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Modeling of biopolymerases: a simple case study

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ABSTRACT

We reviewed the new methods that can be used in the recycling of biomass, with regard to the modeling of enzymes and the new possibilities of their application. Progress has been made in the pretreatment of biomass, as well as in the diversity and application of the enzymes used, but based on molecular modeling, very little progress has been made in development. There is also minimal progress in modifying the enzymes used during biomass processing to adapt to environmental conditions. There are hardly any publications where molecular modeling techniques are used to modify, improve, and adapt enzymes to different environmental conditions. In our opinion, in addition to modern biochemical, enzymatic, and biotechnological methods, enzyme modeling would help in the targeted design and modification of enzymes used in biomass processing.

Introduction

The biopolymers found in plant cell walls can be divided into three main groups: cellulose, hemicellulose and lignin. Their close connection makes the utilization of biomass so difficult. The recycling of biomass is essential nowadays not only because fossil energy sources are gradually being exhausted, but also because it is necessary to reduce environmental pollution caused by increasing energy consumption.

Together with the recently investigated and applied methods, we explored the latest possibilities and results of the utilization of plant structural biomass (Fülöp & Ecker 2020). Progress has been made in all areas, and this area shows continued development. New physical and chemical methods have been developed for the pretreatment of biomass, which can result in more efficient extraction of biomass materials. A wide range of enzymes found in microorganisms have been isolated and used in biomass processing. They studied the working mechanism of enzymes, and developed new and more efficient methods. In our opinion, there are some areas where there has been less progress recently. These include changing the properties of existing enzymes through rational design based on structure modeling, molecular dynamics simulations, enzyme-substrate interactions, and virtual

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mutagenesis.

There are untapped and enormous possibilities for the production of modified enzymes that work more efficiently under the industrial conditions used to process biomass (Fülöp & Ecker 2020). By rationally modifying enzymes, the efficiency of biomass conversion, enzymes or processes can be significantly increased. Physical, chemical, biochemical, biotechnological, and molecular biological methods are available to modify the protein side chains of enzymes for more efficient utilization of biomass, which are more suitable for the applied techniques.

With the wide application of these methods, further development can be expected in this area, and it can only be supported to investigate the function of enzymes in different environmental and/or industrial conditions with in silico modeling, virtual modification of molecules, and molecular simulations (Ecker & Fülöp, 2018). This would help the development of industrial biomass utilization methods used so far without changing the method, only the specific enzymes should be modified so that they would work more efficiently under the given conditions, thus increasing productivity and reducing costs.

Enzymes that break down polysaccharides are often associated with glycosylation, N- and O-linked glycans, the role of which is only partially known. Glycans can influence critical properties of enzymes: N-glycosylation improves thermal and proteolytic stability, O-glycosylation improves Cellulose Binding Module (CBM) binding affinity and stability in addition to proteolytic stability, but their presence does not necessarily affect catalytic activity (Amore et al. 2017). Modeling glycosylated cellulases can improve our understanding of the different functions of glycans.

Chung et al. (2019) described a glycosylated cellulase in which mainly galactose-based disaccharides could be found. This glycosylation dramatically affected the hydrolysis of insoluble substrates, proteolytic and thermal stability, and was shown to be necessary for this enzyme to function in harsh environments, including industrial environments (Chung et al. 2019). It should be noted that immobilized cellulase-polymethacrylate particles show excellent pH adaptability compared to free cellulase (Chan et al 2019).

Material and methods

The following keywords were used during database search: polysaccharides, cellulose hemicellulose lignin biodegradation, modification, immobilization, industry, O-glucosyl hydrolase, cellulase enzyme modeling, molecular modeling, computation, cellulase modification, cellulose enzyme modification modeling. The search was combined with OR and AND expressions. The searches were carried out until 2020. We also reviewed the articles and their references. The articles found to be interesting from the point of view of the problems highlighted above were classified into the following categories: Biological degradation of polysaccharides. Mechanical and physical-chemical modification of substrates. Modification of cellulose, hemicellulose, lignin. Modification of enzymes. Immobilization of enzymes. Molecular modeling of enzymes (Dancs et al. 2023). We compiled our study from these articles (Ecker & Fülöp, 2014; Fülöp & Ecker, 2020) to examine the prevalence of model-based modification of extracellular enzymes and the use of their rational design compared to traditional methods.

Based on the data included in the study, we performed the following enzyme-ligand dockings to be able to outline further possibilities for research related to extracellular enzymes based on their results: a previously studied *Cellulomonas* sp. (Fülöp et al. 1996; Fülöp & Ponyi, 1997) a cellulose and a lignin fragment were docked separately to the catalytic domain of cellulase CelB7. A xylan and a lignin fragment were separately docked

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(Williams et al. 2000) to the catalytic domain of a xylanase (PDB: 1J01). A mannan and a lignin fragment were docked separately to the active center of a mannanase (PDB: 2X2Y) (Hekmat et al. 2010), and for a lignin peroxidase, (PDB: 1B82) (Blodig et al. 2001). A lignin and a cellulose fragment were docked separately to the active center of a lignin peroxidase (Duhovny et al. 2002), both subunits of the enzyme were present. The dockings were performed with the Patch Dock program (Schneidman-Duhovny et al. 2005).

Results and discussions

With simple modeling, we can obtain a lot of valuable data and properties, and set up hypotheses, which do not require a lot of experimental work and energy. We illustrate this with a few examples.

Cellulases

Celluloses are one of the main groups of biopolymers found in plant cell walls, and they are closely connected with the other groups. The bacterial strain we modeled (*Cellulomonas sp.*) was chosen in such a way that this bacterium contains most of the enzymes required for breaking down biopolymers, so we could more easily trace the 3D structure and similarities of the different enzymes. First, we used the catalytic domain and CBM of a previously studied (Fülöp et al. 1996) cellulase for modeling. Their 3D structure can be seen in Figure 1.

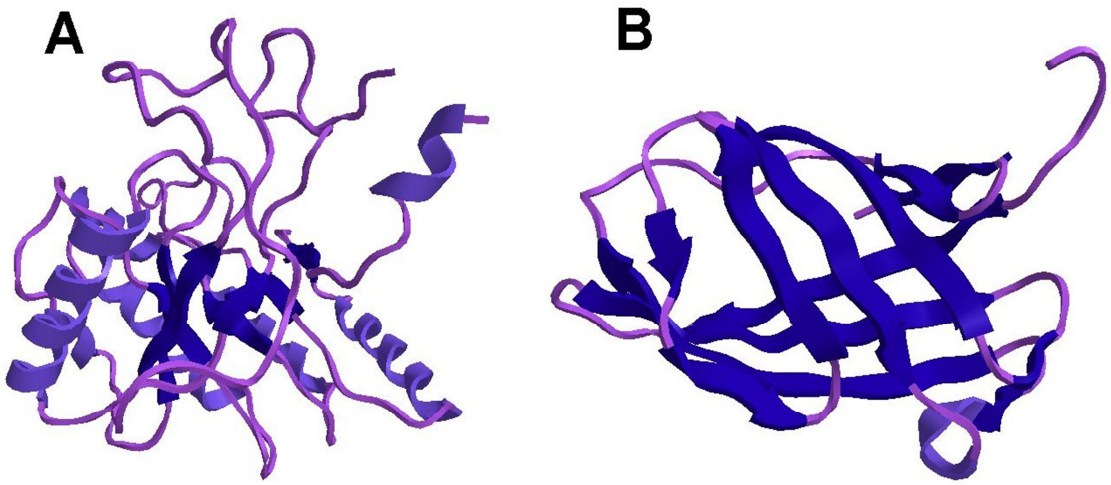

Figure 1. *Cellulomonas* sp. CelB7 cellulase, 3D structure of the catalytic domain "A" and the cellulose-binding domain "B". Cartoon representation mode.

The two types of the domains are fundamentally different. The catalytic domain mainly contains α-helix parts, while the CBM mainly contains the β-sheet structure. This is generally true for the binding and catalytic domains of other O-glycosyl hydrolases as well. In addition to cellulases, many other enzymes are involved in the breakdown of cellulose, including, but not limited to various β-glucanases, β-glucosidases and cellobio-hydrolases. Cellulases are very important in the degradation of biomass (Chen & Wang, 2017), the CBM connects the enzyme to the cellulose fibers, loosening it so that the active center of the catalytic domain can carry out the catalysis (Figure 2). The model of the complex of the

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catalytic domain of cellulase with a cellulose fragment is shown in Figure 2. The ligand fits into the part of the enzyme where the previously described amino acids Asp255 and Glu87, which play an important role in hydrolysis, are found (Saharay et al. 2010).

Due to their activity, the resulting smaller oligomers often inhibit (Agrawal et al. 2021) the further functioning of the enzyme by binding these smaller fragments to the enzyme (Figure 2), thus inhibiting their functioning.

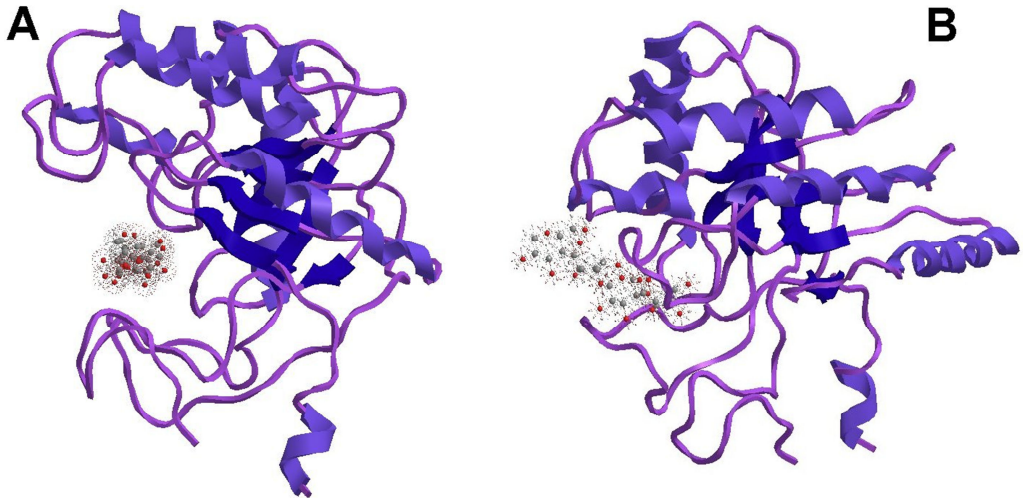

Figure 2. *Cellulomonas* sp. CelB7 docking complex of the catalytic domain with a cellulose fragment from two different viewpoints "A" and "B". The ligand (cellulose part) fits into the active center and fills it.

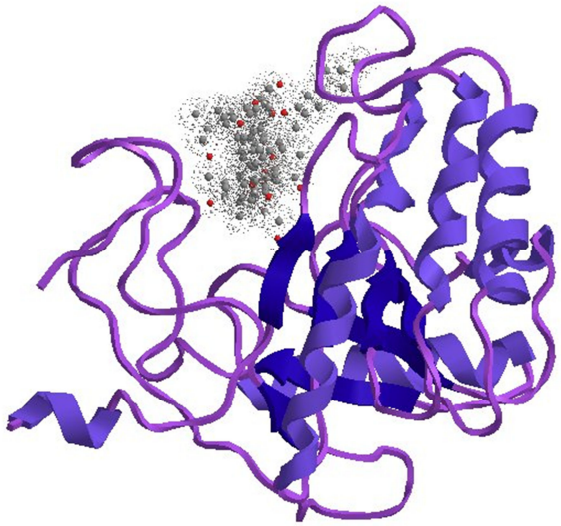

Figure 3. Docking complex of the catalytic domain of *Cellulomonas* sp. cellulase CelB7 with a lignin fragment. The lignin part is connected to the active center and thus could prevent the action of cellulase.

Lignin inhibits cellulase activity in higher concentrations (Botella et al. 2005), this fact has been demonstrated in many cases (Berlin et al. 2006). In some cases through modeling, we proved that lignin can connect (Figure 3) to the catalytic domain of CelB7 cellulase. The obtained docking simulations showed a small percentage of this connection, so this can prove that lignin either inhibits cellulase by a small percentage or that a high concentration is required for lignin to significantly inhibit the cellulase enzyme.

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Figure 3 clearly shows that lignin is in the active center of the cellulase and can bind there. At the same time, we have little information about the strength of binding and about how long the lignin can remain in the active center. With practical experiments, it should be decided whether lignin is simply a competitive inhibitor or permanently inhibits the function of the enzyme.

Xylanases

Another important enzyme group of biopolymerases is the various xylanases. Xylanases break down the xylan parts of hemicellulose in different ways. Xyloglucan side chains are a significant part of hemicellulose, so xylanases are important enzymes in the breakdown of biomass.

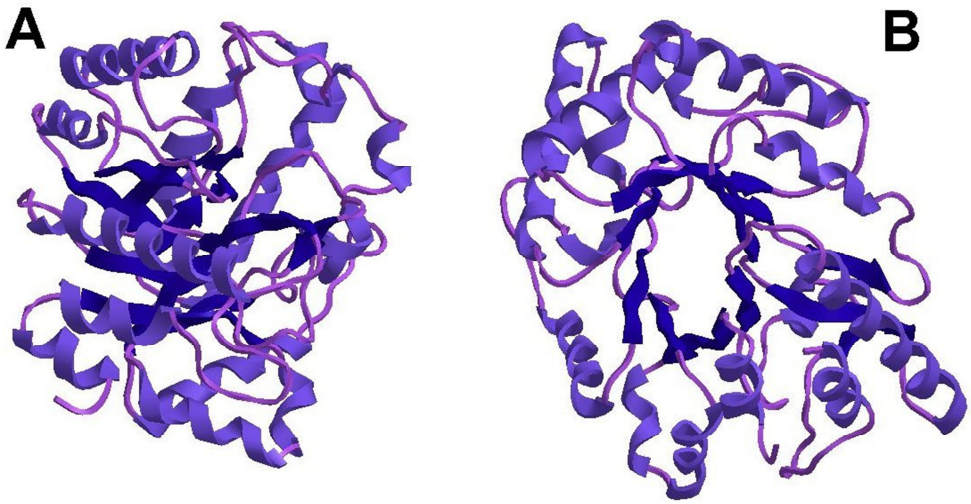

Figure 4. 3D structure of the catalytic domain of *Cellulomonas fimi* xylanase (PDB: 1J01), from two different viewpoints "A" and "B". Cartoon representation mode.

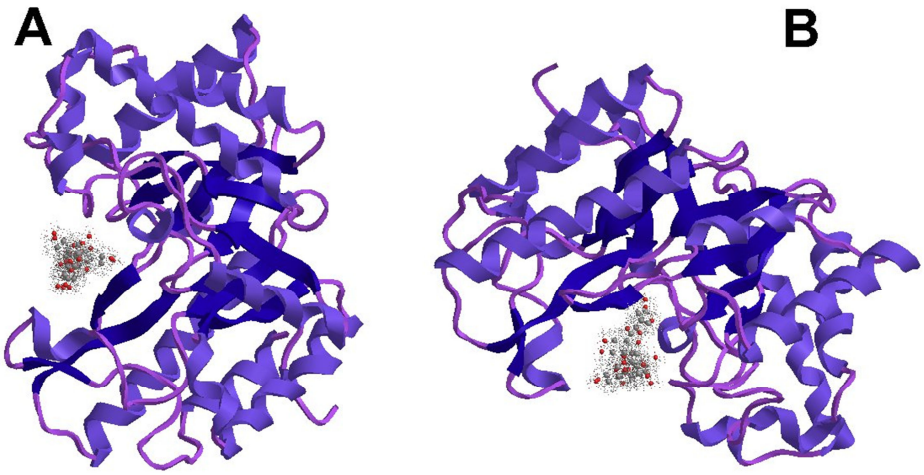

Figure 5. Docking complex of the catalytic domain of *Cellulomonas fimi* xylanase (PDB: 1J01) with a xylan fragment from two different views "A" and "B".

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Figure 4 shows that the catalytic domain of the xylanase enzyme mainly contains α-helix secondary structural elements. A similar conclusion was reached in the case of other microorganisms (Muhammad et al. 2020). There are many similarities between cellulases and xylanases, which is also reflected in their properties. Our modeling, clearly shows the docking complex of the xylanase enzyme with a fragment of xylan (Figure 5). This is like a similar complex of cellulase. Perhaps this explains the fact that among the cellulases, there are several enzymes that also have xylanase activity. The possibility of cooperation between cellulolytic and hemicellulolytic enzymes was investigated (Ross et al. 1992) and mutual synergism was demonstrated.

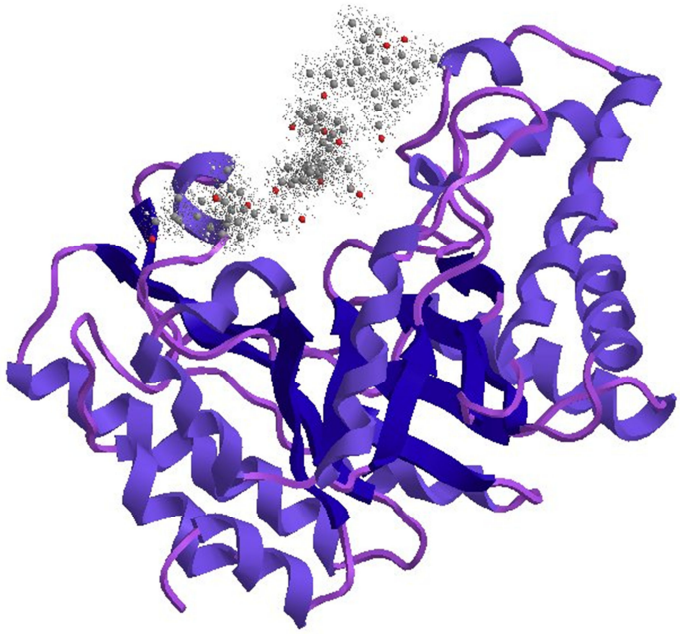

Figure 6. Docking complex of the catalytic domain of *Cellulomonas fimi* xylanase (PDB: 1J01) with a lignin fragment. The lignin fragment connects to the active center and can thus prevent the enzyme from functioning.

The catalytic domain of *Cellulomonas fimi* xylanase shows a strong affinity towards lignin (Figure 6). This was also demonstrated by 3D molecular modeling. The modeling shows a great similarity to the binding of cellulase to lignin, which is not surprising, since many cellulases also have xylanase activity.

The connection can be partly explained by the adsorption of enzymes (cellulase and xylanase) on the surface of lignin, but the detailed mechanisms of the binding are still poorly understood (Kellock et al. 2017).

However, the underlying mechanisms of enzyme-lignin interactions remain unclear (Li & Zheng 2017). The non-specific adsorption of cellulases and xylanase to lignin prevents the enzymatic conversion of biomass. Calculations show that cellulase and xylanase with a negatively charged surface can reduce inhibition by lignin. It is possible to produce highly active cellulases and xylanases that are resistant to lignin-mediated inactivation, although further work is needed to understand this issue (Whitehead et al. 2017).

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Mannanases

Mannans also belong to hemicellulases. Mannanases are involved in the degradation of mannopyranose oligomers attached as side chains to the rhamnogalacturonan parts of hemicellulose. Mannanases can be found in both monomeric and dimer forms (Figure 7). The mannanase-mannose docking complex can be of several types (Figure 8), depending on whether the mannan is attached to the monomeric or to the dimeric enzyme. Figures 7 and 8 show that the mannanase enzyme contains both a catalytic and a substrate-binding part. The docking complex, like the other enzyme-substrate connections discussed so far, is stable and suitable for breaking down the substrate.

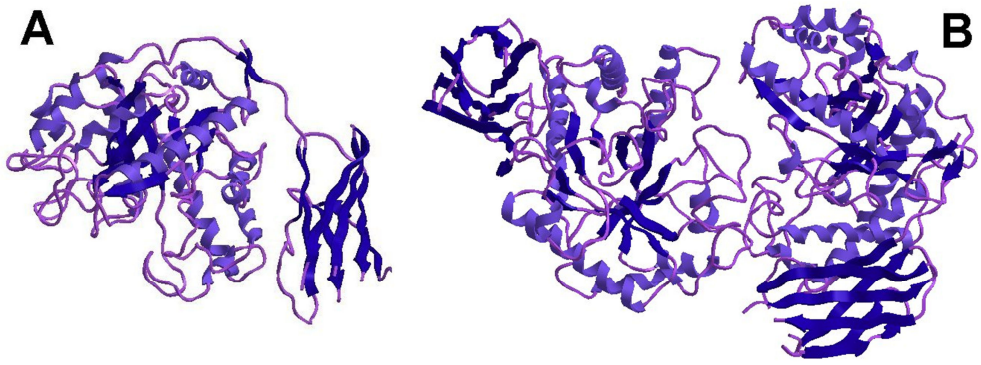

Figure 7. 3D structure of *Cellulomonas fimi* endo-beta-1,4-mannanase (PDB: 2X2Y). "A" is a monomer, "B" is a homodimer. Cartoon representation mode.

A synergistic effect between xylanase and mannanase has been described (Várnai et al. 2011). Nevertheless, the two enzymes show little similarity (Figures 4 and 7), so this cannot be an explanation for their cooperative functioning. With the hemicelluloses and the enzymes involved in their breakdown described so far, the possibilities are endless. In addition to those discussed so far, hemicellulose also contains gluco-pyranoside, galacto-pyranoside, rhamno-pyranoside and arabino-furanoside side chains.

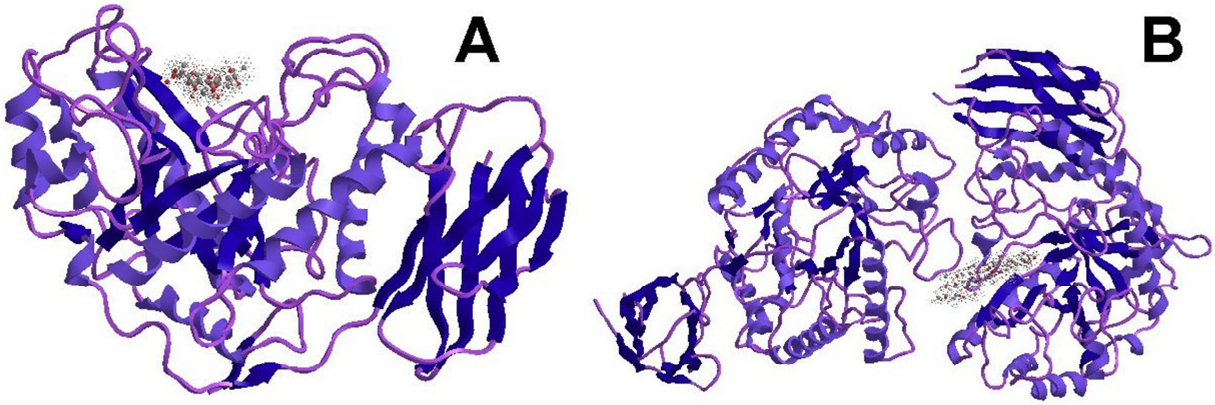

Figure 8. *Cellulomonas fimi* endo-beta-1,4-mannanase (PDB: 2X2Y) and its docking complex with a mannan fragment from two different perspectives. Relationship between "A" monomeric enzyme and mannan, "B" homodimeric enzyme and mannan. In both cases, the ligand (mannan) fits into the active center and fills it.

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Gel-like pectin is a heteropolysaccharide consisting mainly of galacturonic acid and is connected to hemicelluloses at several points. The additional enzymes involved in the breakdown of these side chains are also very diverse. The diverse variety of hemicelluloses and hemicellulases and their modeling will be a later objective, which will not be easy and may present challenging tasks.

There are almost no references in the literature regarding whether the mannanase-lignin relationship exists and what its effects are. We managed to model this relationship (Figure 9). It is clearly visible in the figure how well the lignin fragment matches the active center of mannanase, although the nature of the interaction is not yet fully clarified and further studies are needed.

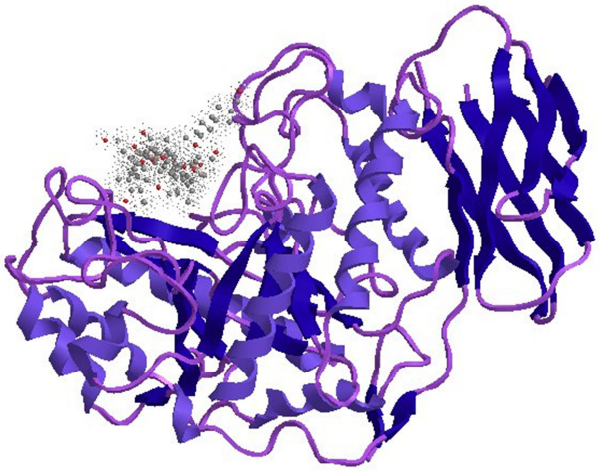

Figure 9. Docking complex of *Cellulomonas fimi* endo-beta-1,4-mannanase (PDB: 2X2Y) with a lignin fragment. The lignin part attaches to the active center and can block the enzyme's function.

Water-soluble (low molecular weight) lignin has been reported to reduce the rate of enzymatic digestibility, however, the catalytic mechanism of the enzyme-lignin interaction remains elusive (Li et al. 2022). Molecular modeling can also help in this (Figures 3, 6 and 9), which may explain the phenomenon. In order to mitigate the negative effects of lignin, extensive research has been conducted to explore the fundamental mechanisms of enzyme-lignin interactions in order to develop technologies that can overcome the negative effects of lignin on enzymatic hydrolysis (Li & Zheng 2017).

Lignin peroxidases

The third group of biopolymers is lignin. Lignin peroxidase is produced by a white rot fungus called *Phanerodontia chrysosporium*. It catalyzes the oxidation of veratryl alcohol, as well as the decomposition of lignin and prevents the depolymerization of lignin (Note: brown rot fungi break down cellulose and hemicellulose in wood. Cellulose and hemicellulose are broken down with hydrogen peroxide (H_2O_2), so no saccharides are produced, no utilization, only oxidation).

Figure 10 shows the 3D model of the lignin peroxidase enzyme, the two monomers are identical, thus forming a homodimer. It is known that both monomers contain a heme skeleton, so these enzymes are called heme peroxidases, which require H_2O_2 as an oxidant.

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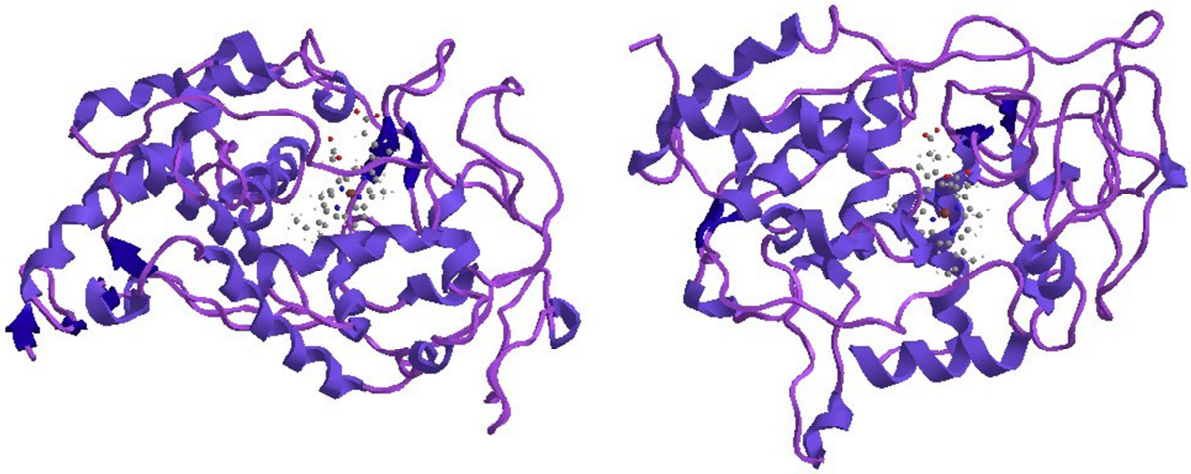

Figure 10. 3D structure of *Phanerodontia chrysosporium* lignin peroxidase (PDB: 1B85). The enzyme is present in a homodimeric state. Cartoon representation mode.

The model of the complex formed by both subunits of the lignin peroxidase enzyme with a lignin fragment is shown in Figure 11. The ligand fits between the two subunits. The possibility arises that the presence of both subunits (with the same sequence) may be necessary for the recognition and degradation of lignin - further measurements are required to establish this. In extremely acidic conditions, lignin peroxidase does not catalyze properly, as Pham et al. described (Pham et al. 2018). In their article, they replaced the lignin peroxidase heme part with the heme in manganese peroxidase (MnP) and thus the catalytic efficiency increased significantly.

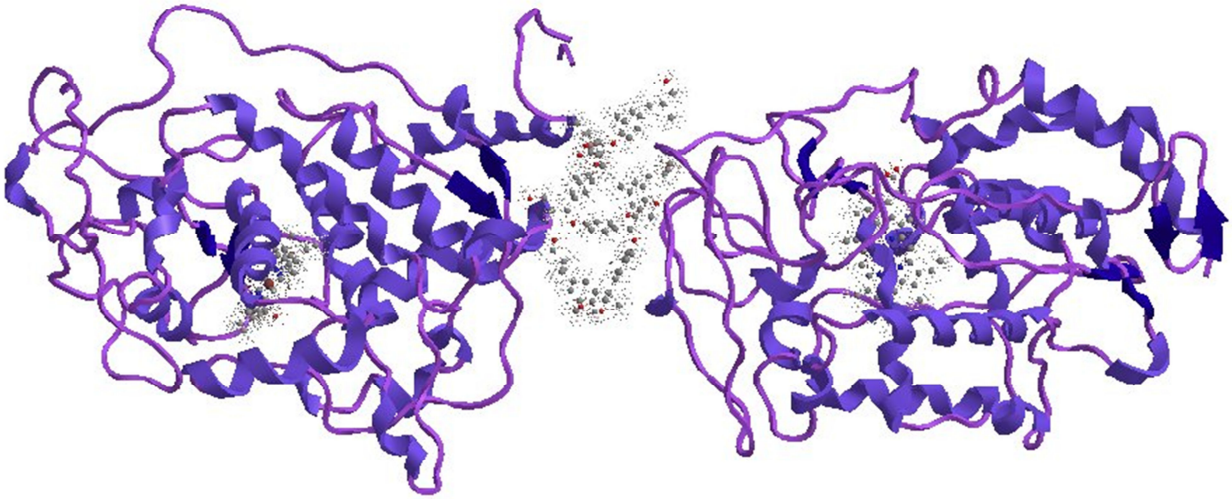

Figure 11. *Phanerodontia chrysosporium* lignin peroxidase (PDB: 1B85) and its docking complex with a lignin fragment. The lignin fragment fits between the two subunits, the 1-1 heme part can be recognized in the subunits.

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One of the goals of our subsequent research is to find out whether random amino acid changes cause a different 3D structure, as well as whether the different 3D structure causes differences in the enzyme's activity and its substrate binding capacity, and whether we can explain these at the molecular level. In addition, it would be interesting to investigate why the presence of lignin causes a significant decrease in the activity of cellulases (and other hydrolases) (Haarmeyer et al. 2017). If it were possible to produce more efficient cellulases by random amino acid changes, it could become possible to learn more about lignin-cellulase interactions.

Conclusions

There are many possibilities to improve the enzymes currently used for biomass processing. Computer design and modeling techniques can be used to modify enzymes so that they work more efficiently under specific physicochemical conditions. By studying the functioning of enzymes and the catalytic conditions, it is possible to intervene in chemical reactions by modifying the structure of the enzyme to better suit the actual operation. For this, it is necessary to know the applied enzymatic processes, and with computer modeling and planning, the function of the enzymes can be modified in the hope of better use.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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