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ORIGINAL PAPER

Hydrolysis of soy isoflavone glycosides by recombinant β-glucosidase from hyperthermophile *Thermotoga maritima*

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Abstract A recombinant *Thermotoga maritima* β-glucosidase A (BglA) was purified to homogeneity for performing enzymatic hydrolysis of isoflavone glycosides from soy flour. The kinetic properties K_m, k_{cat}, and k_{cat}/K_m of BglA towards isoflavone glycosides, determined using high-performance liquid chromatography, confirmed the higher efficiency of BglA in hydrolyzing malonylglycosides than non-conjugated glycosides (daidzin and genistin). During hydrolysis of soy flour by BglA at 80°C, the isoflavone glycosides (soluble form) were extracted from soy flour (solid state) into the solution (liquid state) in thermal condition and converted to their aglycones (insoluble form), which mostly existed in the pellet to be separated from BglA in the reaction solution. The enzymatic hydrolysis in one-step and two-step approaches yielded 0.38 and 0.35 mg genistein and daidzein per gram of soy flour, respectively. The optimum conditions for conversion of isoflavone aglycones were 100 U per gram of soy flour, substrate concentration 25% (w/v), and incubation time 3 h for 80°C.

Keywords Isoflavones · Hyperthermophilic β-glucosidase · *Thermotoga maritima* · Soy

Introduction

Soy isoflavones, the structural homolog of human estrogen, are abundantly found in soy in aglycone forms (daidzein, genistein, and glycitein) and in their glycoside forms: non-conjugated glycosides (daidzin, genistin, and glycitin), acetylglycosides (acetyldaidzin, acetylgenistin, and acetylglycitin), and malonylglycosides (malonyldaidzin, malonylgenistin, and malonyl glycitin) (Fig. 1). Many researchers have revealed that the biological effects of isoflavones are not due to the glycoside form, but mainly to their aglycones, such as daidzein and genistein [1–3]. Isoflavone aglycones, which are hydrolysates of isoflavone glycosides by β-glucosidase (β-D-glucoside glucohydrolase; EC 3.2.1.21), are highly bioactive due to their unimpeded intestinal absorption [4, 5]. Thus, there is more interest in increasing the amounts of isoflavone aglycones in soy products and converting isoflavone glycosides to their aglycones.

Soy cake is an important byproduct produced during soy oil processing and has been shown to be a rich source of isoflavone [6]. Interestingly, the formation of isoflavone aglycone should be associated with the enzymatic hydrolysis of isoflavone glycosides from soy cake in which it is possible to find large amounts of isoflavones to convert it into pharmacologically active compounds [7, 8]. The glycosidases (*O*-glycoside hydrolase, EC 3.2.1.x) are a widespread group of enzymes with significant biochemical, biomedical, and industrial importance that catalyze the hydrolysis of glycosidic bonds in oligo- and polysaccharides. Because of the exceptional number of possible combinations between carbohydrates, there are a large number of glycosidases of varying substrate specificity [9]. A β-glycosidase with a remarkable β-glucosidase activity and wide substrate specificity was purified and characterized from the hyperthermophile *Thermotoga maritima* [10]. The gene coding for this enzyme in our laboratory was overexpressed in *Escherichia coli*. In this paper, we describe the isoflavone glycosides conversion for their aglycones production from a commercially available soy cake or soy

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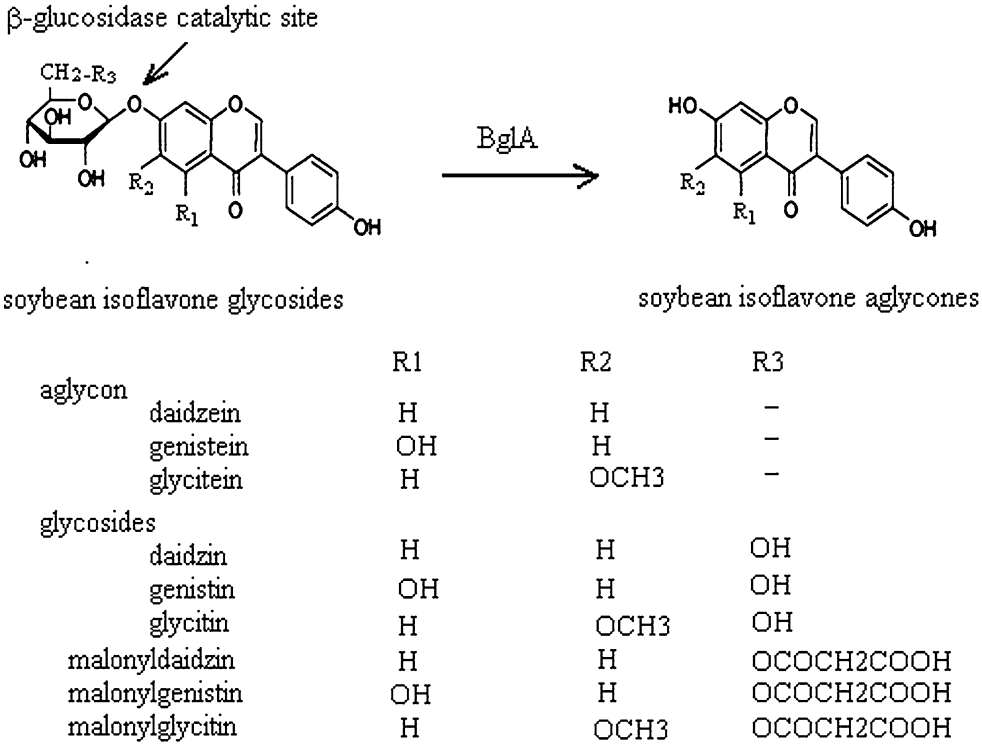

Fig. 1 Structures of soy isoflavonoid glycoside natural substrates of the recombinant *T. maritima* β-glucosidase A

flour by using the recombinant *T. maritima* β-glucosidase A (BglA) and evaluate the potential of BglA for production of isoflavone aglycones by comparing their ability to hydrolyze isoflavonoid glycosides in soy extracts and soy flour suspensions.

Materials and methods

Materials

The *p*-nitrophenyl (*p*NP) substrate *p*NP-β-D-glucopyranoside (*p*NPG) was purchased from Sigma Chemical Co. (St. Louis, MO). High-performance liquid chromatography (HPLC) grade methanol and acetonitrile were purchased from Fisher Scientific (Hanover Park, IL). Isoflavone standards of daidzin, daidzein, genistin, and genistein were purchased from Sigma Chemical Co. (St. Louis, MO); malonylgenistin and malonyl daidzin were purchased from Wako Chemical Co. (Wako Pure Chemical Industries, Ltd., Japan). *T. maritima* (ATCC43589) was grown anaerobically at 80°C in modified Luria-Bertani (LB) medium [11]. *Escherichia coli* JM109 (DE3) (Promega, Madison, WI) and BL21-CodonPlus(DE3)-RIL (Novagen, USA) were used as hosts for the expression of β-glucosidase A gene from *T. maritima* via the T_7 RNA polymerase expression system with pET-20b plasmids (Novagen). Cells were cultured at 37°C in Luria-Bertani (LB) supplemented with 100 μg ampicillin ml^{-1} or 60 μg chloromycetin ml^{-1}.

Preparation of the recombinant β-glucosidase

Based on the DNA sequence of the *bglA* gene (Genbank entry X74163) of *T. maritima*, two PCR primers were designed and synthesized to allow in-frame cloning in the vector pET-20b. The N-terminal primer (5′-CCCCATAT GAACGTGAAAAAGTTCC-3′) included an ATG translational start codon inside the *Nde*I restriction site (CATATG). The C-terminal primer (5′-CCC TCTAGA TCAGTCTTCCAGACCGT-3′) included an *Xho* I restriction site (TCTAGA) and stop codon (TCA). A PCR amplification of 35 cycles with *Pyrobest* DNA polymerase (TaKaRa, China) was carried out in a 50-μl reaction containing 0.2 mM dNTPs each, 20–35 pmol each of the primers, and 0.5 μg chromosomal DNA template. Amplicons were ligated into pET-20b at restriction sites *Nde*I and *Xho* I, resulting in recombinant plasmid pET-20b-bglA. *E. coli* BL21-Codonplus (DE3)-RIL was used as host for the expression of this recombinant plasmid.

Recombinant strain was grown in LB with 30 μg kanamycin ml^{-1} and 50 μg chloromycin ml^{-1} at 37°C to OD_{600} 0.8 and incubated further with IPTG (25 μmol l^{-1}) for 6 h. The cells were harvested by centrifugation, resuspended in 0.1 mM pH 5.0 sodium phosphate buffer, and sonicated for 45 s three times. The overexpressed recombinant protein was purified in three steps, heat treatment, DEAE Sepharose anion-exchange chromatography and TOYOPEARL HW-55F. The cell extracts were heat-treated (75°C, 20 min), then cooled in an ice bath, and centrifuged

(9,600×*g*, 4°C, 30 min). The resulting supernatant was loaded onto a DEAE-Sepharose Fast Flow column (1.5 × 25 cm) previously equilibrated with 50 mM sodium phosphate buffer. The column was eluted with a linear gradient of NaCl from 0 to 0.4 M. Active fractions that eluted were dialyzed against 50 mM sodium phosphate buffer and concentrated by ultrafiltration with an Amicon YM-35 membrane. Then these fractions were applied to a TOYOPEARL HW-55F (2.5 × 60 cm) equilibrated with 25 mM sodium phosphate buffer containing 0.15 mM NaCl. The column was eluted with the same buffer, and fractions were collected and screened for enzyme activity. The fractions that exhibited enzyme activity were dialyzed overnight, concentrated by ultrafiltration with an Amicon YM-35 membrane, and lyophilized. The fraction was used as the purified enzyme for subsequent analysis.

Protein determination and enzyme assay

Protein concentrations were determined by the Bradford method [12] using bovine serum albumin as a standard. Enzyme activity was quantified by *p*-nitrophenol (*p*NP) release from *p*-nitrophenyl *β*-D-glucoside (*p*NPG). The reaction volume was 200 µl, initiated by adding appropriate diluted recombinant *β*-glucosidase into 100 mM pH 5.0 potassium phosphate buffer (PPB) containing 20 µl 20 mM *p*NPG at 70°C. The reaction was stopped by the addition of 600 µl of 1 M Na_2CO_3 after hydrolyzing 5 min; the amount of *p*NP released was measured at 410 nm against a blank. All reactions were done in triplicate. A standard curve was prepared using *p*NP. One unit was defined as the amount of enzyme producing 1 µmol *p*NP per min at 2 mM *p*NPG and 70°C in 0.1 M PPB, pH 5.0.

HPLC analysis

HPLC analysis was used to measure the amounts of daidzein, genistein, daidzin, genistin, malonylgenistin, and malonyldaidzin to quantify changes in isoflavone content of crude extracts treated with BglA. Separation and quantification of isoflavonoids were achieved with an Agilent HC-C 18 (4.6 × 250 mm, 5 µm) reverse phase column on an HP series 1100 HPLC (Agilent Corp., Palo Alto, CA) with the UV detector set at a wavelength of 260 nm in a manner similar to that Chuankayan et al. described for the separation of soy isoflavones [7, 8]. A linear HPLC gradient was employed. Solvent A was 0.1% phosphoric acid in water, and solvent B was acetonitrile. Following the injection of 20 µl of the sample, solvent B was increased from 10 to 35% over 45 min. The solvent flow rate was 0.8 ml min^{-1}.

Peaks of soy isoflavone glucosides (genistin, daidzin, malonylgenistin, and malonyldaidzin) and aglycones (genistein and daidzein) were identified by matching retention times with isoflavonoid standards. Relative amounts were calculated from relative peak areas, because all peaks were well within the linear range of the instrument.

Kinetic studies

The kinetic properties of BglA toward soy isoflavone glucosides were determined by incubating genistin, malonylgenistin, daidzin, and malonyldaidzin at five concentrations in the range of 0.045–1.9 mM, depending on the K_m seen in preliminary experiments and whether substrate inhibition was seen at higher concentrations. The standard reaction mixture (final volume, 50 µl) contained 5% DMSO in 100 mM pH 5.0 PPB with 0.054 µg of BglA at 70°C. The mixture without enzyme was preincubated at 70°C for 5 min. After 5 min, the reactions were stopped by ice water cooling according to Briante et al. [13], and freeze-dried and re-suspended in 50 µl methanol for HPLC analysis. The aglycone product of each soy isoflavonoid glycoside substrate was detected by measuring the absorbance at 260 nm. The amounts of liberated aglycones were calculated from standard curves of aglycone (genistein and daidzein) peak areas over the 0.018–3.7 mmol range. All reactions were done in duplicates, and the relative standard deviation was <10%. The K_m value and maximum reaction velocity (V_{max}) soy isoflavone glucosides were determined by the method of Lineweaver–Burk plots.

Hydrolysis of crude soy flour extract

Soys were purchased from the local market in Nanjing, China, and ground into powder and stored at −20°C for use. Firstly, soy flours (SF) were extracted with three volumes of *n*-hexane by stirring for 30 min at room temperature for defatting, then precipitated by centrifugation at 9,600×*g* for 20 min and air-dried. To compare the hydrolysis efficiencies of BglA toward the isoflavonoid glycosides in crude soy flour extract, 3 g of SF was extracted with six volumes of 80% methanol by stirring 2 h at 80°C and removing the extract supernatant by centrifugation, concentrated by rotary evaporator, dried by speed vacuum and hydrolyzed with 150 U BglA in 12 ml of 0.1 mM PPB (pH 5.0) at 80°C for 2 h. The reaction was stopped by ice water cooling [13]. The stopped reactions were dried by speed vacuum and resuspended in 1 ml methanol. A control reaction of crude extract without enzyme was set up in the same manner.

Hydrolysis of soy flour suspension

Twenty-five percent (w/v) of SF was incubated with the BglA in 0.1 M PPB at pH 5.0 in a thermostatically controlled incubator at 80°C. The enzyme amounts for BglA

were 300 U per 3 g of SF. The reactions were stopped by cooling at −20°C before analysis, and centrifuged at 17,418×*g* and 4°C for 5 min to remove the supernatant. The pellet was extracted 2 h by the addition of six volumes of 80% ethanol at 80°C and ethanol extract removed by centrifugation at 17,418×*g* for 5 min. Reaction supernatant and ethanol extract were dried by speed vacuum and resuspended in 1 ml methanol for HPLC analysis. A control reaction of crude extract without enzyme at room temperature or at 80°C was set up in the same manner. Each assay was done in duplicate, and 20 µl of each sample was injected for HPLC analysis.

Two-approach setup for the production of isoflavone aglycones

For the two-step approach (Fig. 2a), firstly, total soy isoflavones were extracted from SF, and then BglA was used to hydrolyze these soy isoflavones. For the one-step approach (Fig. 2b), the SF was directly hydrolyzed using BglA, and then extracted total soy isoflavones. The incubator in both approaches was thermostated at 80°C. The percent conversion and yield of enzymatic hydrolyze were calculated by the following equations, percent conversion = $R/Q \times 100\%$, yield = G/F (mg/g); R, the increased amount of certain aglycone; Q, the initial amount of corresponding soy isoflavone glucosides and their aglycones before hydrolyzing; G, the amount of certain aglycone; F, the amount of SF been processed.

Results

Enzyme production and purification

The recombinant *T. maritima* BglA, expressed in *E.coli* BL21-Codonplus (DE3)-RIL, made up about 13% of the total soluble proteins on the basis of density scanning of the SDS-PAGE gel (Fig. 3, lane 2). The expressed BglA was purified to homogeneity by column chromatography using DEAE Sepharose anion-exchange and TOYOPEARL HW-55F after heat treatment (Fig. 3, lane 5). The purity of the enzyme was established by SDS-PAGE analysis, which produced a single band with a molecular mass of 47 kDa. The data on the purification are summarized in Table 1. The enzyme was purified 24.4-fold to a specific activity of 1,632 U mg^{-1} protein from the cell with a yield of 12.3%.

Kinetic properties

The kinetic parameters of the purified BglA for daidzin, genistin, malonylgenistin, and malonyldaidzin were determined using HPLC to quantify the amounts of the reaction products. All the determined kinetic parameters are summarized in Table 2. Both k_{cat} and K_m values for malonylgenistin were 99.2 ± 3.5 s^{-1} and 0.97 ± 0.08 mM, respectively, and those for genistin were 348.3 ± 11 s^{-1} and 4.22 ± 0.13 mM, respectively. While the β-D-glucosides of daidzein acted as substrates, their k_{cat} and K_m values for malonyldaidzin were 8.4 ± 0.23 s^{-1} and 0.091 ± 0.008 mM,

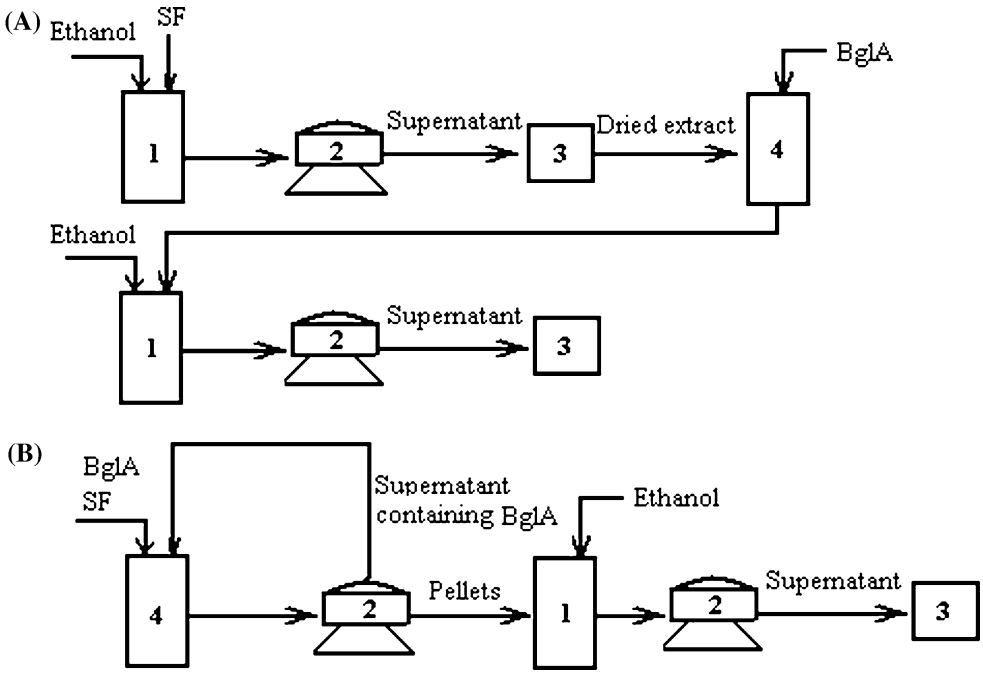

Fig. 2 Two enzymatic methods setup: **a** two-step approach; **b** one-step approach (*1* extraction, *2* centrifugation, *3* rotary evaporator, *4* thermostatically controlled incubator)

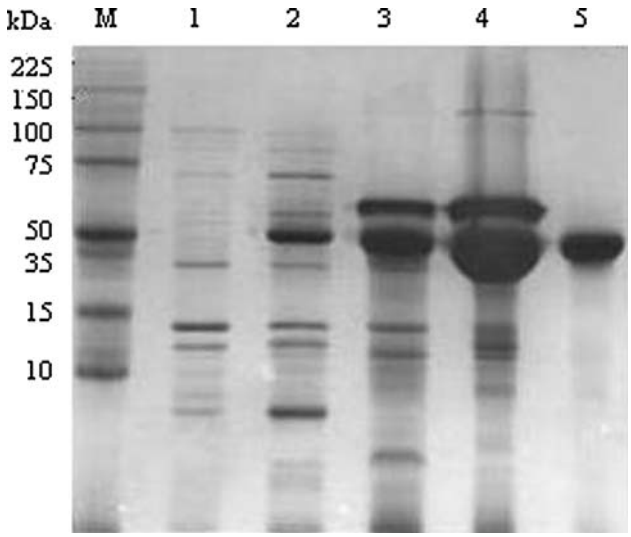

Fig. 3 SDS-PAGE analysis of purification procedure process of the recombinant BglA. Protein samples were separated in 12% SDS-polyacrylamide gels and stained with Coomassie blue. Lane M, low molecular weight marker; *lane 1 E. coli* BL21-Codonplus (DE3)-RIL containing the plasmid pET-20b, *lane 2* crude extract of *E. coli* BL21-Codonplus (DE3)-RIL containing pET-20b-bglA, *lane 3* fraction after heat treatment of *E. coli* BL21-Codonplus (DE3)-RIL containing pET-20b-bglA, *lane 4* fraction after DEAE-Sepharose chromatography, *lane 5* purified BglA after TOYOPEARL HW-55F column

respectively, and those for daidzin were $124.8 \pm 11\ s^{-1}$ and 2.08 ± 0.064 mM, respectively. Both K_m and k_{cat} values of BglA towards malonylgenistin and malonyldaidzin were lower than genistin and daidzin, respectively. The calculated specificity constants (k_{cat}/K_m) for malonylgenistin and malonyldaidzin were higher than the values for genistin and daidzin, respectively.

Enzymatic hydrolysis of soy flour extract and soy flour suspension

To evaluate the usefulness of BglA for the hydrolysis of soy isoflavone glycosides in soy flour extract and soy flour suspension, the methanol extract from SF was separated and analyzed by HPLC. The peaks of daidzin, genistin, malonylgenistin, malonyldaidzin, daidzein, and genistein were identified in SF by comparison with commercial standards. Soy isoflavone glucosides (daidzin, genistin, malonylgenistin, malonyldaidzin) were the most predominant isoflavonoids in the soy flour extract, whereas the peaks for their aglycones (daidzein and genistein) were very small. When hydrolyzed soy flour extract by adding BglA at 80°C, almost all soy isoflavone glycosides were converted to aglycones (data not show).

Subsequently, the effects of thermal condition (heating temperature of 80°C) on aqueous extraction of soy isoflavones from SF are shown in Table 3a. The amounts of isoflavone glycosides in the aqueous solution were higher at 80°C than at room temperature. Thermal treatment resulted in increases of non-conjugated glycosides (2.01-fold daidzin and 1.81-fold genistin) higher than those of malonylglycosides (0.61-fold malonyldaidzin and 0.88-fold malonylgenistin). When hydrolyzing SF by adding BglA compared with no BglA at 80°C, the enzymatic hydrolysis

Table 1 Purification of the recombinant BglA

Purification step	Total protein (mg)	Total activity (U)	Sp activity (U mg^{-1})[a]	Purification (fold)	Recovery (%)
Crude extract	169	11,289	66.8	1.0	100
Heat treatment	27	6,446	238.7	3.6	57.1
DEAE-Sepharose	4.8	3,590	747.9	11.2	31.8
Gel filtration	0.85	1,387	1,632	24.4	12.3

As described in "Materials and methods," the cells from recombinant strain BL21-Codonplus (DE3)-RIL containing pET-20b-bglA were harvested by centrifugation and disrupted with a French press three times. The cell extracts were heat-treated and centrifuged. The resulting supernatant was loaded onto a DEAE-Sepharose Fast Flow column. Active fractions that eluted were dialyzed against 50 mM sodium phosphate buffer and concentrated by ultrafiltration with an Amicon YM-35 membrane. Then these fractions were applied to a TOYOPEARL HW-55F. The fractions that exhibited enzyme activity were dialyzed overnight, concentrated by ultrafiltration with an Amicon YM-35 membrane, and lyophilized

[a] Activity was determined with *p*-nitrophenyl *β*-D-glucopyranoside as substrate (1 mM) in 50 mM SPB (pH 6.2) at 90°C

Table 2 Kinetic properties of the recombinant *T. maritima β*-glucosidase A toward soybean isoflavones

Substrate	K_m (mM)	V_{max} (U mg^{-1})	k_{cat} (s^{-1})	k_{cat}/K_m ($M^{-1}s^{-1}$)
Genistin	4.22 ± 0.13	444.7 ± 14	348.3 ± 11	8.26×10^4
Malonylgenistin	0.97 ± 0.08	126.7 ± 4.5	99.2 ± 3.5	1.03×10^5
Daidzin	2.08 ± 0.064	159.4 ± 14	124.8 ± 11	6.03×10^4
Malonyldaidzin	0.091 ± 0.008	10.73 ± 0.29	8.4 ± 0.23	9.31×10^4

Reactions were done in the standard reaction at 70°C in 0.1 mM SPB (pH 5.0) and stopped at 5 min. The isoflavone aglycones were quantified by HPLC, as described in "Materials and methods," and the rates of product formation were calculated

Table 3 Relative levels of soy glycosides and aglycones before and after soy flour with thermal condition (A) as well as with BglA (B)

Glycoside or aglycone	(A) Relative amount of isoflavone after thermal condition[a]						(B) Relative amount of isoflavone after reaction[b]	
	0 h control		25°C/3 h		80°C/3 h		80°C/3 h with BglA	
	Supernatant	Pellet	Supernatant	Pellet	Supernatant	Pellet	Supernatant	Pellet
Daidzin	100	100	117	107	318	88	1.7	0.8
Malonyldaidzin	100	100	157	109	218	78	0.5	1.9
Genistin	100	100	120	126	301	101	1.2	0.9
Malonylgenistin	100	100	176	111	254	85	0.2	1.2
Daidzein	100	100	108	126	122	367	162	347
Genistein	100	100	142	192	173	263	126	473

Soy flours were incubated in 0.1 M PPB (pH 5.0) at 25 or 80°C for 3 h, respectively, and relative amounts were determined by HPLC, as described in "Materials and methods"

[a] The amounts in 0 time were set at 100%, and relative levels (relative HPLC peak areas) at other time and temperature are reported

[b] The amounts without BglA at 80°C for 3 h were set at 100%, and relative levels (relative HPLC peak areas) with BglA are reported

resulted in increases of 1.62-fold in daidzein and 1.26-fold in genistein in the solution, and in 3.47- and 4.73-fold increases of daidzein and genistein in the pellets, respectively (Table 3b), which hydrolyzed up to 98–99% of isoflavonoid glycosides in the solution and pellets, where all the isoflavonoid glycosides were almost converted to isoflavone aglycones by BglA. The amounts of aglycone product in the pellets were much more than those in the solution.

Application in production of isoflavone aglycones from SF

For the preparation of isoflavone aglycones from SF, we have followed two different approaches, the one-step and two-step approaches (Fig. 2), in order to establish the better strategy for the substrate hydrolysis. The percent conversion and yield of each aglycone for the hydrolysis of soy flour in the one-step and two-step approaches were compared, which approximated to conversion of 94 and 86%, respectively, yielding 0.38 and 0.35 mg genistein and daidzein per gram of SF. In both the one-step and two-step approaches, the amounts of aglycone product were almost the same. Therefore, the one-step approach was chosen to prepare isoflavone aglycones; its biotransformation conditions were modulated for increasing the isoflavone aglycone yield as shown in Fig. 4. A good yield of isoflavone aglycones for SF hydrolyzed by BglA was obtained when treated with enzyme amounts of 100 U per gram of SF at 80°C for 3 h using 25% (w/v) of soy flour in aqueous buffer (see Fig. 4a, b, c).

Discussions

To develop powerful enzyme tools for the production of soy isoflavone aglycones from soy cake, hyperthermophilic enzymes such as *T. maritima* β-glucosidase (BglA) were targeted, which exhibited hyperthermostability and a broad pH profile. In this paper, the recombinant BglA producing catalytically active forms was expressed in *E. coli* and purified to homogeneity. The kinetic properties of BglA towards soy isoflavone glycosides were determined using HPLC. Although both the K_m and k_{cat} values of BglA towards malonylgenistin and malonyldaidzin were lower than those of genistin and daidzin, respectively, the calculated specificity constants (k_{cat}/K_m) for malonylgenistin and malonyldaidzin were higher than the values for genistin and daidzin, respectively. The results showed that BglA efficiently hydrolyzed non-conjugated glycosides (daidzin and genistin) and conjugated glycosides (malonyldaidzin and malonylgenistin). However, the BglA was much less specific for isoflavone glycosides than for β-glucosidase from the roots of soy, which is one of the highest substrate specificities of β-glucosidases described to date [14]. Further work to increase substrate specificity on isoflavone glycosides of *T. matitima* β-glucosidase is required.

To evaluate the usefulness of heating temperature and hyperthermophilic BglA for the hydrolysis of isoflavone glycosides in SF, the relative levels of soy glycosides and aglycones after incubation of SF with or without BglA were investigated. Although recombinant BglA exhibited optimal activities and high stabilities at 90°C, 80°C of high temperature was chosen to study the hydrolysis reaction in order to prevent isoflavone glycosides from interconversion and loss [15]. The experimental results revealed that thermal condition helped the extraction of isoflavone glycosides (soluble form) from SF (solid state) into the aqueous solution. BglA was able to convert almost all the isoflavonoid glycosides to their aglycones (insoluble form) in a short time, where large amounts of isoflavone aglycones existed in the pellet to be separated from the BglA protein in the solution, indicating the utilization of the BglA

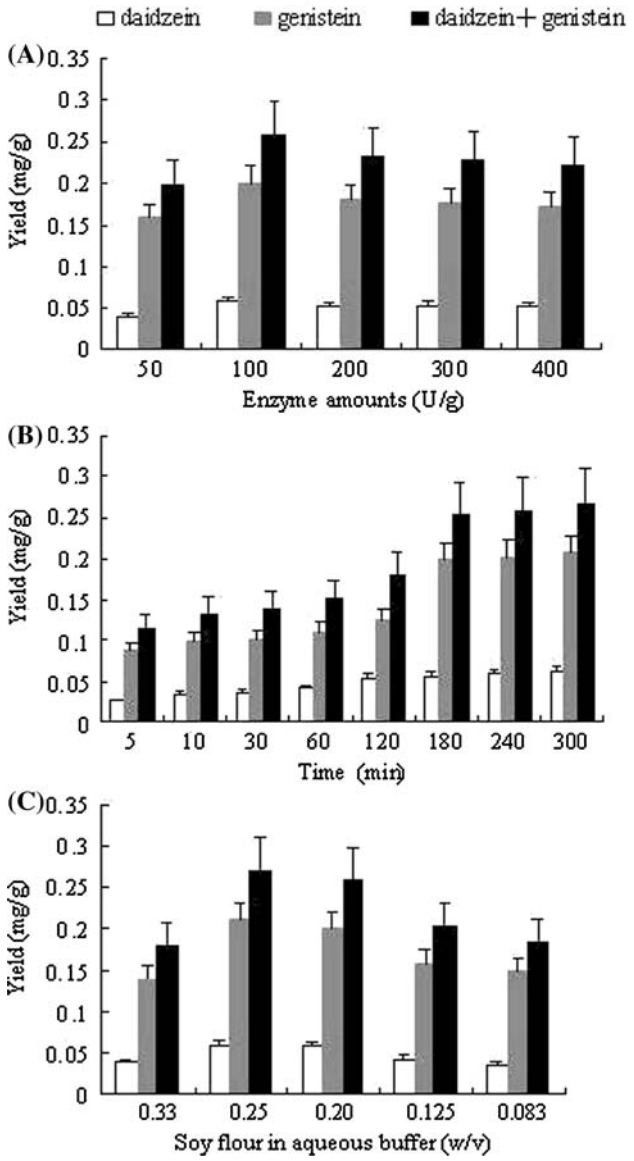

Fig. 4 The effect of biotransformation conditions on the yield of soy isoflavone aglycones by BglA. **a** Effect enzyme amount on yield of isoflavone aglycones at 80°C for 3 h using 25% (w/v) of soy flour; **b** effect of different hydrolysis time on yield of isoflavone aglycones with 100 U BglA per gram of SF at 80°C using 25% (w/v) of soy flour; **c** effect of SF concentration on yield of isoflavone aglycones with the same enzyme amounts at 80°C for 3 h

remained in the reaction supernatant by a recycling approach. Our report is in accord with previous studies on isoflavone aglycone production by display of β-glucosidase from the yeast cell surface. The results showed that a precipitate, which corresponded to be isoflavone aglycone, was observed in the reaction mixtures containing yeast cells due to the insolubility of isoflavone aglycones [1].

In order to examine the application of industrial isoflavone aglycone production using BglA, the hydrolysis experiments were performed in one-step and two-step approaches. The process of enzymatic soy flour extraction was complex and expensive as it needed two steps: firstly, total soy isoflavones in the soy flour were extracted and then enzyme was used to hydrolyze these soy isoflavones [16]. Thus, it is not promising for industrial applications. The BglA in the one-step approach was proved to hydrolyze isoflavone glycosides in SF efficiently. The main product of enzyme hydrolysis, isoflavone aglycones, is known to have bioavailability and health benefits, such as prevention of many cancers [17, 18], lowering the risk of cardiovascular diseases, alleviation of osteoporosis and menopause syndrome, and improvement of bone health [19, 20]. Therefore, the hyperthermophilic *T. maritima* β-glucosidase A constitutes a powerful tool for soy product industry applications. Furthermore, the simple method in which the enzyme hydrolyzed SF by BglA in thermal conditions has a considerable potential application in food and medicine production.

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