was converted to levan and/or dextran, thereby aggregating the cells. However, sucrose-dependent dextran synthesis by *A. viscosus* has not been observed. Sucrose aggregation occurred over a concentration range of 1% to 5% and had a pH optimum of 5.6 to 6.0. The degree of aggregation was found to be a direct function of sucrose incubation time, and 37 and 68 hr old cells aggregated to a greater extent than did 91 hr old cells.

Actinomyces viscosus is a ubiquitous member of the plaque flora; its ability and the ability of its extracellular levan to activate C by the alternate C pathway could be very important in the pathogenesis of periodontal disease. Plaque not only provides the necessary close association of *A. viscosus* to periodontal tissues, but also establishes a continuous levan producing site. Complement components are known to be chemotactic for PMN and macrophages, and be capable of causing release of lysosomal enzymes. Activation of C is also known to create bone resorption through a prostaglandin mediator. A sequence of events generated by C activation by *A. viscosus* or its products could easily cause damage to tissue seen in periodontal disease, and therefore may be at least one etiologic mechanism of the disease. Although the levan from *A. viscosus* is only weakly immunogenic, its ability to activate C and participate in cellular aggregation may make this polysaccharide an important virulence factor for this organism.

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