A catalysis-independent mechanism underlies alginate lyase biofilm dispersion and antibiotic synergy

K.E. Griswold12 and J.W. Lamppa1
1Thayer School of Engineering, Dartmouth College, 14 Engineering Dr., Hanover, NH 03755, U.S.A.
2Molecular and Cellular Biology Program, Geisel School of Medicine, Dartmouth College, Hanover, NH 03755, U.S.A.

Chronic airway infection by Pseudomonas aeruginosa is the primary cause of premature mortality among cystic fibrosis (CF) patients, and this opportunistic, drug-resistant pathogen is also more broadly associated with burn wounds and various hospital acquired infections. During colonization of the CF airway, P. aeruginosa causes a range of clinical complications including stimulation of a hyper-inflammatory response, obstruction of the pulmonary tract, and progressive loss of lung function. The bacteria’s persistence within the CF lung is thought to result in part from a biofilm mode of growth, which contributes to evasion of the host immune response and antibacterial chemotherapies. In contrast to environmental niches, in the CF lung P. aeruginosa generally transitions to a mucoid phenotype characterized by overproduction of the exopolysaccharide alginate. This co-polymer of (1,4)-linked β-D-mannuronic acid and α-L-guluronic acid alters biofilm architecture and function and thereby compounds P. aeruginosa’s persistence in the chronically inflamed airway.

Given alginate’s contribution to mucoid biofilm structure, its function in bacterial virulence, and its role in the persistent nature of lung infections, it has long been considered an attractive target for interventional therapies. In particular, biocatalytic degradation of mucoid P. aeruginosa biofilms using alginate lyase enzymes has been the subject of more than 20 years of research. Alginate lyase treatment has been shown to reduce viscosity in cultures of clinical isolates and in CF sputum, it strips biofilms from abiotic surfaces, it enhances phagocytosis and killing of P. aeruginosa by human immune cells, and it improves the efficacy of various anti-pseudomonal antibiotics. These observations have motivated continued interest in the use of inhaled alginate lyases as prospective treatments for chronic P. aeruginosa infections of the lung, particularly when used in combination with antibacterial chemotherapies.

We have completed a systematic analysis of the solution phase kinetics, biofilm-disrupting potential and antibiotic synergy of two promising alginate lyase therapeutic candidates. Sphingomonas sp. A1 alginate lyase (A1-III) has been shown previously to exhibit high levels of activity towards bacterial alginate, and has also been subjected to molecular engineering with an eye towards therapeutic applications. P. aeruginosa alginate lyase (AlgL) is produced by the CF-associated pathogen itself, and its confirmed role in bacterial alginate biosynthesis suggests the enzyme as an obvious choice for developing alginate-degrading CF biotherapies. On a superficial level, our results are consistent with the encouraging outcomes of prior alginate lyase studies. Our systematic approach, however, has led to the unexpected insight that enzyme-mediated biofilm disruption and antibiotic synergy are completely decoupled from catalytic activity. In fact, in our in vitro model system, equivalent biofilm dispersion and antibiotic synergy can be achieved with non-catalytic proteins or simple amino acids. As presented, our results suggest the need to carefully reexamine fundamental assumptions that have motivated more than two decades of research on therapeutic alginate lyases, and they represent a cautionary tale that may have broader relevance to other examples of biofilm-dirupting enzymes.

Keywords alginate lyase; biofilm dispersion; antibiotic synergy; Pseudomonas aeruginosa; cystic fibrosis

![Figure – SEM images of mucoid P. aeruginosa biofilms. Biofilms were treated (from left to right) with buffer only, 500 μg/ml tobramycin, 1000 μg/ml A1 alginate lyase, or a combination of tobramycin and alginate lyase. Enzyme-mediated disruption of biofilm matrix as well as synergy with the antibiotic are apparent, and these results are consistent with prior analysis of alginate lyases as anti-biofilm agents. This presentation will demonstrate that, surprisingly, these effects are entirely independent of alginate lyase catalytic function.](image-url)