ABSTRACT

Applications involving lectins are frequently limited by the low binding affinity of lectins for glycans. Individual lectin-glycan interactions are typically of low-affinity. However, high avidity is frequently attained in biological systems by virtue of the inherent multivalent presentation of glycans on cell surfaces coupled with the occurrence of high order lectin oligomers and/or tandem repetition of lectin domains in the polypeptide. Part 1 of my thesis is focused on engineering a bacterial F-type lectin domain (FLD) with the aim of increasing its ligand binding strength.

F-type lectins are L-fucose binding lectins with a typical sequence motif, HX(26)RXDX(4)R/K, containing conserved residues that participate in L-fucose binding. My co-workers previously reported the presence of a few eukaryotic F-type lectin domains with partial sequence duplication resulting in the presence of two L-fucose-binding sequence motifs (Bishnoi et al., 2015). I hypothesized that such partial sequence duplication might result in greater avidity of lectin-ligand interactions. Inspired by this example from Nature, I engineered a bacterial F-type lectin domain from *Streptosporangium roseum* to attain avid binding by mimicking partial duplication. The engineered lectin with partial sequence duplication demonstrated 12-fold greater binding strength to multivalent fucosylated glycoconjugates. The increased binding strength stemmed from avid binding that was, interestingly, mediated by an increased tendency for oligomerization rather than duplication of L-fucose binding sites. I believe that this Nature-inspired strategy might be useful for engineering lectins to improve binding strength in various applications.

Many bacterial FLDs are organized in CBM-like fashion with diverse domain architectures (Bishnoi et al., 2015). CBMs are well known for their role in promoting the enzymatic activity of their co-associated carbohydrate-active enzyme by targeting and proximity effects (Guillén et al., 2010; Hervé et al., 2010). With the aim of understanding the role of FLDs in targeting or modulating the activity of co-associated enzyme domains, I studied a *Cellulophaga algicola*

DSM 14237 protein possessing an FLD co-associated with a CBM6_Cellulase like domain and an alginate lyase domain in Part 2 of my thesis. Alginate lyases have tremendous applications in agricultural, biotechnological and pharmaceutical industries, and there is a need to find new alginate lyases with increasing industrial applications. My experimental results demonstrated that the alginate lyase domain of this *Cellulophaga algicola* protein (*Ca*Aly) is a functional alginate lyase, with endotypic mode of action and optimal temperature and pH of 37 °C and pH 7, respectively. It degraded both polyM and polyG alginates with higher preference for polyG. The co-associated FLD (*Ca*FLD) was found to be an active F-type lectin domain with fucose binding property, and preference for binding fucosylated glycans with H-type 2 motif. The FLD did not have any apparent stimulatory effect on the enzyme activity of the co-associated alginate domain.

Alginate lyases are considered potentially useful in disrupting alginate-rich *Pseudomonas* biofilms in infected lungs of cystic fibrosis patients but there is yet no clinically approved alginate lyase that can be used as a therapeutic. The alginate in *Pseudomonas* biofilms is characterized by high levels of O-acetylation and the presence of polyM blocks, making it resistant to many alginate lyases and host immune effectors (Franklin and Ohman, 1993; Pier et al., 2001; Sherbrock-Cox et al., 1984; Skjåk-Braek et al., 1986; Veeranagouda et al., 2011). Another challenge is the deactivation of alginate lyases in the presence of high ion concentrations found in sputum samples of cystic fibrosis patients (Mrsny et al., 1994).

I assessed the potential of *Ca*Aly and other alginate lyase candidates scoured from published literature as agents for inhibition of biofilms formed by mucoid *Pseudomonas* strains isolated from clinical samples. *Ca*Aly demonstrated significant biofilm inhibition ability in an in vitro biofilm assay using biofilms on plastic surfaces, and proved to be the best out of the panel of alginate lyases tested. *Ca*Aly also cleared biofilms of *Pseudomonas* formed over A549 lung epithelial cell lines. Appending an F-type lectin domain, either *Ca*FLD or *Sr*FLD, which has better binding towards Lewis^a/Lewis^x, glycans known to be expressed on cystic fibrosis mucins (Schulz et al., 2007), to *Ca*Aly did not serve to improve

the ability of *Ca*Aly to inhibit biofilm formation, indicating that the alginate lyase was not targeted by these FLDs in this experimental set up. Nevertheless, my study indicates that *Ca*Aly is an efficient alginate lyase domain, with better inhibition of biofilm formation of clinical *Pseudomonas* strains than other previously reported alginate lyases. It is a potential candidate to be tested for *Pseudomonas* biofilm destruction in sputum samples of CF patients.