Role of Cel7A Linker in Enzymatic Hydrolysis of Cellulose Chains: A Simulation Study

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Proposal Title: Computational Studies of Nanoscale Biocatalytic Mechanisms Relevant to Biomass Conversion

Plant biomass represents an abundant source of fermentable carbohydrates, which when converted to fuels such as ethanol, holds the potential for significant environmental, economic, and strategic gains. Currently, chemical or biological conversion of biomass is too costly to permit ethanol to compete as a viable alternative fuel; hence, there is a need to understand and ultimately engineer the nanoscale mechanisms of the depolymerization of cellulose.

Cellobiohydrolase I (CBH_I), from *Trichoderma reesei*, is one of the most active cellulases known. This enzyme hydrolyzes cellulose in a "processive" manner, moving along a cellulose chain liberating cellobiose residues. For this reason, CBH_I has been cited as an example of nature's nanomachines[1,2]. CBH_I is a multi-domain enzyme, consisting of a large catalytic domain containing an active site tunnel and a small cellulose binding domain (CBD) joined to one another by a 26 amino acid linker peptide (Fig. 1). While much is known about the structure and composition of the CBD and catalytic domain a complete CBH I structure has not been solved. The linker domain plays a significant role in enzyme function (mutation studies show reduced or full negation of enzymatic activity on crystalline cellulose when the linker is removed); however, although the amino acid sequence in the polypeptide chain of the linker is known, the biophysical nature of the linker structure, its role in hydrolysis, and its relation to the catalytic and binding domains is unknown.

The primary aim of this work is to elucidate the nanoscale mechanism of action of CBH I on cellulose. It is believed that the enzyme moves along a single cellulose chain in a processive manner oscillating between an extended and compressed state, in a caterpillar like motion. Due to the flexibility of the linker, the CBD can remain bound to one site of the cellulose while the enzyme hydrolyzes the cellulose chain within a 4_nm range. Once

the linker becomes compressed to a very short distance, it has been hypothesized that the energy of the linker will be enough to free the cellodextrin chain from the CBD and allow the enzyme to progress down the cellulose chain[3]. Using computational methods we are probing the validity of the proposed mechanism by which the linker moves along a cellulose chain. Ultimately, we would like to understand the mechanism of binding and catalysis in this system, with the goal of optimizing the nanoscale action of the enzyme, and eventually designing more effective versions of this enzymatic nanomachine. We believe the knowledge gained will also provide insight into nature's design of nanoscale devices, which will be useful in the development of biomimetic nanodevices.

Research Achievement:

Free energy calculations of the linker peptide in water have shown the linker exhibit two stable states at lengths of 2.5 and 5.5 nm during a extension/compression process, with a free energy difference of 10.5kcal/mol between the two states separated by an energy barrier. The switching between these stable states could support the hypothesis that the linker peptide has the capacity to store energy in a manner similar to a spring. Our simulations also indicate that the free energy of the linker depends on the existence of the cellulose substrate. In particular, a free linker shows distinct free energy profiles compared with that of the linker above a cellulose surface, which implies that the interaction with the cellulose surface plays at least a partial role in determining any energy storage feature in the linker[6].

Future Work:

Simulations will be performed to probe the influence of the stretching/compression pathway and the role of the surface and interaction of the sugar residues on the linker backbone with the cellulose surface. We will also be studying mutated linkers that have been studied experimentally at and shown to have a reduced activity.

References:

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Publications:

X. Zhao, T. R. Rignall, C. McCabe, W. S. Adney, M. E. Himmel, "Energy Storage Mechanism of the Trichoderma reesei Cel7A I Linker Peptide from Molecular Dynamics Simulation," *Chemical Physics Letters*, **460** 284–288 (2008).
X. Zhao, C. Taylor, C. McCabe, W. S. Adney, M. E. Himmel, "Role of Cel7A Linker in Enzymatic Hydrolysis of Cellulose Chains: A Simulation Study," *in preparation*, (2009).