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New Dioscin-Glycosidase Hydrolyzing Multi-Glycosides of Dioscin from *Absidia* Strain

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A novel dioscin-glycosidase that specifically hydrolyzes multi-glycosides, such as 3-*O*-α-L-(1→4)-rhamnoside, 3-*O*-α-L-(1→2)-rhamnoside, 3-*O*-α-L-(1→4)-arabinoside, and β-D-glucoside, on diosgenin was isolated from the *Absidia* sp.d38 strain, purified, and characterized. The molecular mass of the new dioscin-glycosidase is about 55 kDa based on SDS–PAGE. The dioscin-glycosidase gradually hydrolyzes either 3-*O*-α-L-(1→4)-Rha or 3-*O*-α-L-(1→2)-Rha from dioscin into 3-*O*-α-L-Rha-β-D-Glc-diosgenin, further rapidly hydrolyzes the other α-L-Rha from 3-*O*-α-L-Rha-β-D-Glc-diosgenin into the main intermediate products of 3-*O*-β-D-Glc-diosgenin, and subsequently hydrolyzes these intermediate products into aglycone as the final product. The enzyme also gradually hydrolyzes 3-*O*-α-L-(1→4)-arabinoside, 3-*O*-α-L-(1→2)-rhamnoside, and β-D-glucoside from [3-*O*-α-L-(1→4)-Ara, 3-*O*-α-L-(1→2)-Rha]-β-D-Glc-diosgenin into diosgenin as the final product, exhibiting significant differences from previously reported glycosidases. The optimal temperature and pH for the new dioscin-glycosidase is 40°C and 5.0, respectively. Whereas the activity of the new dioscin-glycosidase was not affected by Na^+, K^+, and Mg^{2+} ions, it was significantly inhibited by Cu^{2+} and Hg^{2+} ions, and slightly affected by Ca^{2+} ions.

Keywords: Dioscin, dioscin-glycosidase, hydrolyzing multi-glycosides

Dioscorea nipponica is a popular herb in China and widely used in traditional Chinese medicine, functional foods, and cosmetics. The Chinese agricultural name for

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Dioscorea nipponica is Huangjiang, and the rhizome of *Dioscorea nipponica* is used to prevent rheumatic diseases, viral infection, and bronchitis [17]. One of the major physiologically active ingredients in *Dioscorea nipponica* is dioscin, which belongs to the family of steroidal saponins. Dioscin has been proven to be physiologically active, improving the cardiovascular function and exhibiting antitumor [11, 13], anticancer [3, 10], antidiabetic [12], and antiviral [6] activities. It is also an important ingredient for the chemical synthesis of steroidal drugs.

The main dioscin in the rhizome of *Dioscorea nipponica* is 3-*O*-α-L-rhamnopyranosyl-(1→4), [α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranosyl-diosgenin {*i.e.*, 3-*O*-α-L-Rha*p*-(1→4), [α-L-Rha*p*-(1→2)]-β-D-Glc*p*-diosgenin}, which contains three glycosides [14, 15]. In general, saponins in their natural form (*i.e.*, dioscin in *Dioscorea nipponica*) cannot be directly absorbed and utilized by the human body. After oral intake of *Dioscorea nipponica*, the glycosides of dioscin are hydrolyzed by digestive enzymes and/or intestinal bacteria into low-sugar-saponin and aglycone, which are absorbed slowly in the gastrointestinal tract to exhibit physiological activity [1, 8]. Therefore, the modification of natural saponins by enzymes *in vitro* to produce more active second saponins that can be easily absorbed and utilized by the human body would add great value to functional foods and medicines made from herbs such as *Dioscorea nipponica*.

Accordingly, to obtain physiologically active saponins that can be easily absorbed by the human body, the current authors previously reported on saponin-glycosidases, isolated and extracted from microorganisms, plants, and livers, that hydrolyze the saponin-sugar moiety to produce more active low-sugar-moiety saponins [9, 14, 18, 19]. Three types of enzymes hydrolyzing dioscin-glycosides were previously reported: dioscin-α-L-rhamnosidase from pig liver [14] and

dioscinase from a microorganism [9]. The enzyme from pig liver [14] hydrolyzes 3-*O*-α-L-(1→2)-rhamnopyranoside and 3-*O*-α-L-(1→4)-rhamnopyranoside from dioscin to produce 3-*O*-β-D-glucopyranosyl-diosgenin, whereas the dioscinase from the microorganism (sp.s00c strain) [9] hydrolyzes 3-*O*-α-L-(1→2)-Rha*p* and 3-*O*-α-L-(1→4)-Rha*p* from dioscin to produce 3-*O*-β-D-glucopyranosyl-diosgenin; it also hydrolyzes 3-*O*-α-L-(1→2)-Rha*p* and 3-*O*-β-L-(1→3)-Glc*p* from 3-*O*-α-L-(1→2)-Rha*p*, [3-*O*-β-L-(1→3)-Glc*p*]-β-Glc*p*-diosgenin to produce 3-*O*-β-D-glucopyranosyl-diosgenin. Feng *et al.* [4, 5] also reported a glucoamylase with steroidal saponin-rhamnosidase activity from a *Curvularia lunata* strain. This enzyme is specific to the terminal α-1,2-linked rhamnosyl residues of the sugar chain at the 3-*O*-position of dioscin; that is, the enzyme only hydrolyzes the α-L-(1→2)-rhamnoside from dioscin to produce 3-*O*-α-L-Rha*p*-(1→4)-β-D-Glc*p*-diosgenin.

Therefore, this study isolated, purified, and characterized a new dioscin-glycosidase from the *Absidia* sp.d38 strain that specifically and gradually hydrolyzes either 3-*O*-α-L-(1→4)-Rha or 3-*O*-α-L-(1→2)-Rha from dioscin to produce 3-*O*-α-L-Rha-β-D-Glc-diosgenin, rapidly hydrolyzes the other α-L-Rha from 3-*O*-α-L-Rha-β-D-Glc-diosgenin into the main intermediate products of 3-*O*-β-D-Glc-diosgenin, and subsequently hydrolyzes these intermediate products into aglycone as the final product (Fig. 1).

MATERIALS AND METHODS

Materials
The *Absidia* sp.d38 strain isolated from Chinese traditional *koji* (Daqu in Chinese) [19] was obtained from the Food and Fermentation Culture Collection at Dalian Polytechnic University, Dalian, P. R. China. The herb, *Dioscorea nipponica,* was purchased from Xianyang, Shannxi Province, P. R. China, and the dioscin extracted following existing procedures [15]. Standard dioscin, diosgenin, 3-*O*-[α-L-Rha-(1→2), α-L-Ara-(1→4)-]-β-D-Glc-diosgenin, 3-*O*-α-L-Rha-β-D-Glc-diosgenin, and 3-*O*-β-D-Glc-diosgenin were all obtained from Prof. Baiping Ma (Academy of Military Medical Science, Beijing, P. R. China) and Sino-Herb Bio-Tech Co. Ltd, Xian, P. R. China. Silica gel (Kiesel gel 60 F-254, Merck) was used as the thin-layer chromatography (TLC) plate, and the protein molecular markers were from GE Healthcare Life Science Products, LMW (14,000–97,000). All the other reagents in the study were of analytical grade and used without further purification.

Microorganism Culturing
The *Absidia* sp.d38 strain was cultured at 28–30°C with shaking in a medium (200 ml in 1,000 ml conical flask) containing 5% malt extract and 0.75% plant extract from *Dioscorea nipponica* for 84–96 h. The maltose, cell growth, and enzyme activity in the fermentation were determined following existing procedures [7].

Crude Enzyme Extraction
The culture was centrifuged to remove the cells, and $(NH_4)_2SO_4$ was then slowly added to the cell-free culture with stirring to 40% saturation and the mixture stored at 4°C for 4 h. After removing the protein precipitated by the centrifugation, more $(NH_4)_2SO_4$ was added to 75% saturation and the mixture stored at 4°C overnight. Thereafter, the mixture was centrifuged to harvest the crude enzyme, which was then dialyzed against a 0.02 M acetate buffer (pH 5.0) and subsequently diluted to 1/20 (v/v) with the 0.02 M acetate buffer (pH 5.0). The non-dissolved fraction was removed by centrifugation, and the resulting crude enzyme solution subjected to further enzyme purification.

Purification of New Dioscin-Glycosidase
Ten ml of the crude enzyme solution was eluted on a DEAE-cellulose DE-52 (Whatman) column (Φ2.0 cm×11 cm) and the proteins were
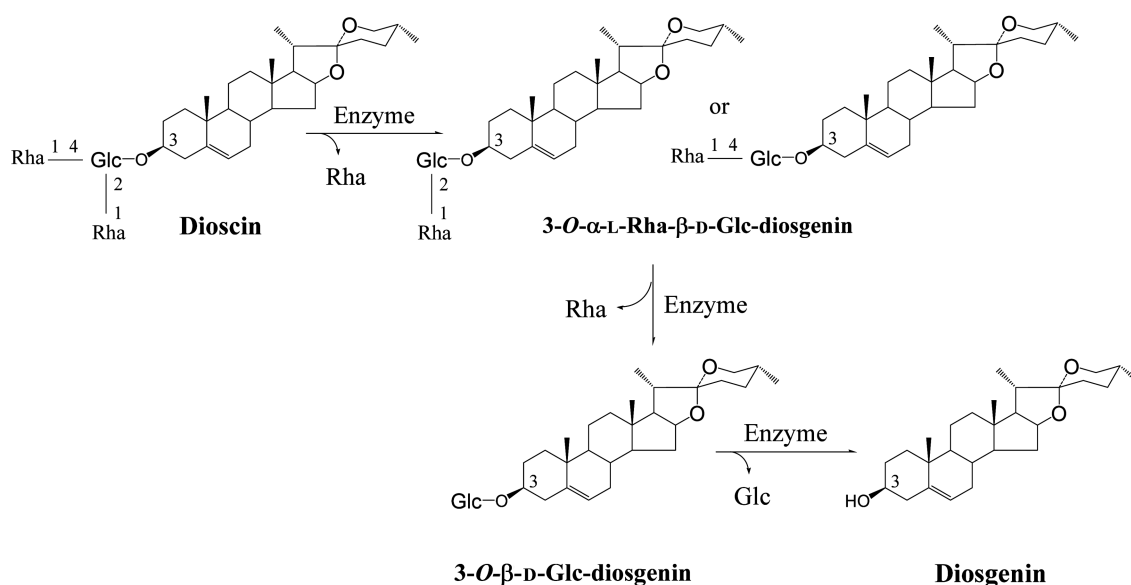

Fig. 1. Action patterns of novel dioscin-glycosidase in dioscin hydrolysis.

fractionated stepwise with 0.06, 0.12, 0.18, 0.24, 0.3, 0.4, 0.5, and 0.6 M KCl in a 0.02 M acetate buffer (pH 5.0, 3.0 ml/fraction). The enzymatic activity of each fraction was then evaluated based on the hydrolysis of dioscin, and the fractions that exhibited hydrolyzing activity were respectively dialyzed against a 0.02 M acetate buffer (pH 5.0), freeze-dried, and dissolved in 1/10 (w/v) distilled water. Vertical slab polyacrylamide gel electrophoresis was also used for further purification of the special dioscin-glycosidase. The resulting enzyme band on the vertical slab polyacrylamide gel was cut and mashed in an acetate buffer, and the non-dissolved materials were removed by centrifugation, leaving a pure enzyme solution that was evaluated for its molecular mass and enzymatic properties. The enzyme protein purity was also examined by HPLC using a C8 column.

Assays of Enzymatic Activity

The enzymatic activity of the dioscin-glycosidase was assayed using 2.0 mg/ml dioscin in a 0.2 M acetate buffer (pH 5.0) as the substrate. A dioscin solution (0.1 ml) was added to the same volume of the enzyme solution and allowed to react at 40°C for 30 h. Next, 0.2 ml of n-butanol saturated by water was added to the reaction mixture to stop the reaction. The reaction product in the n-butanol layer was then analyzed by TLC with chloroform:methanol:H$_2$O [70:30:5 (v/v/v)] as the developing solvent. The spots on the silica plate were scanned using a Shimadzu CS-930 spectrophotometer [1]. One unit of enzyme activity was defined as the amount of enzyme that hydrolyzed 1 μmol of the substrate per hour.

HPLC Method

HPLC (Waters 2695 Separations Module with Waters 2996 Photodiode Array Detector) was used to examine the enzyme protein purity and analyze the enzymatic reaction product.

A C-18 Hypersil 5 μm ODS2 (Φ4.6×250 mm) column was used to analyze the enzymatic reaction product, where the measuring wavelength was 203 nm and the injected volume was 10 μl. The mobile phase was A (acetonitrile) and B (water): 0−2 min, A from 0% to 70%; 2−10 min, A from 70% to 80%; 10−20 min, A from 80% to 90%; 20−40 min, A 90%; and 40−60 min; the column was eluted by 100% A.

The enzymatic reaction product sample used for the HPLC was obtained through pretreatment of the reaction product with an AB-8 Diaion resin column. One ml of a dioscin−enzyme reaction mixture was eluted on a 10-ml column of AB-8 Diaion resin (from Tianjin Chemical Plant of Nankai University, P. R. China). The resin column was first washed with 80 ml of a 0.01 M acetate buffer (pH 5.0) and 50 ml of 20% alcohol, and then eluted with 60 ml of 83% alcohol to separate and collect the reaction products. These products were dried by vacuum distillation, and dissolved in 1 ml of methanol before the HPLC analysis.

A Kromasil C8 (Φ4.6×200 mm) column was used to examine the enzyme protein purity, where the measuring wavelength was 215 nm, the flow rate was 1 ml/min, and the injected volume was 10 μl. The mobile phase was A (acetonitrile and 1% trifluoracetic acid) and B (water and 1% trifluoracetic acid): 0−20 min, A from 20% to 50%; 20−40 min, A from 50% to 80%; 40−50 min, A from 80% to 20%.

Estimation of Enzyme Molecular Mass

The purity and molecular mass of the resulting dioscin-glycosidase were determined by SDS−polyacrylamide gel electrophoresis (SDS−

PAGE) using α-lactalbumin (14.4 kDa), a trypsin inhibitor (21.1 kDa), carbonic anhydrase (30 kDa), ovalbumin (45 kDa), albumin (66 kDa), and phosphorylase b (97 kDa) as the standard proteins under the same conditions [16]. The enzyme molecular mass was determined from the standard curve constructed using the standard proteins.

Determination of Protein Concentration

The protein concentration was measured using the Folin reagent method of Bradford with bovine serum albumin as the standard [2].

### Assays of Effects of pH, Temperature, and Reaction Time on Dioscin-Glycosidase Reaction

To determine the optimal pH, temperature, and reaction time for the new dioscin-glycosidase, various pHs, temperatures, and reaction times were investigated for the enzymatic reaction, and the products analyzed by TLC. The optimal pH for the special dioscin-glycosidase was determined at 40°C with different buffers at 0.2 M. An acetate buffer and phosphate buffer were used for pH 4.0−5.0 and 6.0−8.0, respectively. The optimal temperature was determined between 30°C and 80°C using a standard enzymatic assay at pH 5.0 (0.2 M acetate buffer), and the optimal reaction time was examined between 3 h and 39 h at 40°C and pH 5.0 (0.2 M acetate buffer).

RESULTS

Enzyme Fermentation

To examine the production behavior of dioscin-glycosidase during the fermentation of the *Absidia* sp.d38 strain, the cell growth, enzyme production, and maltose reduction were all measured, as shown in Fig. 2.

The highest dioscin-glycosidase production by the *Absidia* sp.d38 strain occurred after fermentation for 84 to 96 h, at which point the enzyme production reached about 13 U/ml. Even after 96 h, the enzyme production was still maintained on a high level. Meanwhile, the maltose rapidly reduced from 60 to 84 h, and became stable after 96 h, whereas the cell concentration rapidly increased from 60 to
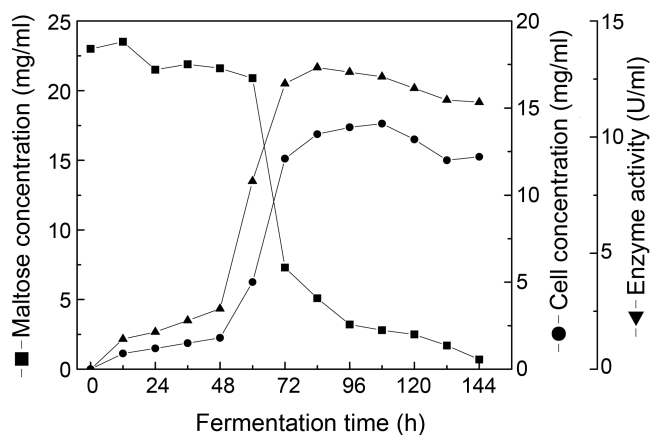
Fig. 2. Enzyme fermentation behavior.
Fermentation was carried out at 28−30°C with shaking.
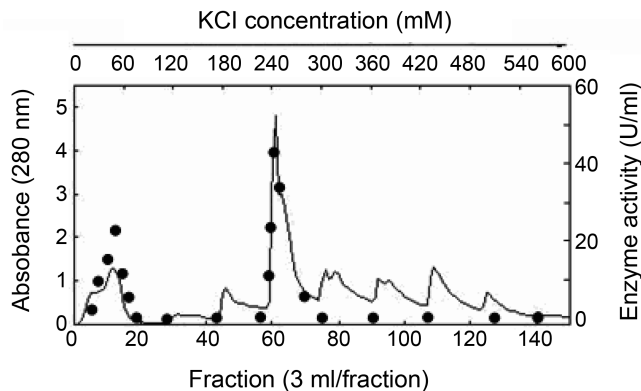
Fig. 3. Purification of new dioscin-glycosidase on a DEAE-cellulose DE-52 column.
Φ2.0 cm×11 cm, 3 ml/fraction; elution buffer, 0.06, 0.12, 0.18, 0.24, 0.3, 0.4, 0.5, and 0.6 M KCl in 0.02 M acetate buffer (pH 5.0). –, Protein absorbance at 280 nm; ●, Enzyme activity.

84 h, and became stable after 84 h, indicating that the maltose was quickly utilized in the cell growth to produce the enzyme during the fermentation process. Thus, since the enzyme production was found to be closely related to the cell growth, the enzyme fermentation time was defined as 84 to 96 h in the experiments.

Enzyme Purification
A cell-free culture of the *Absidia* sp.d38 strain was treated with 40% saturated $(NH_4)_2SO_4$ to remove any precipitate, and further treated with 75% saturated $(NH_4)_2SO_4$ to harvest the enzyme. The crude enzyme solution was then eluted on a DEAE-cellulose DE-52 (Whatman) column (Φ2.0 cm×11 cm) and fractionated to collect different fractions, as shown in Fig. 3.

The dioscin-hydrolyzing activity of each fraction was assayed with dioscin as the substrate, and fractions 61 and 62 were found to hydrolyze dioscin effectively, indicating that these fractions contained dioscin-glycosidase.

Further purification of the above fractions was performed using vertical slab polyacrylamide gel electrophoresis: the enzyme in fractions 61 and 62 eluted by a 0.24 M KCl solution formed one single band on the gel. The enzyme band on the gel was then cut and dissolved in a 0.02 M acetate buffer (pH 5.0) to obtain the pure enzyme. The
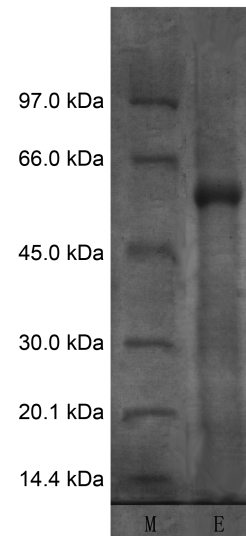
Fig. 4. SDS–polyacrylamide gel electrophoresis of new dioscin-glycosidase.
M, Markers: α-lactalbumin (14.4 kDa), trypsin inhibitor (21.1 kDa), carbonic anhydrase (30 kDa), ovalbumin (45 kDa), albumin (66 kDa), and phosphorylase b (97 kDa); E, fraction 61 special dioscin-glycosidase.

yield of the special dioscin-glycosidase after the DEAE-cellulose column separation was about 2.6%, and the specific activity of the enzyme was increased 7.8 times compared with that of the cell-free culture. To confirm the purity of the enzyme, further separation was conducted using polyacrylamide gel electrophoresis, however, the enzyme specific activity was not changed and the yield was only 0.66%, as shown in Table 1. HPLC was also used to check the purity of the dioscin-glycosidase. Only one peak appeared on the HPLC, at 15.857 min, indicating that the dioscin-glycosidase separated by the DEAE and PAGE was already a pure enzyme. This datum was omitted. Thus, since the enzyme acquired through the DEAE-cellulose column separation was already concluded to be a pure dioscin-glycosidase, it was evaluated for its molecular mass and enzymatic properties.

Molecular Mass of New Dioscin-Glycosidase
SDS–polyacrylamide gel electrophoresis was used to estimate the molecular mass of the new dioscin-glycosidase from the *Absidia* sp.d38 strain. The purified enzyme from

Table 1. Results of enzyme extraction and purification.

Step	Volume (ml)	Total activity (U)	Total protein (mg)	Specific activity (U/mg)	Purification fold (χ fold)	Yield (%)
Fermentation	400	18,500	1246	14.8	1.0	100
$(NH_4)_2SO_4$ precipitation	40	11,816	609.1	19.4	1.3	64.9
DEAE-cellulose (4 times)	12	486	4.2	115.7	7.8	2.6
Electrophoresis	2	122	1.05	116.2	7.9	0.66

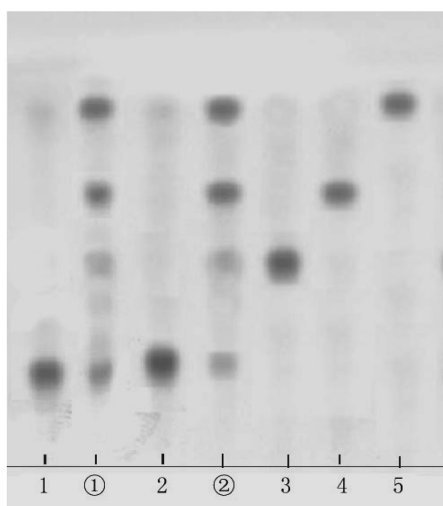fraction 61 formed one band on the SDS–polyacrylamide gel, as shown in Fig. 4.

Standard proteins were run together with the enzyme, and the enzyme molecular weight calculated using a standard curve and plotting the log of the molecular weights of the standard proteins vs. the relative mobility of the proteins. The molecular mass of the new dioscin-glycosidase was determined to be about 55 kDa.

Enzymatic Hydrolysis of Dioscin

The products of the hydrolysis of dioscin by the new dioscin-glycosidase are shown in Fig. 5.

The hydrolysis products of the dioscin-glycosidase on dioscin had the same Rf values as 3-O-β-D-Glc-diosgenin and diosgenin (aglycone), and a small amount of 3-O-α-L-Rha-β-D-Glc-diosgenin, indicating that the new dioscin-glycosidase gradually hydrolyzed either 3-O-α-L-(1→4)-Rha or 3-O-α-L-(1→2)-Rha from dioscin into 3-O-α-L-Rha-β-D-Glc-diosgenin, while also rapidly hydrolyzing the other α-L-Rha from 3-O-α-L-Rha-β-D-Glc-diosgenin into the main intermediate products of 3-O-β-D-Glc-diosgenin, and then hydrolyzing these intermediate products into aglycone as the final product.

To investigate the ability of the enzyme to hydrolyze another glycoside-diosgenin, 3-O-[α-L-Rha-(1→2), α-L-Ara-(1→4)-]-β-D-Glc-diosgenin was reacted with the enzyme, as shown in Fig. 5. The dioscin-glycosidase also gradually hydrolyzed the 3-O-(1→4)-α-L-arabinoside, 3-O-(1→2)-α-L-rhamnoside, and β-D-glucoside on diosgenin into low-glycoside intermediate products and then hydrolyzed these intermediate products into diosgenin.

The hydrolysis products of the new dioscin-glycosidase on dioscin were further purified using an AB-8 Diaion resin and subjected to HPLC analysis. The results are shown in Fig. 6, where peak 1 is dioscin at 5.517 min, peak 2 is 3-O-α-L-Rha-β-D-Glc-diosgenin at 8.036 min, peak 3 is 3-O-β-D-Glc-diosgenin at 14.02 min, and peak 4 is diosgenin (aglycone) at 27.70 min. The HPLC results for the enzyme reaction products were similar to the TLC results for the dioscin reaction in Fig. 5.
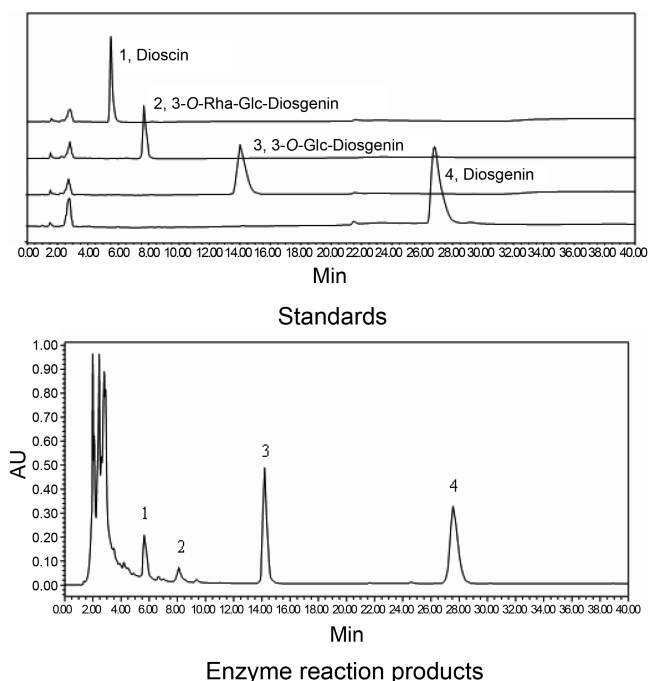
Properties of New Dioscin-Glycosidase

The effects of the pH, temperature, and reaction time on the new dioscin-glycosidase-catalyzed dioscin hydrolysis were evaluated, and the results are shown in Fig. 7.

Fig. 7A shows that the optimal pH was 5.0, are Fig. 7B shows that the optimal temperature was 40°C, and Fig. 7C shows that the optimal reaction time was 30 h.

The effects of various metal ions on the new dioscin-glycosidase were also investigated and are listed in Table 2.

It appeared that the activity of the new dioscin-glycosidase was not affected by Na$^+$, K$^+$, and Mg^{2+} ions as high as 50 mM, but was significantly inhibited by Cu^{2+} and Hg^{2+} ions at 10 mM, and slightly affected by Ca^{2+} ions at 10 mM.

Fig. 5. Hydrolysis of dioscin by new dioscin-glycosidase.
1, Standard dioscin; ①, Enzyme reaction product from dioscin; 2, Standard 3-O-[α-L-(1→4)-Ara, α-L-(1→2)-Rha]-β-D-Glc-diosgenin; ②, Enzyme reaction product from 3-O-[α-L-(1→4)-Ara, α-L-(1→2)-Rha]-β-D-Glc-Diosgenin; 3, Standard 3-O-α-L-Ara-β-D-Glc-diosgenin; 4, Standard 3-O-β-D-Glc-diosgenin; 5, Standard diosgenin (aglycone); Substrate, 2.0 mg/ml and reacted at 40°C for 20 h; Solvent, chloroform:methanol:water =7:3:0.5 (underlayer).

Fig. 6. Enzyme hydrolysis products from dioscin analyzed by HPLC.
1, Dioscin; 2, 3-O-α-L-Rha-β-D-glc-diosgenin; 3, 3-O-β-D-Glc-diosgenin; 4, diosgenin (aglycone). Enzyme, 20 U/ml; Substrate, 4.0 mg/ml dioscin, reacted at 40°C for 20 h.
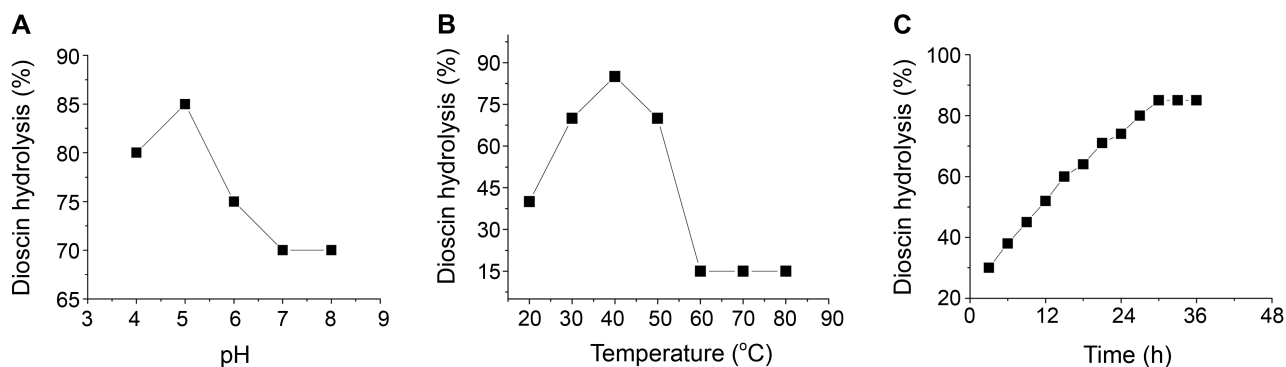
Fig. 7. Effects of pH (**A**), temperature (**B**), and reaction time (**C**) on new dioscin-glycosidase.
Enzyme, 10 U/ml; substrate, dioscin concentration 2.0 mg/ml. **A**. reacted for 24 h at 40°C; **B**. reacted for 24 h at pH 5.0; **C**. reacted at 40°C and pH 5.0.

DISCUSSION

The new dioscin-glycosidase was compared with previously reported dioscin-glycosidases, such as glucoamylase [4, 5], dioscin-α-L-rhamnosidase from pig liver [14], and dioscinase from a microorganism [9], as shown in Table 3.

The glucoamylase from *Curvularia lunata* is strictly specific: it only hydrolyzes the 3-*O*-α-L-(1→2)-rhamnoside from dioscin into 3-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-diosgenin, its molecular mass is about 66 kDa, its optimal temperature is 50°C, and its optimal pH is 4.0 [4, 5]. The dioscin-α-L-rhamnosidase from pig liver displays a wider specificity spectrum: it not only hydrolyzes the 3-*O*-α-L-(1→2)-rhamnoside from dioscin, but also hydrolyzes the 3-*O*-α-L-(1→4)-rhamnoside from dioscin (*i.e.*, the dioscin-α-L-rhamnosidase hydrolyzes dioscin into 3-*O*-β-D-glucosyl-diosgenin), and the molecular mass of this dioscin-α-L-rhamnosidase is about 47 kDa, its optimal temperature is 42°C, and its optimal pH is 7.0 [14]. The dioscinase from the microorganism sp.s00c strain hydrolyzes 3-*O*-α-L-(1→2)-Rha*p* and 3-*O*-α-L-(1→4)-Rha*p* from dioscin to produce 3-*O*-β-D-glucopyranosyl-diosgenin, and it also hydrolyzes 3-*O*-α-L-(1→2)-Rha*p* and 3-*O*-β-L-(1→3)-Glc*p* from 3-*O*-α-L-(1→2)-Rha*p*, [3-*O*-β-L-(1→3)]-β-Glc*p*-diosgenin to produce 3-*O*-β-D-glucopyranosyl-diosgenin, and the molecular mass of this dioscinase is about 59 kDa, its optimal temperature is 40°C, and its optimal pH is 5.0 [9]. In contrast, the new dioscin-glycosidase from the *Absidia* sp.d38 strain gradually hydrolyzes either 3-*O*-α-L-(1→4)-Rha or 3-*O*-α-L-(1→2)-Rha from dioscin into 3-

O-α-L-Rha-β-D-Glc-diosgenin, further hydrolyzes the other α-L-Rha from 3-*O*-α-L-Rha-β-D-Glc-diosgenin into the main intermediate products of 3-*O*-β-D-Glc-diosgenin, and subsequently hydrolyzes these intermediate products into aglycone as the final product. The new dioscin-glycosidase also gradually hydrolyzes 3-*O*-(1→2)-α-L-rhamnoside, 3-*O*-(1→4)-α-L-arabinoside, and β-D-glucoside on diosgenin. The molecular mass of the enzyme is about 55 kDa. When using dioscin as the substrate, the enzyme reaches its highest transformation rate at pH 5.0, a temperature of 40°C, and reaction time of 30 h. Thus, the reaction pathway catalyzed by this enzyme differs from those ascertained for previously identified enzymes with dioscin-hydrolyzing activity, implying potentially new applications for the biotransformation industry.

The new dioscin-glycosidase isolated from the *Absidia* sp.d38 strain hydrolyzes multi-glycosides, such as α-L-(1→2)-rhamnoside, α-L-(1→4)-rhamnoside, α-L-(1→4)-arabinoside, and β-D-glucoside on diosgenin, such as dioscin, into aglycone (diosgenin) as the final product. The reaction characteristics of the new dioscin-glycosidase are similar to those of another saponin-glycosidase, Ginsenosidase type I, which hydrolyzes multi-glycosides of ginsenoside Rb1, Rb2, and Rc [18]. Like Ginsenosidase type I, the multi-glycoside nature of the new dioscin-glycosidase significantly differs from what is considered the norm for glycosidases, as described in Enzyme Nomenclature by NC-IUBMB (Nomenclature Committee of the International Union Biochemistry and Molecular Biology, described in http://www.qmul.ac.uk/iubmb/enzyme): one enzyme hydrolyzes

Table 2. Effects of metal ions on new dioscin-glycosidase (relative activity, %).

Metal ions	None	Na$^+$		K$^+$		Ca^{2+}		Mg^{2+}		Cu^{2+}	Hg^{2+}
Concentration (mM)	0	10	50	10	50	10	50	10	50	10	10
Enzyme activity (%)	100	99	102	103	100	107	67	108	110	13	4

Enzyme, 10 U/ml; substrate, dioscin concentration 2.0 mg/ml; reacted at 40°C and pH 5.0 for 20 h.

Table 3. Comparison of dioscin-glycosidases.

Enzyme	Enzyme source	M (kDa)	Hydrolyzing glycoside type of dioscin	Final enzyme products	Optimal temperature (°C)	Optimal pH
Dioscin-glycosidase in this paper	*Absidia* sp.d38 stain	55	α-L-(1→2)-Rha α-L-(1→4)-Rha β-D-Glu	Aglycone	40	5.0
Glucoamylase[a]	*Curvularia lunata* strain	66	α-L-(1→2)-Rha	3-O-Rha-Glc-diosgenin	50	4.0
Dioscin-α-L-rhamnosidase[b]	Pig liver	47	α-L-(1→2)-Rha α-L-(1→4)-Rha	3-O-Glc-diosgenin	42	7.0
Dioscinase[c]	Microorganism sp.s00c strain	59	α-L-(1→2)-Rha α-L-(1→4)-Rha β-D-(1→3)-Glc	3-O-Glc-diosgenin	40	5.0

[a], [4, 5]; [b], [11]; [c], [8].

one type of glycoside. Thus, further study of the new dioscin-glycosidase will undoubtedly bring new insights to the understanding of glycosidases.

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