INTRODUCTION

β-glucosidase is a general term of glycoside hydrolases that specifically catalyze the hydrolysis of oligosaccharides (usually containing 2 to 6 monosaccharide residues), alkyl and aromatic group terminal nonreducible β-D-glucosidase, thereby releasing monosaccharides and corresponding ligands (Lan et al., 2019). So far, β-glucosidase is widely found in archaea (Schröder et al., 2014), bacteria (Akram et al., 2018), eukaryotes (B. Li & Renganathan, 1998), and plants (Ketudat Cairns et al., 2015), and it performs extensive and vital physiological functions. According to the consistency of the amino acid sequence, glycoside hydrolases were divided into several families, and the classification information was entered into the CAZy website for real-time updates (http://www.cazy.org). There are up to now 133 families of glycoside hydrolases. According to this classification, β-glucosidase is distributed in families 1, 3, 5, 9, 30, and
Most β-glucosidases belong to broad substrates specific β-glucosidases, which can simultaneously hydrolyze disaccharide or oligosaccharide substrates, alkane glycoside substrates, and aromatic glycoside substrates. β-glucosidase is widely used in industry (Ferreira et al., 2018), agriculture (Vazquez et al., 2019), food, and medicine fields. In food processing, as a part of the food flavor enzyme, β-glucosidase can produce gentian oligosaccharide for coffee, chocolate, and other products. Because of the use for improving the flavor or taste, β-glucosidase can digest the flavor precursors in fruits (Gueguen et al., 1996), tea (Su et al., 2010), wine (Hemingway et al., 1999), and then release the flavor. In the research and development of food and health care products, β-glucosidase can release functional aglycones because of its hydrolytic activity to a variety of bioactive substances, such as isoflavone aglycones (Horii et al., 2009) and sapogenins (Huq et al., 2016).

Saponins are complex compounds in glycosides which composed of sapogenin and glycosyl. They are mainly distributed in many herbal medicines, such as ginseng, Platycodon grandiflorum, and liquorice (Zhang et al., 2020). Modern pharmacological research has found that the main active component of ginseng is ginsenoside (Attele et al., 1999). Currently, more than 100 kinds of single ginsenoside have been isolated and identified (Chen et al., 2020). Some ginsenosides with low content and high medicinal activity are called rare ginsenosides (J.-E. Kim et al., 2019; Yang et al., 2020). At present, it has been confirmed that the ginsenoside Rb1, which has the highest content in ginsenoside, can be hydrolyzed by β-glucosidase to remove the glucose unit in its chemical structure and generate rare ginsenosides with simpler structure and easier absorption by human body (M. Kim et al., 2005). The transformation of ginsenosides in vitro is mainly through the β-glucosidase enzymatic hydrolysis of the main ginsenosides (Cui et al., 2019), resulting in different types of ginsenoside subtypes (Liu et al., 2019). Therefore, it is of great significance to study the substrate specificity or selectivity of β-glucosidase for its effective utilization. According to the literature, although a variety of glycoside hydrolases have been reported, the ability of ginsenoside transformation, such as β-D-glycosidase and α-L-arabinopyranosidase (T.-H. Kim et al., 2018), similar studies mainly focus on the characterization of enzymatic properties and transformation properties. Nevertheless, the study of the interaction and its mechanism between glycosidase and ginsenoside molecules is rarely reported.

The correct understanding of the interaction between macromolecules and small molecules of compounds can be carried out through the mutual corroboration of multispectral experimental results and the visualization of molecular dynamics simulation. In previous studies, for the important human serum albumin (HSA) in human body, the preparation method of berberine nanoparticles (nano-BER) was studied to improve its solubility in aqueous phase and the formation of its complex with human serum albumin (HSA) and total transferrin (HTF) (Sharifi-Rad et al., 2020). The effect of nano-cur binding on the interaction of hsa-HTF binary system and ternary system was studied by multi spectral and molecular dynamics simulation (Mokaberi et al., 2020). In addition, the interaction between hemoglobin (HB) and lomefloxacin (LMF) was also determined by fluorescence spectroscopy, and the molecular simulation results were used as evidence (Mokaberi et al., 2019). DNA is the main target in organism and participates in important intercellular processes. Small molecules can bind with histone DNA and damage the division, growth, inhibition, and apoptosis of cancer cells. The interaction between histone H1 calf thymus DNA (CT-DNA) complex and propyl acridone (PA) was studied using multispectral, viscosity, and molecular simulation techniques (Shakibapour et al., 2019). As an important class of proteins, enzymes play an important catalytic role in biochemical reactions. The effects of three silver nanoparticles with different particle sizes on the binding of curcumin with lysozyme under physiological conditions were studied by spectroscopic and zeta potential techniques (Kamshad et al., 2019).

Given the above, this work will consider the interaction between ginsenoside Rb1 and β-glucosidase. For Paenibacillus polymyxa with ginsenoside transformation ability, we synthesized its β-glucosidase gene which was the key to the catalytic process and then constructed it on the pET-28a (+) vector. The recombinant expression vector pET-28a (+)-bg1L was transformed into E. coli BL21 (DE3) for expression in order to obtain high purity β-glucosidase.

In this work, we reported the interaction between ginsenoside Rb1 and β-glucosidase by spectroscopic method such as UV, fluorescence, and CD and evaluated the effect of Rb1 on the conformation of β-glucosidase. LSPR would be used to determine the specific binding force of their interaction. For it is difficult to achieve the binding conformation in the micro state by conventional experimental means, molecular docking will provide visually simulate the best binding site of β-glucosidase and ginsenoside Rb1 and expected to reveal the important hydrogen bond force and important amino acid residues in the interaction. We aimed to explain or give some insights into the interaction mechanism of β-glucosidase and ginsenoside Rb1.

2 | MATERIALS AND METHODS

2.1 | Strains, vectors, and reagents

Escherichia coli DH5α was preserved in our laboratory for gene cloning and large amplification vector plasmids and recombinant plasmids and used as the cloning host. E. coli BL21 (DE3) was preserved in our laboratory and served as the expression host. Plasmids containing the target gene were synthesized and connected to the prokaryotic expression vector pET-28a (+) for protein expression by Sangon biological engineering (Shanghai, China) Co., LTD. All restriction endonucleases and ligases were purchased from Takara (Dalian, China).
Absorbance value (OD 600) measured at the wavelength of 600 nm was used for purification and removed the heteroprotein by eluate containing 20 mM imidazole. The target protein was collected with 150 mM imidazole eluent and dialyzed overnight in 20 mM pH = 7.0 disodium hydrogen phosphate–citric acid buffer. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) was performed to verify protein purity. The protein was then concentrated, freeze-dried, and stored at −20 °C for future use.

2.2 Design and synthesis of β-glucosidase gene

Using NCBI to query β-glucosidase from Paenibacillus polymyxa, which belongs to the first family of glycoside hydrolases (GenBank: M60211.1), the size of the β-glucosidase gene was 1,344 bp, the amino acid sequence was 448 aa, and its molecular weight was predicted to be 52 kDa. In view of its origin in bacteria, it can be expressed in E. coli. In order to insert the target gene into the expression vector, restriction enzyme sites were introduced into the 5’ and 3’ ends of the foreign gene, respectively. At the same time, the amino acid sequence encoded by the original β-glucosidase gene was not changed. The optimized gene is named bgIB. The gene sequence was entrusted to Sangon Biotechnology Co. Ltd. to complete the whole gene synthesis. The fragment was digested with NdeI and XhoI and then cloned into similarly digested plasmid pET-28a (+). The plasmid DNA was extracted using the Wizard® Plus SV Minipreps DNA Purification System (Promega). After the synthesis, the whole gene was sequenced to ensure the fidelity of the target gene.

2.3 Expression and purification of recombinant β-glucosidase

The 2.0 ml engineered bacteria E. coli BL21(DE3) were added into 100.0 ml Luria-Bertani (LB) liquid medium containing 30 μg/mL kanamycin and incubated for 12 hr at 37 °C at 180 r/min. Then, the medium was transferred to 2.0 L LB liquid medium containing 30 μg/mL kanamycin and incubated for 120 r/min until the final concentration was 0.5 mM and induced overnight at 298 K. At 298 K, the absorption changes of the complex were evaluated at 280 nm. The contribution of β-glucosidase and ginsenoside Rb1 mixture to compare the contribution of different concentrations of ginsenoside Rb1 to the spectrum.

2.4 Absorption spectrum of β-glucosidase and ginsenoside Rb1

The UV spectrum of the β-glucosidase and ginsenoside Rb1 mixture samples was obtained using a UV Visible (UV-Vis) spectrometer (Cary series, Agilent Technologies). The UV-Vis absorption study of 10 μM β-glucosidase was performed in 50 mM phosphate buffer at pH 7.0 to reach the final ginsenoside Rb1 concentrations from 0–100 μM. Put the two universal UV cuvettes into the UV spectrometer, and the constant temperature is 298 K. One was a β-glucosidase solution containing 3 ml as the control group, and the other was the experimental group with a 3 ml buffer. The parameters were as follows: spectral scanning speed 400 nm/s; wavelength range 200–400 nm. At 298 K, the absorption changes of the complex were evaluated at 280 nm. The contribution of β-glucosidase was subtracted from β-glucosidase and ginsenoside Rb1 mixture to compare the contribution of different concentrations of ginsenoside Rb1 to the spectrum.

2.5 Fluorescence spectrum of β-glucosidase and ginsenoside Rb1

The fluorescence spectrum of the mixture of β-glucosidase and ginsenoside Rb1 was obtained by a fluorescence spectrophotometer (Hitachi f-7000). The instrument was connected to a constant temperature control tank to characterize the molecular interaction at 298 K, 304K, and 310K. The formation of the complex was estimated using the fluorescence quenching method described above, with some minor modifications. At the same time, the excitation wavelength of excitation and emission was set to 280 nm, and the slit width was 5 nm. In the range of 300 to 500 nm, the emission spectrum was collected to increase the equal part of 100 μM ginsenoside Rb1 solution to the fixed initial volume (2.0 ml) of 10 μM β-glucosidase solution to prepare a series of solutions. Stern-Volmer equation can be used to study the quenching mechanism.

\[
F_0/F = 1 + K_q \tau_0 [Q] = 1 + K_q [Q]
\]

In the process of fluorescence quenching type analysis, an important and unavoidable problem was the fluorescence internal filtering effect (Kamshad et al., 2019). The internal filtering effect would affect the quenching data, which made the calculated quenching constant have an error. Therefore, the fluorescence intensity used in the research process is corrected by the following formula.
2.6 | Nonradiative energy transfer of \( \beta \)-glucosidase and ginsenoside Rb\(_1\)

10 \( \mu \)M \( \beta \)-glucosidase was accurately prepared. The fluorescence spectrum of \( \beta \)-glucosidase was obtained by scanning in the range of 280 nm to 600 nm with the excitation wavelength of 280 nm by the fluorescence spectrophotometer. The concentration of 10 \( \mu \)M of ginsenoside Rb\(_1\) was accurately prepared, and the UV-visible spectrum was determined in the range of 200 nm – 430nm. The integral area of the overlapped peak of the characteristic fluorescence spectrum of \( \beta \)-glucosidase with that of the characteristic ultraviolet absorption spectrum of Rb\(_1\) was obtained by MATLAB program. Then, the R-value was calculated by the formula of binding distance, and the binding distance between \( \beta \)-glucosidase and ginsenoside Rb\(_1\) was obtained. The calculation formula of the combined distance is as follows.

\[
E = \frac{R_0^6}{R_0^6 + r^6} = 1 - \frac{F}{F_0}
\]

2.7 | Circular dichroism (CD) spectrum of \( \beta \)-glucosidase and ginsenoside Rb\(_1\)

To discover the effects of binding on the secondary structure of \( \beta \)-glucosidase induced by Rb\(_1\), circular dichroism (CD) spectra (200–260 nm) of 10 \( \mu \)M \( \beta \)-glucosidase were collected on a MOS-500 Circular Dichroism Spectrometer (Bio-Logic Science Instruments, Grenoble, France). The optimal concentrations of ginsenoside Rb\(_1\) were confirmed through the preliminary experiment which is 100 \( \mu \)M in 50 mM phosphate buffer at pH 7. In the presence or absence of ginsenoside Rb\(_1\), both samples were evaluated with a 1 mm cell, under constant nitrogen flush at 298 K. The scan rate was 200 nm \( \times \) \( \text{min}^{-1} \); the response time was 1 s, and the bandwidth was 1 nm. The changes in the percentage of secondary structure elements of \( \beta \)-glucosidase were computed by using CDNN software.

2.8 | Localized surface plasmon resonance (LSPR) of \( \beta \)-glucosidase and ginsenoside Rb\(_1\)

To determine the kinetics of the interaction between \( \beta \)-glucosidase and ginsenoside Rb\(_1\), we used the LSPR method (open SPR XT, Nicoya Life sciences, on, Canada). COOH chip was installed according to the standard operation procedure of the open sprtm instrument. The first step was to start to run PBS buffer (pH 7.4) at the maximum flow rate (150 \( \mu \)l/min). After reaching the signal baseline, 200 \( \mu \)l of 80% IPA (isopropanol) was injected for 10 s to discharge bubbles. After reaching the baseline was to wash the sample ring with buffer solution and empty it with air. After the signal reached the baseline, adjusted the buffer flow rate to 20 \( \mu \)l/min. Added 200 \( \mu \)l EDC / NHS solution to wash the sample ring with buffer solution, and made it empty with air. The purified \( \beta \)-glucosidase was diluted to 2 \( \mu \)M and added into the activation buffer to fix the protein on the COOH sensor chip. All experiments were conducted with filtered and degassed PBS buffer (NaCl 137 mM, KCl 2.7 mM, Na\(_2\)HPO\(_4\) 4.3 mM, and KH\(_2\)PO\(_4\) 1.4 mM) at a continuous flow rate of 20 \( \mu \)l/min at 20°C. The analyte which concentrations of ginsenoside Rb\(_1\) were 0.5 to 2 \( \mu \)M was immobilized by \( \beta \)-glucosidase. The surface of the sensor chip will be regenerated by flowing buffer for a long time. The association and dissociation phases of 240 s and 600 s were recorded, respectively. Data analysis included a dissociation curve of up to 400 s. The analyte was passed through the blank COOH sensor chip to measure the background response of ginsenoside Rb\(_1\) combined with the sensor chip. The analysis software used for the results of this experiment was trace tracer (Ridgeview Instruments AB, Sweden), and the analysis method was one to one analysis model.

2.9 | Molecular docking simulation

The crystal structure of \( \beta \)-glucosidase was studied by molecular docking and downloaded from the protein database (www.rcsb.org, PDB: 2Z1S). The initial structure of ginsenoside Rb\(_1\) was modeled by a molecular simulation package Sybyl 7.3, and Tripos force field and Gasteiger-Marsili were used to charge optimizes molecular geometry. The main modeling steps were as follows: (a) Using blast or PSI-BLAST to search the template of the target sequence. (b) Using structure alignment method to compare and overlap the template. (c) Using sequence alignment method to compare the target sequence with the sequence of the template structure. (d) Using modeler to generate the model of target sequence. The docking program was applied to the structure after modeling to get the Ramachandran diagram. The molecular docking of \( \beta \)-glucosidase and ginsenoside Rb\(_1\) were carried out with AUTODOCK Vina software. Lamarckian (LGA) genetic algorithm was used to calculate the possible conformation of ginsenoside Rb\(_1\) molecule bound to protein bglB. In the docking process, at most 10 conformations of the compound were considered, and the conformation with the lowest binding free energy was taken for further analysis.

2.10 | Statistical analysis

Data were repeated at least thrice and expressed as the mean values ± standard deviations (SD). The Tukey test was used to identify significant differences between means (\( p < 0.05 \)) utilizing a one-way ANOVA test. Typical spectra and data were presented as figures.
3 | RESULTS

3.1 | Gene synthesis and expression vector construction of β-glucosidase

The recombinant vector pET-28a (+)-bglB was identified by agarose gel electrophoresis. According to the Figure 1a, the recombinant vector PET-28a (+)-bglB was verified by double digestion of recombinant vector by agarose gel electrophoresis. The size of the 1.356 + 5.3K bp was PET-28a (+)-bglB, which was neat and bright on the edge. Therefore, it can be preliminarily confirmed that the recombinant plasmid has been successfully constructed. The correct recombinant plasmid pET-28a (+)-bglB was introduced into the receptive cells for transformation, and the required monoclonal was screened after overnight culture in LB plate.

3.2 | Expression and purification of β-glucosidase

The β-glucosidase gene of bglB was PCR amplified, then cloned into the pET-28a (+), and transformed in E. coli BL21 (DE3) for the expression of target enzyme. In the presence of IPTG, large amounts of a protein were produced, at a molecular mass of 52 kDa, corresponding to that expected for the full-length protein encoded by bglB. When whole E. coli cells were lysed in a buffer solution, the β-glucosidase was soluble and catalytically active. As shown in Figure 1b, purification of β-glucosidase was achieved by Ni-NTA Agarose and a single band protein identified by SDS-PAGE can be obtained. Therefore, on the one hand, it could be determined that under the condition of 30°C, 200 rpm oscillation induction, the desired inducible expression product, that is, prokaryotic expression of target protein β-glucosidase, can be better obtained in the precipitate induced overnight. On the other hand, it can be determined that the gene fragment size of β-glucosidase induced by prokaryotic expression is 52 kDa. The predicted expression molecular weight of bglB was consistent with other research result from the Paenibacillus polymyxa (Huang et al., 2019).

3.3 | UV-Vis absorption studies of β-glucosidase and ginsenoside Rb1

UV-Vis absorption spectrophotometry is regarded as an effective method to study the structural change of target protein. There are two important absorption bands of β-glucosidase: (a) the absorption band at about 280 nm is composed of aromatic amino acids such as tryptophan, tyrosine, and phenylalanine. Under the action of the enzyme, the absorption peak of β-glucosidase is negatively related to the activity of the enzyme. (b) Soret absorption band at 405 nm. The absorption peak at about 280 nm is composed of benzene heterocyclic structure of aromatic amino acid residues. Therefore, we can analyze and judge the interaction between ginsenoside Rb1 and β-glucosidase protein solution according to the change of the UV absorption peak intensity and the displacement of the maximum absorption peak. Due to the benzene ring structure of Rb1 containing UV absorption peak, it is necessary to add the ginsenoside Rb2 of equal concentration on both sides of the experimental group and the blank group. The Figure 2a showed the influence of ginsenoside Rb1 on the UV-Vis absorption spectrum of β-glucosidase. According to the change of absorption spectrum of β-glucosidase, it could be inferred that ginsenoside Rb1 bound to β-glucosidase and changed its conformation with the protein. It could be seen that the absorption intensity of β-glucosidase at about 280 nm raised with the increase of ginsenoside Rb2 concentration, indicating that Rb2 caused the hydrophobic groups of aromatic amino acids (Trp) in β-glucosidase to be more wrapped and the polarity of corresponding structure to be weakened (Agrawal et al., 2016). In addition, this process led to the formation of a new conjugation system between β-glucosidase and small drug molecules, and the addition of a new π-π* transition. The energy of the π-π* transition increased, resulting in a blue shift of the absorption peak at about 280 nm and the formation of a drug protein ground state complex. The ginsenoside Rb2 is a major panaxadiol from ginseng root, and it can gradually remove the glucose groups at C3 and C20 sites by glycosidase catalysis to generate secondary ginsenosides (Tian et al., 2016).

The shift and intensity of UV absorption peak are related to the surrounding environment. The absorption peak of β-glucosidase at
280 nm was mainly caused by the $\pi-\pi^*$ transition of aromatic heterocycles in aromatic amino acids. With the addition of ginsenoside Rb1, the intensity of the absorption peak at 280 nm was enhanced, which indicated the interaction between ginsenoside and $\beta$-glucosidase. The addition of the small molecular compound led to the extension of peptide chain in protein molecule, which exposed the tryptophan residues in the subdomain and increased the hydrophilicity. It was confirmed that ginsenoside Rb1 formed a complex with $\beta$-glucosidase. However, the microenvironment was not significantly changed by observing the UV Vis absorption spectrum of the complex.

3.4 | Fluorescence quenching studies of $\beta$-glucosidase by ginsenoside Rb1

The binding mechanism of ginsenoside Rb1 and $\beta$-glucosidase was further studied by fluorescence experiment. Fluorescence quenching is a common tool to study the interaction between ligands and proteins as well as substrate and enzyme (L. Li et al., 2016). It provides valuable information about quenching mechanism, binding sites, and binding constants (Günther et al., 2018). Fluorescence quenching refers to the reduction of fluorescence intensity, which may lead to collision quenching, energy transfer, formation of ground state complexes, molecular rearrangement, and other process of many other types of molecular interactions(Wang et al., 2020). The intrinsic fluorescence of $\beta$-glucosidase was from three aromatic amino acid residues (tyrosine, tryptophan, and phenylalanine) (Luo et al., 2019). In the fluorescence study, when the excitation wavelength was set to 280nm, the emission fluorescence of $\beta$-glucosidase was mainly attributed to its intrinsic fluorescence residues Tyr and Trp, while when the excitation wavelength was set to 295nm, it minimized the emission of Tyr residues, which selectively excites Trp residues. The quenching mechanism of ginsenoside Rb1 on $\beta$-glucosidase and other binding parameters were studied by using the excitation wavelength of 280nm. At 298K, the maximum fluorescence emission intensity of natural enzyme was 349 nm. As shown in the Figure 3a, different concentrations of ginsenoside Rb1 (0, 10, 20, 40, 60, 80, and 100 $\mu$M) quenched $\beta$-glucosidase at 298K in a phosphate buffer of 50mM pH of 7.0. The measurements were also made at 304 K and 310 K at the same concentration conditions as shown in Figure 3b and Figure 3c. With the increase of ginsenoside Rb1 concentration, the fluorescence intensity of $\beta$-glucosidase decreased. A red shift was observed with the increase of ginsenoside Rb1 concentration at the excitation wavelength of 280nm. These results indicate that the microenvironment of tyrosine, tryptophan, and phenylalanine residues in $\beta$-glucosidase has changed. Therefore, ginsenoside Rb1 had a significant effect on the conformation of enzyme, and its quenching mechanism was usually divided into static quenching mechanism and dynamic quenching mechanism. The higher the temperature led to the faster diffusion, and more collisions happened. Therefore, the dynamic quenching constant would increase with the increase of temperature. As shown in Figure 3d, in order to determine the fluorescence quenching mechanism, the fluorescence quenching data were analyzed according to the known Stern–Volmer equation. A linear nature of the Stern–Volmer plot ($y = 0.00754x + 1.0984$, $R^2 = 0.9941$) was found in the Rb1 interaction with $\beta$-glucosidase. It could be seen that ginsenoside Rb1 had a good linear relationship between $F_0/F$ and [Q] at 298K. $K_{SV}$ values of Rb1, as the quenching constant $K_{SV}$ value is $8.37 \times 10^3$ L/M, and the rate constant $K_q$ value is $8.37 \times 10^{11}$ L/M·s. In the Stern–Volmer plot, $F_0$ was the maximum fluorescence intensity when $\beta$-glucosidase solution was without dropping anything, and $F$ was the maximum corrected fluorescence intensity when $\beta$-glucosidase solution was added with ginsenoside Rb1. $K_{SV}$ was Stern–Volmer quenching constant. [Q] was the molality concentration of ginsenoside Rb1. $K_q$ was the rate constant of biomolecular quenching process. $\tau_0$ was the fluorescence lifetime of the biomacromolecule, which was about $10^{-8}$s. Therefore, the $K_{SV}$ and $K_q$ values differ by $10^{-8}$ times.
The experiment was carried out at three temperatures at 298K, 304K, and 310K, respectively. The data were shown in the Table 1. The $K_{SV}$ of the interaction between ginsenoside Rb$_1$ and β-glucosidase increased with the increase of temperature, and $K_{SV}$ was greater than the dynamic quenching constant of $2.0 \times 10^{10}$ L M$^{-1}$s$^{-1}$. It can be preliminarily inferred that the fluorescence quenching of β-glucosidase by Rb$_1$ belongs to static quenching (Kayukawa et al., 2019).

3.5 | Binding distance of ginsenoside Rb$_1$ and β-glucosidase

Energy transfer can be divided into radiation energy transfer and nonenergy transfer, among which nonradiation energy transfer is also called fluorescence resonance energy transfer (FRET) (Hemachandran et al., 2017). If there is radiation energy transfer between donor and acceptor, the fluorescence spectrum of the fluorescent material will be deformed, and the fluorescence intensity of the fluorescent material will be quenched. The main chromophores of β-glucosidase are tryptophan (Trp) and tyrosine (Tyr). The fluorescence intensity and the maximum emission peak shift of this residue can directly show the microenvironment changes of excellent amino acid and tyrosine residues. It can be seen from the Figure 2b that β-glucosidase had a strong fluorescence intensity at the excitation wavelength of 280 nm and had a large degree of overlap with the UV absorption spectrum of Rb$_1$. The main light-emitting group of β-glucosidase was tryptophan residue, and when the UV absorption spectrum of Rb$_1$ was at the binding position of β-glucosidase, the binding distance $r$ is less than 7 nm, and the tryptophan residue of β-glucosidase can emit fluorescence. The binding distance $r$ of Rb$_1$ to β-glucosidase was 1.79 nm, and the binding distance of Rb$_1$ to β-glucosidase was less than 7 nm. Therefore, the nonradiative energy transfer between Rb$_1$ and β-glucosidase can be preliminarily determined. In conclusion, the energy of β-glucosidase was transferred to Rb$_1$, and the fluorescence intensity of β-glucosidase on tryptophan residue was reduced, and then, fluorescence quenching occurred.

3.6 | Conformation changes of β-glucosidase induced by ginsenoside Rb$_1$

CD spectroscopy is used to be a convenient and precise technique, and it has been widely used to screen the changes in protein conformation based on these characteristics (Bhagyalekshmi et al., 2019). The secondary structure of the target protein was monitored by CD spectroscopy (Jahromi et al., 2020), and the effect of ginsenoside Rb$_1$ on the secondary structure of β-glucosidase was analyzed. 0 and 100 μM Ginsenoside Rb$_1$ and 10 μM β-glucosidase were incubated in 50 mM pH 7.0 and 298 K phosphate buffer for 3 min. The CD of the samples was recorded in the range of 190-260nm. As shown in the Figure 4, the spectrum of β-glucosidase showed two negative bands at 198 and 218 nm, which are the characteristics of α helix and α helix / random helix in β-glucosidase structure. With the addition
of 100 μM ginsenoside Rb1, the CD intensity of β-glucosidase at 198 and 218 nm decreased significantly, which indicated that the secondary structure of β-glucosidase changed significantly with the increase of α-helix content. The content of different secondary structure of β-glucosidase was calculated by CDNN program. The secondary structure of β-glucosidase includes 10.3% β-sheet, 36.8% α-helix, 14.3% β-bend, and 38.6% random coil. With the increase of ginsenoside Rb1 concentration, the content of α-helix increased, while the content of β-sheet decreased, and random coil did not change significantly. According to these results, we think that the structure of β-glucosidase was stable by increasing the content of α-helix. In addition, the decrease of β-sheet structure indicated that ginsenoside Rb1 had an important interaction with hydrophobic contact when compared with the same type of research (Matsuo & Gekko, 2019). The circular dichroism diagram of β-glucosidase had a positive peak near 204nm, and an obvious negative peak near 218nm, which was the characteristic peak of the α-helix in the protein. With the addition of ginsenoside Rb1, the strength of positive and negative peak increased, indicating that the content of α-helix increased continuously. The formation of this phenomenon was consistent with the change trend of enzyme activity, suggesting that α-helix a necessary structure to maintain the conformation of the active center of enzyme molecules. After the ginsenoside Rb1 interacting with β-glucosidase, the hydrogen bonding occurred with polar amino acid residues in protein molecules, which improved the stability of the complex.

3.7 Calculation of Binding Parameters between β-Glucosidase and ginsenoside Rb1

Localized surface plasmon resonance (LSPR) is an optical phenomenon, which can be used to track the interaction between biomolecules in natural state in real time (Elsawy et al., 2016; P. Li et al., 2020). This method has no damage to biological molecules and does not need any markers. We further confirmed the interaction between β-glucosidase and ginsenoside Rb1 by LSPR. As shown in Figure 5, LSPR data clearly showed that β-glucosidase interacted with ginsenoside Rb1 to form a stable 1:1 complex, and the equilibrium dissociation constant (KD) was $5.24 \times 10^{-4}$ ($\pm 2.35 \times 10^{-5}$)M. LSPR data also showed a high correlation the dissociation rate of this interaction, the Ka was $29.7 \pm 6.62 \times 10^{2}$/M/$s$ and the Kd was $1.56 \times 10^{-2}$ ($\pm 2.17 \times 10^{-5}$)$/s$.

3.8 Molecular Docking analysis

The molecular docking analysis of β-glucosidase and its substrate was carried out by using AUTODOCK Vina program. Except for the size of docking box, all docking parameters were default and chose the conformation with the best affinity (i.e., the lowest affinity value, which was ~8.9kcal/M in this docking case) to be selected as the docking conformation for subsequent molecular dynamics simulation. The binding energy of molecular docking was slightly higher than that of spectral experiments, possibly because molecular docking was conducted in a simulated vacuum environment, while spectral experiments were conducted in a solvent environment, which was consistent with other studies that have been reported (Dehghani Sani et al., 2018; Shakibapour et al., 2019).

As shown in Figure 6b, it could be seen from the docking results that the substrate binding site located in a barrel structure formed by β-sheet around the glycosidase center. However, the ginsenoside Rb1 was not completely in the barrel, because its structure was somewhat extended, most of the groups were in the upper part of the barrel structure and interacted with multiple loop structures of receptor protein. The loop structures were also important for the combination of substrates. In addition, because the binding site was somewhat shallow to the barrel structure, it had certain hydrophilicity. The glycoside substrate had many hydroxyl groups and strong hydrophilicity. Therefore, the hydrophilicity of the binding pocket was also important for substrate binding, which conformed to the basic principal of energy matching. It could be inferred that there were many hydrogen bonds between the glycoside substrate and the receptor protein to maintain their binding. Due to its special extension, one part of the substrate was bound to protein β-sheet, and the other part was exposed to solvent environment. As shown in the Figure 6a, a hydrogen bond interaction was formed between the substrate hydroxyl and a plurality of active pocket residues. These residues included Gly45 (skeletal oxygen), Lys46 (side chain amino group), Glu180 (side chain carboxyl group), His181 (side chain N), Gin22 (side chain amide group), Glu116 (side chain carboxyl group), Tyr298 (side chain hydroxyl group), and Glu356 (side chain carboxyl group). These hydrogen bonds were essential for the binding of substrates and further catalysis. Therefore, these residues could also be regarded as hot spots which affect the substrate binding, and their functions could be further verified by point mutation in subsequent experiments. As mentioned in the literature, Glu167 and Glu356 were
essential for the catalysis of glycosidases (Zhou et al., 2019). The substrate involved in this study had a relatively extended spatial conformation, which led to a large steric hindrance in the process of entering the active pocket. This made the distance between glycoside bond (oxygen atom) and catalytic residue larger. The distance between Glu167 and its carboxyl group is 6.1 Å. To some extent, this distance is crucial for the catalytic efficiency of β-glucosidase. The hydrophilicity of β-glucosidase binding pocket had a binding effect on ginsenoside Rb1.

**TABLE 1** Quenching constants and linear equations of interaction between ginsenoside Rb1 and β-glucosidase at different temperatures

| Agent         | T(K) | Equations  | $R^2$   | $K_{SV}$ (L·mol$^{-1}$) | $K_q$ (L·mol$^{-1}$·s$^{-1}$) |
|---------------|------|------------|---------|--------------------------|-------------------------------|
| Ginsenoside Rb1 | 298  | $y = 0.00754x + 1.0984$ | 0.9941  | $8.37 \times 10^3$      | $8.37 \times 10^{11}$       |
|               | 304  | $y = 0.00741x + 1.0778$ | 0.9891  | $8.45 \times 10^3$      | $8.45 \times 10^{11}$       |
|               | 310  | $y = 0.00640x + 1.1471$ | 0.9994  | $8.58 \times 10^3$      | $8.58 \times 10^{11}$       |

**FIGURE 5** Local surface plasmon resonance (LSPR) of the interaction between β-glucosidase and ginsenoside Rb1. The representative curves of the ginsenoside Rb1 concentrations (blue, 0 mM; green, 0.5 mM; yellow, 1 mM; orange, 2 mM;) show the association and dissociation phases of the interaction between β-glucosidase and ginsenoside Rb1. Thin lines of the same color represent the fit between the data and the 1:1 binding model.

**FIGURE 6** (a) MD simulation results showing that the hydroxyl group of ginsenoside Rb1 and several active pocket residues of β-glucosidase all formed hydrogen bond interaction and the amino acid residues Glu167 and Glu356 are very important for the catalysis of β-glucosidase. (b) MD simulation results showing possible binding sites of ginsenoside Rb1 on β-glucosidase. The hydrophilicity of β-glucosidase binding pocket had a binding effect on ginsenoside Rb1.
extent, the occurrence of catalysis was limited. In the later stage, in order to improve the enzyme activity, the substrate could be mutated to combine with the pocket residue, so that the distance would be reduced. Based on the understanding of the sequence and structure of β-glucosidase, it could be possible to improve the activity of β-glucosidase by site directed mutation in the future, as to realize the efficient catalysis of ginsenosides.

4 | DISCUSSION AND CONCLUSION

In order to obtain the enzyme protein with clear gene sequence, in this study, β-glucosidase gene sequence was optimized and synthesized by codon. The total length of bglB gene was 1,344 bp. With pET-28a (+) as the expression vector, the recombinant vector pET-28a (+)-bglB was constructed and transferred into E. coli BL21 (DE3). After induction, the target protein was obtained and purified by Ni-NTA column. The molecular weight and purity of the target protein were identified. The purified β-glucosidase was identified as a single band by SDS-PAGE. The obtained high purity enzyme could be used for subsequent assays to explore its activity and binding effect.

The mechanism of interaction between β-glucosidase and ginsenoside Rb1 was studied by a series means of spectroscopy. With the increase of ginsenoside Rb1 concentration, the UV spectrum showed that there was a slight blue shift (279–277 nm) which indicated that the ginsenoside Rb1 caused the hydrophobic group of aromatic amino acid (Trp) in β-glucosidase to be more wrapped. Thus, the polarity of corresponding structure was weakened and became a new π-π* transition, so that the complex of substrate and enzyme was formed. The fluorescence quenching spectra showed that ginsenoside Rb1 significantly inhibited the intrinsic fluorescence of β-glucosidase, which indicated that there was a strong interdependence between the two molecules, which was consistent with the previous UV spectrum results and the quenching type was the static quenching mechanism. The decrease of the surface hydrophobicity of β-glucosidase indicated that the Rb1 bound to the hydrophobic groups on the protein surface. The circular dichroism spectrum showed that the binding of ginsenoside Rb1 to β-glucosidase resulted in the change of enzyme conformation. LSPR results showed that β-glucosidase and ginsenoside Rb1 had strong binding power, and the specific KD value was determined.

In the molecular docking analysis, the molar stoichiometry of β-glucosidase and ginsenoside Rb1 complex (1:1) showed that it was composed of a ginsenoside Rb1 molecule and each β-glucosidase protein molecule. Molecular models provided valuable insight into the nature of functional groups and forces involved in the combination, as well as the formation of visible complexes. Through the results of molecular docking, the hydrophobic force and hydrogen bond were evaluated, and the important amino acid residues to the protein in the process of β-glucosidase catalyzing ginsenoside were found in the simulation.

In general, we systematically and innovatively studied the binding interaction of β-glucosidase and ginsenoside Rb1 by combining various spectroscopic methods, LSPR and molecular docking. Those results provided a new idea for the gaps in research of ginsenosides and glycosidases which only involve in biotransformation findings in the past. Thus, our findings consist a substantial and definite addition to the present understanding of the interaction between ginsenoside Rb1 and β-glucosidase from GH1 family. It also provided a research method basis for the binding mechanism of enzymes and their atypical substrates in the catalytic process.

5 | INFORMED CONSENT

Informed consent was obtained from all individual participants included in the study.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHORS CONTRIBUTIONS

Shuning Zhong involved in methodology development or design of methodology, writing—original draft preparation, software programming, and software development. Mi Yan involved in investigation. Haoyang Zou involved in data curation. Ping Zhao involved in validation verification. Haiqing Ye involved in supervision and visualization. Changhui Zhao involved in writing—reviewing and editing. Tiehua Zhang* involved in conceptualization ideas, resources, and project administration.

ETHICAL STATEMENTS

This article does not contain any studies with human or animal subjects.

ETHICAL APPROVAL

This article does not contain any studies with animals or human participants performed by any of the authors.

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