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An In Silico Approach to Enzymatic Synthesis of Fucooligosaccharides Using α-L-Fucosidase from *Thermotoga maritima* †

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Abstract: Fucooligosaccharides comprise the primary group of human milk oligosaccharides. Due to their beneficial properties, a series of synthetic methods have been proposed to obtain them. Enzymatic methods show great promise, and α-L-fucosidase from *Thermotoga maritima* has emerged as a powerful catalyst for their production. Nonetheless, the enzyme's limited substrate scope has delayed its wider application. The present work aims to compare the relative reactivity of fucose, pNP-fucose, and ethyl-fucose, while also exploring the molecular interactions of these fucosyl-donors with the enzyme through a combination DFT and docking analysis. The HOMO-LUMO band gaps range from −7.14571 to −4.24429 eV, with α/β-pNP-fucose and α-fucose being the three most reactive compounds. Moderate association energies between −6.4 to −5.5 kcal·mol^{-1} were found in the docking analysis, with α-pNP-fucose and both anomers of ethyl-fucose demonstrating the poorest affinity. In the case of α/β-lactose affinity to the β-fucose/enzyme complex, no significant differences were shown. We conclude that the best fucosyl-donors for transfucosylation are those that maintain an enzyme affinity and reactivity similar to pNP-fucose.

Keywords: Fucooligosaccharides; α-L-fucosidase; DFT study; molecular docking

1. Introduction

Fucooligosaccharides (FucOS) are the main oligosaccharides in human milk comprising 65–77% of the total oligosaccharide content [1]. Due to their antimicrobial, immunomodulatory, and prebiotic activities, as well as their promise to function as developmental cognitive enhancers, their incorporation into commercial formulations has become highly desirable [1,2]. As their isolation is complex due to their low abundance in animal milk, attention has turned to synthesis [1,2]. Fermentation is the most efficient, with generally recognize as safe (GRAS) certification from the U.S. Food and Drug Administration (FDA) permitting the addition of 2′-fucosyllactose (2′FL) to infant formula [2]. Another synthetic alternative with recent promising results is the use of isolated enzyme, which requires the yield optimization of FucOS by fucosyl-transferases. Unfortunately, this approach presents the inconvenience of requiring nucleotide sugars as fucosyl-donors, which are more expensive than those used for fucosidases [1,3]. Consequently, FucOS synthesis by fucosyl-hydrolases like the α-L-fucosidase from *Thermotoga maritima* has gained importance, as this pathway allows the use either of less expensive fucosyl-donors or even agro-industrial waste [3–8]. However, this enzymatic route provides lower yields than the transferase, or involves the release of toxic compounds such as *p*-nitrophenol [5–8]. Alternatives are highly desirable. Thus, the present work aimed to determine the relative reactivity of three non-classical fucosyl-donors through an in silico study to propose substrate alternatives for the enzymatic synthesis of FucOS.

2. Methods

2.1. Geometry Optimization and HOMO-LUMO Parameters

All compounds were totally geometry optimized through the Density Functional Theory (DFT) with the B3LYP/6-311++G(2d,2p) basis set using water as solvent. The highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) density surfaces were visualized with Gabedit 2.5.0. [9].

2.2. Molecular Docking for Hydrolysis and Transfucosylation Process

The A chain of the α-L-fucosidase crystal from *Thermotoga maritima* (PDB: *1ODU*) was prepared with the DockPrep tool implemented in Chimera 1.13.1 [10], prior to its use as the receptor for molecular docking. All molecular dockings were performed by Autodock VINA [11] through the PyRx software [12], taking as reference the amino acids from the active site cited by Sulzenbacher et al. [13], with coordinates for the search space centered on x: −20.63, y: 19.03, and z: 63.32, with a grid cube with dimensions of 25.00 Å. In the case of the hydrolysis, a single docking step was performed for each fucosyl-donor and the receptor. Meanwhile for the transfucosylation process, a sequential docking sequence was employed. First, β-fucose was docked to the enzyme in order to form the pre-complex, then docking was performed again with lactose. The best binding mode for each interaction was obtained and its interactions were processed with the BIOVIA Discovery Studio Visualizer© v19.1.0.18287 [14].

3. Results and Discussion

3.1. HOMO-LUMO Parameters

The HOMO-LUMO frontier orbitals and the bandgaps were calculated for both anomers of fucose, ethyl-fucose, *p*-nitrophenyl (pNP)-fucose, and lactose (Figure 1). In general, β-anomers showed a lower bandgap compared with the α-anomers, except for the pNP-fucose anomers, which showed similar bandgaps. As the magnitude of the HOMO-LUMO gap directly relates to chemical reactivity, where larger bandgaps predict lower reactivity [15,16], we predict that the β-anomers of fucose, ethyl-fucose, and lactose should prove less reactive than their α-anomers; in contrast, both anomers of pNP-fucose should demonstrate similar reactivity. Ranking the series, α/β-pNP-fucose with its electron withdrawing nature, unsurprisingly, is predicted to be the most reactive followed by α-fucose. In general, the α-anomers promise greater reactivity than the β anomers (again, with the exception of the hot pNP electrophile) based on the distribution of density in the frontier molecular orbitals.

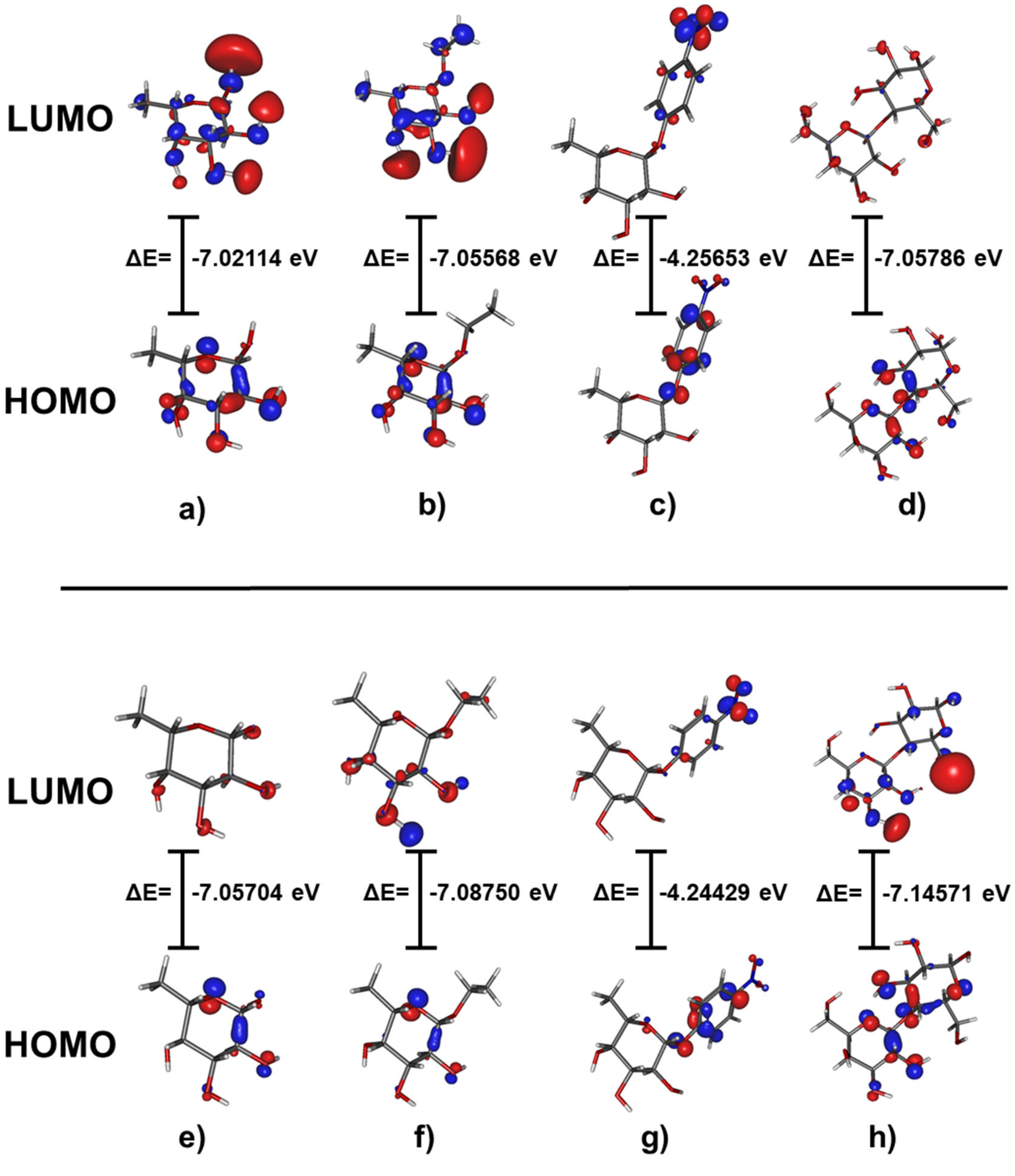

Figure 1. HOMO and LUMO surfaces (blue: positive and red: negative) of (**a**) α-fucose, (**b**) α-ethyl-fucose, (**c**) α-pNP-fucose, (**d**) α-lactose, (**e**) β-fucose, (**f**) β-ethyl-fucose, (**g**) β-pNP-fucose, and (**h**) β-lactose, as well as their HOMO-LUMO band gap.

3.2. Molecular Docking for Hydrolysis Processes

Single molecular docking simulated the hydrolysis catalyzed by *T. maritima's* α-L-fucosidase. Docking scores varied between −6.4 and −5.5 kcal·mol^{-1}, with β-pNP-fucose showing the best affinity to the receptor, while α-ethyl-fucose presented the worst (Table 1). A lower substrate–enzyme affinity could be correlated with lower complex stability and a tendency to destroy it [17]. Thus, among the six different tested molecules, α-ethyl-fucose, β-ethyl-fucose, and α-pNP-fucose appear to be the fucosyl donors most readily hydrolyzed once bound to the enzyme.

Table 1. Coupling energies obtained for each fucosyl-donor and the α-L-fucosidase from *Thermotoga maritima*.

Fucosyl-Donor	Coupling Energy (kcal·mol^{-1})
α-fucose	−6.0
β-fucose	−6.3
α-ethyl-fucose	−5.5
β-ethyl-fucose	−5.8
α-pNP-fucose	−5.9
β-pNP-fucose	−6.4

The key non-covalent interactions were identified (Figure 2). Those identified for β-fucose/enzyme docking (Figure 2d), agree with those reported by Sulzenbacher et al. [13]. All showed essential π-interactions between the C-5 methyl group and aromatic residues on the enzyme. The last interactions propitiate that the sugar ring takes a perpendicular position to this hydrophobic region, favoring van der Waals interactions observed with the rest of the molecule sites [13]. An important change was found for the interaction with the Asp224, the amino acid responsible for the nucleophilic attack that forms the covalent glycosyl-intermediate [13]. This pre-reaction interaction is a hydrogen-bond for β-fucose; but is van der Waals type for α-fucose and α/β-pNP-fucose; is an unfavorable repulsive interaction for α-ethyl-fucose; and a hydrogen bond with the C-4 OH for β-ethyl-fucose, indicating the different binding modes of these two substrates. These insights could explain the differential docking energies and suggests that hydrolysis would prove more challenging.

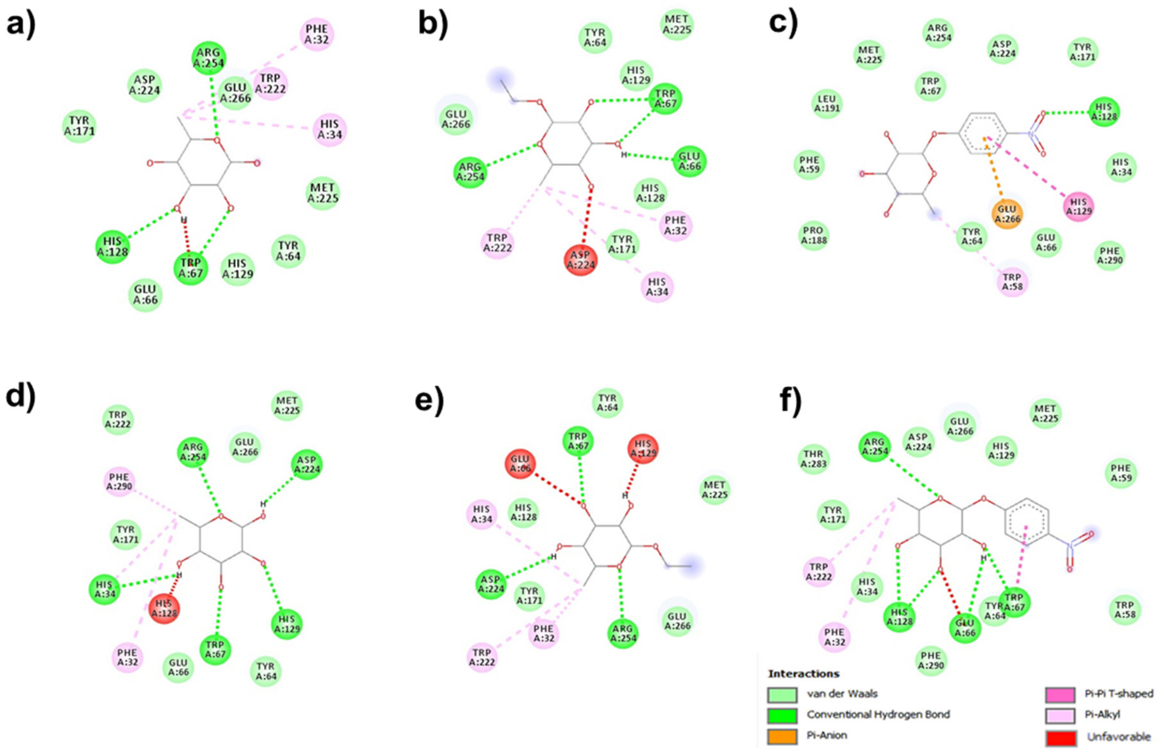

Figure 2. Mappings in 2D of the binding interactions among fucosyl-donors and the α-L-fucosidase from *Thermotoga maritima*: (**a**) α-fucose, (**b**) α-ethyl-fucose, (**c**) α-pNP-fucose, (**d**) β-fucose, (**e**) β-ethyl-fucose, and (**f**) β-pNP-fucose.

3.3. Molecular Docking for Studying Transfucosylation

In order to simulate a transfucosylation process, a sequential docking was performed. First, the β-fucose/enzyme complex was established by a single docking. The results were consistent with the reports of Sulzenbacher et al. [13], with α- or β-lactose, providing docking scores of −5.7 and −5.8 kcal·mol^{-1}, respectively. However, the conformation was different for each (Figure 3). While α-lactose adopts a position near the β-fucose binding site, β-lactose adheres preferentially to a more distal site. β-lactose has only a series of weak van der Waals interactions holding it in place, including with the key Glu266 residue, while α-lactose forms strong hydrogen bonds with both Glu66 and Glu266, the amino acids responsible for the activation of acceptor groups for transfucosylation [13]. This suggests that the alpha anomer should be far more reactive, and indicates that mutarotation likely forms this anomer prior to transformation.

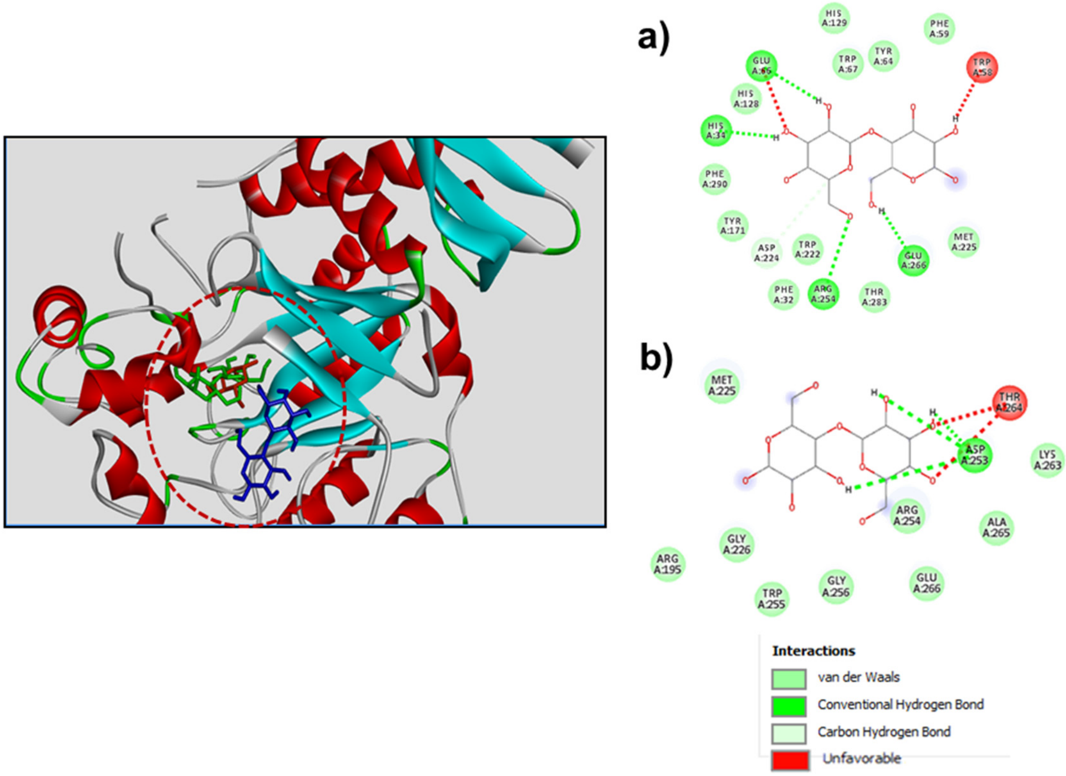

Figure 3. Binding position of α-lactose (green colored) and β-lactose (blue colored) in the complex β-fucose (orange colored)/enzyme, as well as the molecular interactions of (**a**) α-lactose and (**b**) β-lactose to the receptor.

On the other hand, the effect of the fucosyl-donors on the transfucosylation process can be related to the reactivity showed in the HOMO-LUMO gap, because previous in vitro studies have shown the effective transference of pNP-fucose to lactose to synthesize FucOS [5,6], while other studies have found low yields or long process when fucose itself is used as the donor [7,8]. Thus, ethyl-fucose could show similar results to those obtained with fucose mainly due to both molecules showing similar reactivity. Finally, according to *in vitro* results obtained earlier and the in silico insights found here, it is possible to hypothesize that fucosyl-donors with similar structure and/or reactivity to that of pNP-fucose could act as good substrates for transfucosylation with the α-L-fucosidase from *Thermotoga maritima*.

4. Conclusions

In silico insights of the reactivity and molecular interactions with the α-L-fucosidase from *Thermotoga maritima* obtained for each fucosyl-donor and *in vitro* results from literature allowed us to conclude that the best fucosyl-donors for transfucosylation reaction will be those with similar reactivity to pNP-fucose, so that the synthesis of compounds with similar structures, but lower toxicity, should be prioritized to find next generation fucsosyl transfer agents.

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