Inhibition Resistance Mechanism for the Product of Beta-Glucosidases, a Computational Approach

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Biofuels are a renewable energy source that are garnering global attention to the optimization and utilization of them. Such fuels have great relevance for decreasing dependence on fossil fuels, but may represent a obstacle to compete with the production of food from the sugar industry. Fortunately, the production of biofuels from by-products rich in cellulose of that industry, the second generation biofuels, show a potential to overcome that obstacle. They are produced from the use of a set of lignocellulitics microbial proteins. Such proteins degrade cellulose to fermentable sugars by an intricate chain of events. The last link in this chain is proving the biggest challenge in optimizing the production of biofuels, the conversion of cellobiose, coming from the previous steps, in free glucose. Most of the proteins responsible for this catalysis, the beta-glucosidases, are inhibited by the product, drastically reducing the yield of the process as a whole. However, GH1 beta-glucosidase family reports of resistance to inhibition may be observed, but such a mechanism is poorly understood. In this work, we try to understand the factors that influence the inhibition and resistance to inhibition of beta-glucosidases utilizing modeling and molecular dynamics tools. For this, we performed molecular dynamics of equilibrium simulation for two beta-glicosidases, a resistant protein (GH1) and a non-resistant protein (GH3) in the presence of cellobiose, docked glucoses and glucoses manually lysed, totalling six systems. These simulations were performed under CHARMM force field using the free software NAMD. Initially, the energy analysis suggests that the portion of the cellobiose close to catalytic triad (monomer -1) has an major affinity for the active site in GH3 than GH1, but this difference can not be observed when cellobiose is converted into glucose. Moreover, structural analysis seems to show that in GH1, cellobiose -1 monomer tends to be shifted close to sub-site +1 of the protein (more aromatic), reflection of the pi-stacking interactions between the +1 monomer and the same sub-site of the protein, thus losing several contacts between the monomer -1 and the sub-site -1 (more hydrophilic), but during the simulations with glucose (dock and lysis), the monomer -1 is freed to immediately interact with the sub-site -1, reflecting an increase in hydrogen interactions and decrease in hydrophobic contacts when compared to simulation with cellobiose and simulations with glucose. There were no significant energy differences among +1 glucoses in GH1 and GH3, suggesting that the bottleneck for catalysis is present in glucoses -1. Normal modes and principal components analysis are being made, seeking to find structural changes that might point to a possible opening backdoor in beta-glucosidase of the GH1 family. It is expected at the end of this work, we can contribute to a better understanding of the mechanism of inhibition and resistance to inhibition by product in beta-glucosidases.

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