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Screening and characterization of novel carbohydrate binding modules (CBMs) with high affinity regarding the lignocellulosic biomass

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Problématique:

The structure of many cellulases and xylanases comprises a catalytic domain and a carbohydrate binding module (CBM). The role of the catalytic domain is to hydrolyze the polysaccharide substrate while that of the CBM is to target the catalytic domain to the substrate thereby increasing the efficiency of catalysis. Although ligand recognition in CBMs can be highly specific, most CBMs display broad specificity, being able to bind multiple polysaccharides with similar affinities. Broad specificity of CBMs can be detrimental to applications where a single polysaccharide from a complex mixture such as paper pulp needs to be hydrolyzed. In addition, CBMs that preferentially bind the β-glucan linkages of cellulose do so with higher affinity (*Kd* values in the micromolar range) than those prefer the β-xylan linkages of xylan (*Kd* values in the millimolar range). The low binding affinity of CBMs to xylan results in decreased degradation efficiency of this important polysaccharide. To date, the structural determinants which govern the specificity and affinity of CBMs for carbohydrates are not fully understood, limiting their application in industries. This lack of understanding refrains the development of new CBMs displaying highly specific binding to either cellulose or xylan. Such CMBs are crucially needed to bolster their application in several industries, including pulp and paper and biofuel industries.

L'objectif de la recherche

To create novel CBMs capable of binding specifically to a single desired polysaccharide (cellulose or xylan) with high affinity (*Kd* in the low µM range).

Méthodologie

To do so, we are using a Computational Protein Design (CPD) algorithm to predict mutant sequences of CBMs that will display high binding affinity and specificity for cellulose or xylan, respectively. CPD works by predicting amino acid sequences that are energetically compatible with the target protein structure yet also possess a new property such as increased thermostability, improved binding affinity, or altered ligand specificity. The CPD will enable us to evaluate *in silico* generated CBM mutant sequences and identify those that should bind more tightly to either cellulose or xylan. Following CPD, the resulting mutant libraries (predicted to contain CBM sequences able to bind the desired polysaccharide with high affinity) will be built using combinatorial mutagenesis. The resulting CBM genes will be cloned into an appropriate expression system. Later, a high-throughput screening of CBM will allow us to screen these mutant libraries and determine their binding specificities against a model compounds such as cellulose and xylan and selected derivatives.

Applications potentielles et retombées industrielles

This knowledge will accelerate the development of clean technologies, potentially stimulating the bio-economy of Québec and abroad too. For example, these CBMs will be fused to the catalytic domains of enzymes in order to yield improved biocatalysts for the degradation of polysaccharides in the pulp and paper industries but also for biofuel production. The new biocatalysts resulting from this work will provide an alternative to existing chemical processes and thus this proposed research can also positively impact our environment.