Biodegradation mechanism of anionic polyacrylamide in coal preparation using molecular simulation

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Abstract: Biodegradation of anionic polyacrylamide (HPAM) and polyacrylate (PAA) by key enzymes, such as amidase and bacterial laccase, have been reported. However, the interaction mechanism between HPAM or PAA and enzymes is still poorly unclear. Here, docking study was undertook to demonstrate the binding modes and interaction details for degradation of HPAM or PAA. Then, bioactivities between PAA and HPAM were compared with frontier orbital theory. The docking results showed that HPAM completely buried in pocket of Rhodococcus sp. N-771 amidase (Rh Amidase), while most of PAA molecule exposed outside pocket of Bacillus subtilis laccase (B. subtilis laccase), further suggesting PAA was much more difficult to degrade than HPAM. Hydrophobic interactions and hydrogen bonds were necessary for stabilizing HPAM-Rh Amidase or PAA-B. subtilis laccase complex. The frontier orbital analysis indicated that bioactivity of PAA was higher than that of PAA. These findings provide an insight into enzyme-catalyzed degradation of HPAM. It is helpful in designing highly efficient enzymes against HPAM or PAA to protect environment.

Keywords: HPAM; PAA; biodegradation; docking; frontier orbital; bioactivity; bacterial laccase; amidase.

1. Introduction

Polyacrylamide can be divided into anionic polyacrylamide (HPAM), cationic polyacrylamide (CPAM) and non-ionic polyacrylamide (NPAM) according to their charged properties [1]. Among them, HPAM is often used in selective flocculation flotation of fine coal [2]. Due to high molecular weight and hydrophobic carbon chain, HPAM has hydrophobic interaction with fine coal particles. Moreover, calcium ions can often act as a bridge between the anion groups of HPAM and electronegative coal
particles through electrostatic interaction [3, 4]. In modern coal preparation plant, the process water is generally recycled back to the flotation process after solid-liquid separation operation [5]. With the increasing amount of flocculant, the accumulation of HPAM in the circle water of coal preparation is enormous, which has an adverse impact on the flotation of coal. In addition, the release of coal preparation wastewater containing HPAM poses a potential threat to the ecological environment and human health [6]. Thus, it is urgent to develop effective degradation methods for residual HPAM in slime water.

Microbial degradation is an effective method to remove HPAM, which has many advantages, such as environmental friendliness, mild reaction conditions and non-secondary pollution [6, 7]. Bao et al. [8] isolated two HPAM degradation bacteria from produced water of polymer flooding. They were identified as Bacillus cereus and Bacillus sp., respectively, and the aerobic degradation mechanism was assumed. The experimental results showed that the amide side chain of HPAM was converted into carboxyl group under the action of amidase. Meanwhile, it was found that there was an extra peak at the retention time 2.85 min in the degraded samples through HPLC analysis and it may be polyacrylate (PAA), which was the metabolism product of HPAM [9]. Wen et al. [10] reported that Bacillus cereus and Bacillus flexu were also isolated from the activated sludge and oil soil, respectively. The results showed that microorganisms could attack amide groups from the main chain of HPAM, further indicating that bacteria used HPAM as a nitrogen source. The PAA was formed by the deamination of HPAM and it was used as a carbon source for microbial growth. The smaller PAA molecules had been proven to be more easily degrade than the larger ones [11]. Based on the differential results of starch cadmium iodide and total organic carbon (TOC), it was found that the PAA was more difficult to degrade than the amide group. GPC analysis showed that HPAM carbon chains were degraded into small molecular fragments [12].

The essence of biodegradation is enzymatic reaction, and the activity of key enzymes had been detected in the HPAM fermentation broth [13]. Zhang et al. [7] found that the efficiencies of HPAM removal were positively correlated with the activity of
dehydrogenase and urease, respectively. Dehydrogenase facilitated the oxidation and electron transfer of HPAM, while urease accelerated the metabolism of the amide groups in the process of HPAM biodegradation, and played an important role in the nitrogen transformation. Song et al. [14] studied the relationship between bacterial laccase activity and HPAM concentration. The results showed that laccase activity was not affected by HPAM concentration, and laccase activity remained above 80 nmol ABTS oxidized g\(^{-1}\)h\(^{-1}\). Furthermore, hypothesis about biodegradation mechanism of HPAM was proposed. It was pointed out that the amide side chain of HPAM was hydrolyzed by amidase readily, and PAA molecule was produced. Afterwards, the PAA oxidation catalyzed by oxygenase was similar to the \(\alpha\)-oxidation of fatty acid. However, the interaction details between degrading enzymes and HPAM or PAA cannot be obtained through only experiments. But the interaction information between enzymes and substrates can be used to design enzyme mutants with enhancing enzymatic activities. Furthermore, it also helps us to further understand the enzymatic mechanism [15].

Molecular docking, a kind of molecular simulation method, is able to optimize the binding conformation of ligand to the active pocket of a receptor [16-18]. Recently, there are many reports about the interaction analysis between enzymes and environmental organic pollutants by molecular simulation or docking [19, 20]. The three-dimensional crystal structure of bacterial laccase or amidase has been analyzed through experimental technologies such as X-ray diffraction and nuclear magnetic resonance, providing the possibility to further explore the interaction between degrading enzymes and HPAM or PAA at the molecular level [21, 22]. Moreover, according to the frontier molecular orbital theory, HOMO and LUMO have the most important effect on bioactivity [23, 24]. HOMO is more likely to provide electrons, while LUMO has prior to accept electrons. Thus, the analysis of frontier orbital can predict the ability of charge transfer taking place with molecules [25, 26].

The key enzymes and intermediate products had been detected in the degradation process of HPAM through experiments. Additionally, the hypothesis of degradation pathway had also been proposed [27]. However, how PAA or HPAM interacts with
bacterial laccase or amidase is unknown, and researches about revealing biodegradation mechanism of HPAM using molecular docking combined frontier orbital have also been rarely reported. Thus, we used the molecular docking and frontier orbital to analyze binding for enzymes to substrates and bioactivity, respectively. This specific aim was to provide a basis for the interpretation of HPAM metabolisms, and design the HPAM degrading enzymes with better activities.

2. Materials and methods

2.1. Structural model of selected enzymes

The 3D structure of *Rhodococcus sp. N-771* amidase (Rh amidase) [28] and *Bacillus subtilis* laccase (*B. subtilis* laccase) [29] were downloaded from the Protein Data Bank (PDB) (http://www.pdb.org/pdb/home/home.do). The detailed information about selected degrading enzymes is shown in Table1. Before docking, the selected enzymes were prepared with the prepare protein program to repair and perfect the missing loop regions, remove water, add hydrogen, and delete bound ligands [30].

| Enzymes         | Organism       | PDB ID | Chains | Resolution (Å) |
|-----------------|----------------|--------|--------|----------------|
| Amidase         | *Rhodococcus* sp. N-771 | 3A1I   | A      | 2.32           |
| Bacterial laccase | *Bacillus subtilis* | 1GSK   | A      | 1.70           |

2.2. Preparation of HPAM and PAA structures

HPAM, also called as partially hydrolyzed PAM, is named as anionic polyacrylamide, because the carboxyl side chain of HPAM is negatively charged in an alkaline environment [14]. PAA is actually formed by deamination of HPAM. Enzyme-catalyzed degradation essentially belongs to a chemical reaction between functional groups, so only reactive functional groups on HPAM or PAA molecules will be considered. Their dimers contained all the reactive groups, which were selected as the structural models to further study the enzymatic degradation mechanism. The 3D structural models of the HPAM and PAA were generated by Discovery Studio 2020 (DS 2020) software, and both terminals were sealed with methyl groups [31]. Then, the
energy was minimized using the CHARMM force field, as shown in Fig.1.

![Fig.1 Stick structural model of HPAM (a) and PAA (b).](image)

(Elements: gray: carbon; white: hydrogen; red: oxygen; blue: nitrogen.)

2.3. Docking

The partial flexibility CDOCKER program was chosen to perform the docking operation [32]. The CDOCKER program generated ligand conformation sets based on high temperature dynamics. Meanwhile, simulated annealing was adopted to optimize the flexible conformation of each ligand at the active site of the receptor protein. The default parameter was set to retain the first 10 conformations of the optimal ligand. The binding sphere of Rh amidase was created using coordinates x: -21.7011, y: 6.34921 and z: -4.82216 and a radius: 12.021 Å, while the binding sphere of B. subtilis laccase was produced with coordinates x: 98.2429, y: 60.7646 and z: -8.708 and a radius: 10.954 Å. The optimal docking poses were obtained and selected as further analysis based on the highest standard of \textit{-CDOCKER} energy scores.

2.4. Frontier orbital

Geometry optimization and frontier orbital of HPAM or PAA were calculated using Dmol³ program in Material Studio 2017 software, while electronic exchange correlation was described with a Becke-Lee-Parr hybrid three parameter function (B3LYP) [33]. The core electrons were treated with All Electron and the basis set was double numerical with polarization (DNP), polarizing p-orbitals on hydrogen atoms [1].
The k-point sampling was set as only Gamma point, and the convergence threshold was used with $1.0 \times 10^{-6}$ eV/atom for self-consistent field (SCF). The long-range dispersion was corrected by Grimme scheme [34], and the convergence criteria were: $0.004$ Ha/Å for max force; $5 \times 10^{-3}$ Å for max displacement; and $2.0 \times 10^{-5}$ Ha for energy. All the structures were calculated using a COSMO solvation model, and the dielectric constant of water was 78.54 [31].

3. Results and discussion

3.1. Validation of docking accuracy

The docking accuracy of semi-flexible CDOCKER program was validated with the original ligand benzamide docking into the active site of Rh amidase. We selected the highest CDOCKER Energy score poses for further study. We used the pose of original ligand benzamide in crystal structure of Rh amidase as reference ligand. It can be found that the docking conformation had a good superposition with reference ligand, as shown in Fig.2. Root-mean-square deviation (RMSD) between the selected docking pose and reference ligand was 0.04033 Å. The RMSD value was lower than 2.0 Å, meaning that the docking results from semi-flexible CDOCKER protocol could be accurate and acceptable [15].

![Fig.2 The comparison of docking conformation with original conformation in the crystal structure. Carbon atom is represented by original ligand pose (green) and docking pose (gray).](image)

3.2. Binding mode analysis

The docking study of the HPAM or PAA at the active site of degrading enzymes were performed by DS 2020 software. The binding modes of the ligand are potentially
important for the stability and catalytic activities of enzymes [28]. Clearly, the binding mode of HPAM inside Rh amidase was different from that of PAA bound to \textit{B. subtilis} laccase, as can be seen in Fig.3, which suggested the enzymatic performance was influenced by its properties and substrates [35]. The value of Rh amidase-HPAM from -CDOCKER_ Energy score was higher than that of \textit{B. subtilis} laccase-PAA, as can be seen from Table2, which indicated that the Rh amidase-HPAM complex had the higher strength of the affinity [30]. The -CDOCKER_ interaction_ Energies of Rh amidase-HPAM and \textit{B. subtilis} laccase were analyzed in Table2. When making comparisons, HPAM showed the lower interaction energy with Rh amidase, which suggested more stable with the binding site of Rh amidase than the other \textit{B. subtilis} laccase-PAA [36].

![Fig.3 The binding model of Rh Amidase-HPAM (a) and \textit{B. subtilis} laccase-PAA (b)](image)

| Complex                | -CDOCKER_ Energy score (kcal mol\(^{-1}\)) | -CDOCKER_ Interaction_ Energy (kcal mol\(^{-1}\)) |
|------------------------|--------------------------------------------|-----------------------------------------------|
| Rh amidase-HPAM        | 27.9636                                    | 34.4263                                       |
| \textit{B. subtilis} laccase-PAA | 19.0103                                    | 21.6948                                       |

The receptor active pocket is visualized according to the property of hydrogen bond,
as can be seen in Fig.4. From Fig.4(a), HPAM was completely buried in the binding pocket of Rh amidase. The pocket of Rh amidase was very deep, and its entrance was small, which indicated that it could make HPAM difficult to detach from the active site. Most of the PAA molecule was located outside the active pocket, with only a small part of it embedded in the pocket, as shown in Fig.4(b). It can be seen that the entrance of the active pocket was very wide and large, and its bottom was empty. PAA was not completely embedded in the active pocket, indicating that PAA was not closely bound to the active site.

From analysis above, it was concluded that the degradation of PAA was difficult with *B. Subtilis* laccase, compared with the degradation of HPAM with Rh amidase. Therefore, the amide group of HPAM were more inclined to be hydrolyzed by microorganisms into carboxyl group, and \(-\text{NH}_2\) was released into the solution as a nitrogen source. But it was difficult for bacteria to use PAA, the metabolism product of HPAM, as the carbon source for growth. This result showed a consistency experimental results with Sang et al [12].

3.3. Interactional analysis

We visualized and analyzed the interactions between ligand and receptor
surrounding residues in the Rh amidase-HPAM and *B. subtilis* laccase-PAA complexes using the DS 2020 software. Fig.5 and Table3 showed that HPAM interacted by hydrogen bond with residues GLN192, GLY193, GLY194, ALA195 and CYS145 in Rh Amidase. It was worth noting that HPAM simultaneously formed two hydrogen bonds with GLN192 residue, so a total of 6 hydrogen bonds formed between HPAM and Rh Amidase. Moreover, the 6 hydrophobic interactions of ALA332, LEU447, ILE450, ILE223, PHE146 and TRP328 with Rh Amidase also contributed to the stabilization of the HPAM–Rh Amidase complex. We further observed that the carboxyl groups of HPAM did not combine with amino acid residues of Rh Amidase and only amide side chain of HPAM interacted with Rh Amidase. This means that HPAM was hydrolyzed by Rh Amidase at the initial stage, and the amide group of HPAM was preferred to contact with the active center of Rh Amidase, which was verified by tests. Wen et al. indicated that the amido group of HPAM was picked off from the carbon skeleton of HPAM through FT-IR analysis [10].

![Fig.5 The 3D interaction of Rh Amidase and HPAM](image)
Table 3 Interaction analysis of the most stable Rh Amidase-HPAM complex

| interaction residues     | distance (Å) | type            |
|--------------------------|--------------|-----------------|
| A: GLN192:HN - HPAM: O14 | 2.52534      | hydrogen bond   |
| A: GLY193:HN - HPAM: O14 | 1.96463      | hydrogen bond   |
| A: GLY194:HN - HPAM: O14 | 3.07224      | hydrogen bond   |
| A: ALA195:HN - HPAM: O14 | 2.39533      | hydrogen bond   |
| HPAM: H18 - A: GLN192: OE1| 2.98359      | hydrogen bond   |
| HPAM: H19 - A: CYS145: O  | 2.04661      | hydrogen bond   |
| A: ALA332 - HPAM: C24    | 4.32567      | hydrophobic     |
| HPAM: C20 - A: LEU447    | 4.33127      | hydrophobic     |
| HPAM: C20 - A: ILE450    | 4.1072       | hydrophobic     |
| HPAM: C24 - A: ILE223    | 4.63434      | hydrophobic     |
| A: PHE146 - HPAM: C24    | 3.61948      | hydrophobic     |
| A: TRP328 - HPAM: C24    | 4.8151       | hydrophobic     |

From Fig. 6 and Table 4, we found that PAA presented three H-bonds with LYS135 and TYR133 of B. subtilis laccase and LYS135 participated in the formation of two H-bonds with PAA. The ARG487 took part in the hydrophobic contact with PAA, and formed two hydrophobic interactions. The hydrogen bonds and hydrophobic contacts played a vital role in stabilizing the structure of the non-catalytic enzyme–substrate complex. These results showed that B. Subtilis laccase-PAA was not closely bound, and the non-bond interaction was weak than Rh Amidase-HPAM, similar to a previous report that the PAA was much more difficult to degrade than amide group of HPAM by experiments [12].
Table 4 Interaction analysis of the most stable B. subtilis laccase-PAA complex

| interaction residues | distance (Å) | type            |
|----------------------|-------------|-----------------|
| A: LYS 135: HZ2-PAA: O5 | 1.7324     | hydrogen bond   |
| A: LYS 135: HZ3- PAA: O13 | 1.83499   | hydrogen bond   |
| PAA: H9- A: TYR 133: O  | 2.00797    | hydrogen bond   |
| PAA: C19- A: ARG 487 | 3.73269    | hydrophobic     |
| PAA:C23- A: ARG 487  | 4.31484    | hydrophobic     |

3.4. Bioactivity analysis

The frontier orbital energy can provide useful information about HPAM or PAA biodegradation [37]. The lower the energy gap between HOMO and LUMO, the higher the ability of charge transfer, when the molecules interact with enzymes [38]. The HOMO and LUMO of HPAM and PAA are listed in Table 5. From Table 5, we can find that the energy gap (ΔE) of HPAM was lower than that of PAA, accounting for the higher electron transfer of HPAM. Thus, the bioactivity of HPAM was stronger than that of PAA. It was further indicated that HPAM was inclined to degrade than PAA,
providing nitrogen source for bacteria, which was consistent with the results of docking.

Table 5 Frontier orbital energy of HPAM and PAA

|       | HPAM       | PAA       |
|-------|------------|-----------|
| E (Ha)|            |           |
| $E_{HOMO}$ | -0.261221 | -0.28566  |
| $E_{LUMO}$  | -0.001997 | -0.0077   |
| $\Delta E$ | 0.259224  | 0.27796   |

$\Delta E = E_{LUMO} - E_{HOMO}$

From what showed in Fig. 7, we found that the HOMO of HPAM was located on amido group, while the LUMO of it was mainly located on carboxyl group. The HOMO and LUMO of PAA located on two carboxyl group, mainly distributing on both side chain of the carbon skeleton (Fig. 8). The electron orbitals around the carbon chain of the polymer happened a huge change, when the amide group of HPAM was hydrolyzed into carboxyl group, that is, PAA. This also implies that the introduction of amide group can improve bioactivity.

![Fig. 7 The distribution of frontier molecular orbitals for HPAM.](image)

(The isosurface value are 0.05 electrons/Å³.)
4. Conclusions

This study explored the molecular basis of Rh Amidase-HPAM and *B. subtilis* laccase-PAA for HPAM bacterial degradation thoroughly. Meanwhile, bioactivity of HPAM and PAA were predicted through frontier orbital theory. Frontier orbital analysis showed that the bioactivity of HPAM much more than that of PAA. We had successfully identified and analyzed the binding modes and interaction details of Rh Amidase-HPAM and *B. subtilis* laccase-PAA complexes, which was helpful in understanding HPAM-degrading mechanisms by bacteria in an aerobic environment. In addition, our study provided the basis to design new enzyme mutants for high HPAM-degrading ability in the field of coal preparation.

Compliance with ethical standards.

Consent to Participate and Publish

Authors Contributions

Performed the experiments, analyzed the data, and wrote the paper: WANG F. L.

Conceived and designed the experiments: ZHANG D. C. Contributed molecular
simulation software (Discovery Studio 2019), and provided helpful discussion: WU X. F. and DENG S. S.

Funding

The study was financially supported by the National Natural Science Foundation of China (no. 51274012).

Competing Interests

All the authors declare no competing financial interests.

Availability of data and materials

Not applicable

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