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Purification of ginsenoside glycosidase-BglPm using ITC of Elastin like protein-tagged system

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ABSTRACT

Rare ginsenosides are known to have many medicinal values such as anti-tumor and anti-oxidant activity. Rare ginsenosides can be obtained by hydrolyzing saponin glycosides in ginseng with ginsenoside glycosidase. Glycosidases with high purity and activity are necessary to increase the efficiency of saponin conversion. A good example of effective purification is to utilize the elastin-like polypeptide (ELP) system. This purification method has the advantages of high purity, low cost and simple operation. In this study, we optimized the purification conditions of BglPm-ELP by the inverse transition cycle (ITC) method. In addition, we also investigated the enzymatic characteristics of the enzyme.

Keywords: Elastin-type polypeptides, Glycosidases, Ginsenoside, Protein purification

1. INTRODUCTION

Panax ginseng is an important medicinal plant; it has been known as the most efficacious herb from ancient times due to its medicinal properties. It can be used as tonic for immunopotentiation, excitation of the central nervous system, enhancement of hematopoiesis and stimulation of digestion (Leung & Wong, 2010). It contains a great number of components. Examples are saponins, flavonoids, alkaloids, amino acids, polysaccharides, polypeptides and essential oil.

The main bioactive compounds are ginsenoside. It exerts various pharmacological activities such as anti-inflammatory, anti-tumor, cardiovascular protection and anti-aging (Christensen et al., 2006; Wang et al., 2020). To date, more than 180 ginsenosides were discovered from ginseng species. According to their structural characteristics, ginsenosides can be divided into two major groups: dammarane-type tetracyclic triterpene saponins and oleanane-type pentacyclic triterpene saponins. Dammarane-type triterpene saponins are divided into protopanaxadiol (PPD) and protopanaxatriol (PPT) type saponins according to the position of glycosylation. Ginsenosides compose of 62% of PPD, 21% of PPT and 17% of oleanane-type saponins (Shi et al., 2019). Depending on the degree and the type of glycosylation at the C-3, C-6, and C-20 positions of the dammarane skeleton, different kinds of saponins with various structures and pharmacological effects are formed. Saponins with high degree of glycosylation account for more than 90% of the total ginseng

saponins. On the other hand, saponins with low degree of glycosylation are known as rare ginsenoside (Kim et al., 2014; Wang et al., 2019b).

In recent years, it has been proved that rare ginsenosides have significant anti-tumor activity through various mechanisms. Although most ginsenosides have good inhibitory effects on tumor cells, the anti-tumor activity of rare ginsenosides is particularly remarkable, which exerts anti-tumor effects through mechanisms that induce apoptosis and cycle arrest of tumor cells, inhibit neoangiogenesis of tumors, and enhance the immunomodulatory ability of cells (Zhu et al., 2023). Rare ginsenoside F2 has anti-tumor and anti-obesity activities. The mechanisms include induction of apoptosis in neuroblastoma and down-regulation of keratins such as Krt2, Krt10 and Krt16 in gastric cancer cells (Mai et al., 2012; Shin et al., 2012).

Ginsenosides are currently extracted from roots, stems and leaves of ginseng. However, these resources are not reliable because of the long life cycle and various environmental factors. Furthermore, ginsenosides are secondary metabolites of plants, which means that it takes some years to be accumulated in plants. Due to the very low content of rare ginsenosides in plants, they are mainly made from the general ginsenosides (Wong et al., 2015; Yang et al., 2018).

Recently, many scientists are trying to synthesize rare ginsenosides efficiently by modern biotechnology. First, rare ginsenosides are synthesized by reconstitution of the biosynthetic pathway in microorganisms such as *E.coli* and *Saccharomyces cerevisiae* (Han et al., 2015; Dai et al., 2013; Dai et al., 2014; Zhao et al., 2016; Zhao et al., 2017; Wang et al., 2019a). Some microorganisms can also be used as a biocatalyst for the purposeful conversion of rare ginsenosides. For example, *Aspergillus niger sp. J7* efficiently converts ginsenoside Rb1 to produce CK through the pathway of Rb1→Rd→F2→CK (Park et al., 2010; Song et al., 2017; Cao et al., 2020). Enzymatic conversion is the method to convert saponin glycosides, which are abundant in plants, into rare ginsenosides by hydrolyzing the glycosyl groups using specific enzymes (Shin et al., 2016; Shin et al., 2019).

Glycosidases, the glycoside hydrolases (GH, EC 3.2.1), are enzymes that catalyze the hydrolysis of O-glycosides or S-glycosides. Glycosidases are classified into exo-type and endo-types depending on the catalytic sites, whether they act at the terminal or middle of the oligosaccharide/saccharide chain. General ginsenosides are converted into rare ginsenosides after the hydrolysis by glycoside hydrolases (Yoo et al. 2011; Quan et al., 2012).

Ginsenoside glycosidases are classified as I, II, III, IV, V type based on the modified positions of the glycosyl groups and the enzyme activity (Quan et al., 2012). Among them, type I glycosidases can hydrolyze the glycosyl group linked to the C-20 and C-3 positions in PPD-type ginsenoside, for example BglPm and BglMm. Type II glycosidases hydrolyze the glycosyl group linked to the C-20 position of PPD and PPT-type ginseng saponins, respectively. Type III glycosidases are classified as type III-i and type III-ii, which can convert PPD-type ginsenosides into rare ginsenosides, F2 and CK. Type IV can hydrolyze the glycosyl group linked to the C-6 position in PPT-type ginsenosides (Wang et al., 2011; Quan et al., 2013). In conclusion, the application of glycosidases is a prospective technique for the efficient conversion of general ginsenosides into rare ginsenosides. For practical applications, a simple and low-cost purification method should be developed. (Cui et al., 2013).

Although the recombinant DNA technology has been widely used in purification of enzyme, there are still challenges in the large-scale isolation and purification of these enzymes with high purity and activity (Mithieux & Weiss, 2005). Therefore, it is very important to develop an efficient purification method.

Among the various purification methods, the purification system based on the elastin-like polypeptides (ELPs) is an effective way to separate and purify the enzymes without using affinity chromatography (Urry et al., 1974). Elastin-like polypeptides (ELPs) are functional polymers which respond to stimulation of external environment through reversible phase transition. Elastin is a kind of extracellular matrix protein. It is mainly present in animal connective tissue. It is stretched when there is a stimulation and restored after removal of stimulus. Elastin peptides were artificially synthesized by genetic engineering (McPherson et al., 1992). This peptide exhibits reversible phase transitions in response to temperature changes.

In recent years, researchers have started to study about ELPs and their physicochemical properties and the polypeptide sequence. ELPs are synthetic thermosensitive polypeptides (Zhao et al., 2020). Its phase transition temperature (Tt) can be controlled by controlling the sequence composition, length and concentration of the peptide chain of ELPs (Varanko et al., 2020). Below the specific phase transition temperature, ELPs exhibit aeolotropic and hydrophilic structure, are easily soluble in aqueous solution, and exist in monomeric form. This temperature is called as low critical solubilizing temperature (LCST). In contrast, at higher temperatures than Tt, ELPs form a β-helix that exhibits dynamic and regular structure. Besides the intrinsic thermal-sensitive properties of ELPs, external factors such as salt in solution also affect solubility. The type and concentration of salt have a significant effect on the phase transition

temperature (Tt) of ELPs. For example, hydrophilic anions such as Cl^- can reduce the Tt of ELPs. (Meyer & Chilkoti, 1999; Lüdeke et al., 2022; Qin et al., 2020).

Thermal response characteristics of ELPs can be used as inverse transition cycle (ITC) (Shi et al., 2013; Shi et al., 2017; Fletcher et al., 2019). ITCs have been widely utilized in the purification of ELPs without an affinity chromatography. In this process, the ELP-tagged proteins can be purified effectively by adding soluble salts to the ELPs solution or by adjusting the appropriate temperature. Due to the stability of the ELPs fusion protein system, it has been already used for the expression and purification of fused proteins in plant expression systems such as tobacco. In addition, it has been mainly used for the purification of fused proteins expressed in *E. coli*. Some researchers reported results of fusion of interferon (IFN) with ELPs and His, respectively, showing that the efficiency of purification of ELPs-fused protein was similar to that of His-fused protein (Floss et al., 2010). It does not affect the kinetic parameters, secondary structure and activity of the enzyme, which significantly increases the stability of the enzyme (Patel et al., 2007; Zhang et al., 2010; Hassouneh et al., 2010; Hollingshead & Liu, 2020; De Haas et al., 2023).

In this study, we optimized the purification method of ginsenoside glycosidase BglPm using the properties of ELPs. We also investigated the enzymatic properties to verify the potential of ELPs. The aim of this study was to develop a new, simple and low-cost purification method of rare ginsenosides using the ITC of Elastin like protein-tagged system.

2. MATERIALS AND METHODS

2.1. Materials

E. coli Rosetta (DE3) with insertion of the recombinant expression vector pET22b-BglPm-ELPs was used in the experiments. For the investigation of ITC purification of ginsenoside glycosidase BglPm-ELPs and enzymatic characteristics, Tris-base, ginsenoside standard (Rc, Rb1, Rb2, Rd, F2, Rg3, Rh2, CK, PPD), isopropylthio-β-D-galactoside(IPTG) were provided from Beijing Suolaibao.Co.Ltd. Acrylamide, N, N'-methyl bisacrylamide, sodium dodecyl sulfate (SDS), β-mercaptoethanol, Coomassie blue R250, tetramethylenediamine (TEMED), ammonium persulfate (APS) were purchased from Sigma.co.ltd, USA and p-nitrophenyl-b-D-pyranoglucose (pNPG) was purchased from Shanghai aladine.co.ltd. Glycerin, methanol, ethanol, butanol, and glacial acetic acid were purchased from Beijinghuaxueshiji.co.ltd. Chromatographic-grade methanol and chromatographic-grade acetonitrile were purchased from Merk.co.ltd, Germany. Bradford protein-concentration assay kit was purchased from Shanghaibiyuntian.co.ltd. Sodium chloride, potassium dihydrogen phosphate, and bisodium hydrogen phosphate were purchased from Guoyaojituan.co.ltd. Protein marker was purchased from Thermo.co.ltd.

2.2. Induced expression and extraction of BglPm-ELPs

The recombinant strain was inoculated in 100ml of LB medium containing 100 μg/ml of ampicillin at the rate of 2% (v/v). Cultivation was stopped when OD reached at 0.6~0.8 after shaking at 37°C, 180 rpm for 4~5 h. 0.1-0.5 mM of IPTG was added and shaken-cultured for 16-18h at 16°C, 28°C and 37°C. After completion of fermentation, the biomass was collected by centrifugation at 12,000 rpm for 10 min at 4 °C. The 50 mM of PBS buffer solution was added until OD reached 10. This solution was homogenized in ice bath. The soluble protein and precipitate were separated by centrifugation at 10,000 rpm for 10 min.

2.3. Purification of BglPm-ELPs using ITC

After the mycelia were ultrasonically crushed, 1 mL of supernatant was dispensed into 2 mL centrifuge tubes. The tubes were placed in a thermostatic water bath at 40 °C and then incubated for 40 min. When the solution was turbid, it was centrifuged at 37°C for 10 min at 12, 000 rpm to remove the supernatant rapidly. The PBS solution was added to the centrifuge tube. Then, it was placed on ice for 40 min and then centrifuged at 12,000 rpm for 10 min at 4 °C. The supernatant obtained by centrifugation was defined as the supernatant obtained after the first ITC purification. This procedure was repeated three times. To characterize the phase transition with different kinds of salts and concentrations, the ITC phase transition purification was carried out by adding 1.5M, 2M, 2.5M and 3M of NaCl and Na_2CO_3, respectively.

2.4. Measurement of in vitro enzymatic activity of BglPm-ELPs

First, 400μL of 10% methanol was added to 1mL of total ginsenoside solution. Then, 100μL of total ginsenoside and 100μL of crude enzyme were added in 2 mL centrifuge tubes, respectively. It was reacted at 37°C for 24-48 h. After completion of the reaction, the equal amount of n-butanol was added to supernatant at room temperature. Then, it eas extracted for 1h and then centrifuged at 12,000rpm for

1 min. The supernatant was collected. This process was repeated twice. The extracted solution was evaporated at 55°C, dissolved in chromatographic-grade methanol and stirred for 1 h at room temperature. After filtration through a 0.22μm filter, UPLC analysis was performed.

2.5. Determination of the optimal reaction temperature of BglPm-ELPs

The purified BglPm-ELPs was diluted in PBS buffer solution. After mixing homogeneously, 200μL was added to 10μL of 5 mM pNPG of the reaction medium, the thermostatic reaction was carried out in 30°C, 40°C, 50°C, 60°C, 70°C, and 80°C of a water bath for 30 min, respectively. The absorbance was measured at 400nm of wavelength after the completion of the reaction.

2.6. Determination of optimal pH values of BglPm-ELPs

After appropriate dilution of the enzyme solution with buffer solution pH 4.0, 5.0, 6.0, 7.0, 8.0, and 9.0, 5mM pNPG was added to the reaction medium and reacted for 30min at the optimum reaction temperature. The relative enzyme activity was calculated at different pH.

3. RESULTS

3.1. Measurement of extracellular enzyme activity of BglPm-ELPs crude enzyme

BglPm-ELPs crude enzymes were compared with BglPm. Total ginsenosides were used as substrate. The result was described in Fig 1.

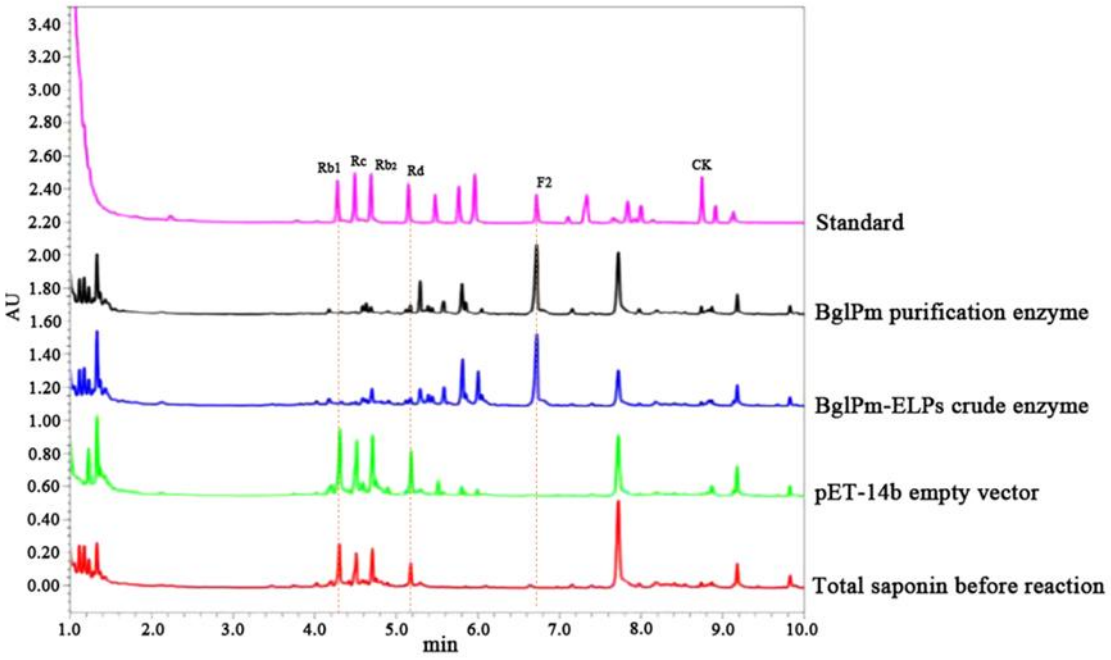

Fig 1. HPLC analysis of the enzymatic reaction products of the crude enzyme and BglPm

As shown in the figure, the ELP-tagged BglPm-ELPs showed the same activity to the non-tagged BglPm. It was found that BglPm-ELPs hydrolyzed the glucose outside at the C-3 and C-20 positions of saponins, mainly hydrolyzing Rb1 and Rd to produce F2. The results showed that the ELP marker did not affect the hydrolytic function of the glycosidase BglPm. It could be used in subsequent experiments.

3.2. Optimization of induced expression conditions of BglPm-ELPs

To increase the soluble enzyme expression of BglPm-ELPs, the induction of the expression strain was tested at three different temperatures: 16°C, 28°C, and 37°C. The results of SDS-PAGE analysis of fused proteins at different temperatures are shown in Fig 2.

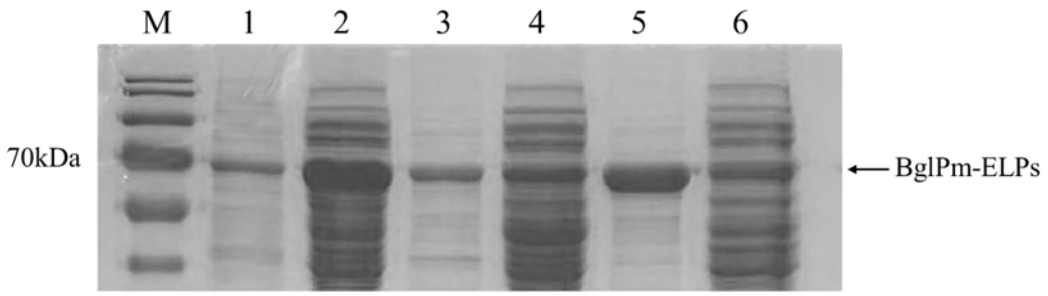

Fig 2. SDS-PAGE analysis of BglPm-ELPs induced at different temperatures.
M: marker, 1: supernatant at 37°C, 2: precipitate at 37°C, 3: supernatant at 28°C, 4: precipitate at 28°C, 5: supernatant at 16°C, 6: precipitate at 16°C

The results showed that fused protein BglPm-ELPs exhibit soluble enzymatic expression. However, the expressing amount was relatively low. It existed as inclusion bodies in the precipitate at 37°C. In contrast, the soluble expression amount of BglPm-ELPs protein was the highest at 16°C. in addition, the amount of inclusion bodies was the lowest. Thus it was confirmed that 16°C was the optimum induction temperature of BglPm-ELPs protein. To increase the soluble enzyme expression amount of the BglPm-ELPs, the expression strain was induced at different IPTG concentrations. Expression amount was examined at different IPTG concentrations of 0.1, 0.2, 0.3, 0.4, and 0.5mM. The changes in IPTG concentration did not significantly affect the soluble enzyme expression amount of the glycosidase BglPm-ELPs (Fig S1). Finally, 0.5mM IPTG was set as the optimum condition for induced expression.

3.3. Purification of ginsenoside BglPm-ELPs using ITC

To purify BglPm-ELPs using the ITC characteristics of ELPs, ITC purification was performed according to the experiment method. SDS-PAGE electrophoresis was performed on the supernatant and the precipitate of the samples collected at each step. The result was described in Fig 3. Based on previous data that NaCl can promote phase change of ELPs, ITC purification tests were carried out using 1.5M NaCl.

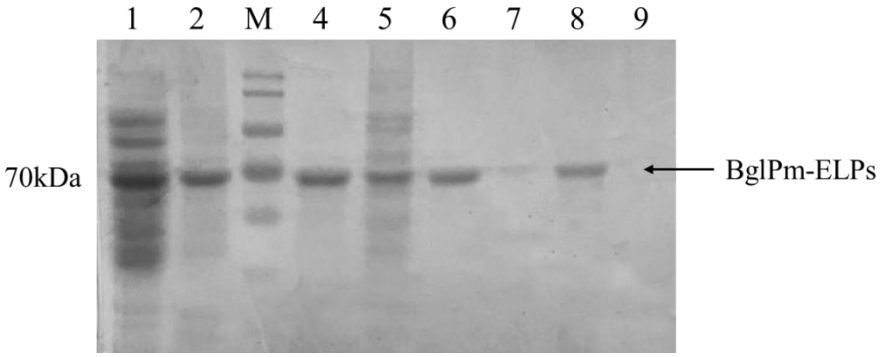

Fig 3. SDS-PAGE analysis of BglPm-ELPs after three-step ITC purification.
1: supernatant after induction of pET14b-BglMm, 2: pET14b-BglMm precipitation after induction, M: protein marker, 4: first supernatant of pET22b-BglPm-ELPs, 5: first precipitation of pET22b-BglPm-ELPs, 6: second supernatant of pET22b-BglPm-ELPs. 7: second precipitation of pET22b-BglPm-ELPs 8: third supernatant of pET22b-BglPm-ELPs, 9: third precipitation of pET22b-BglPm-ELPs.

As shown in the figure, BglPm-ELPs were not detected as non-target proteins by SDS-PAGE electrophoresis after tertiary purification. The results of evaluating total activity, total protein, specific activity, purified drainage and recovery after each ITC purification step of BglPm-ELPs are summarized in Table 1.

As shown in the table, the purification efficiency of BglPm-ELPs were 1.42 times higher and recovery was 65% compared to the crude enzyme. In order to investigate the effect of NaCl and Na_2CO_3 on purification of BglPm-ELPs, SDS-PAGE result was analyzed (Fig S2, S3). The results showed significant phase change under 1.5, 2, 2.5, and 3M NaCl concentrations during the purification of BglPm-ELPs.

Table 1. Activity and purification efficiency after step-by-step ITC purification of BglPm-ELPs

Class	Total activity (U)	Total protein (mg)	Specific activity (U/mg)	Purification times	Recovery (%)
Crude enzyme	852.47	0.85	1007.18	1.00	100%
First supernatant	697.75	0.71	985.16	0.98	82%
First precipitation	160.86	0.51	312.65	0.31	19%
Second supernatant	648.63	0.60	1087.83	1.08	76%
Second precipitation	123.48	0.32	389.23	0.39	14%
Third supernatant	553.40	0.39	1431.05	1.42	65%
Third precipitation	8.23	0.25	32.77	0.03	1%

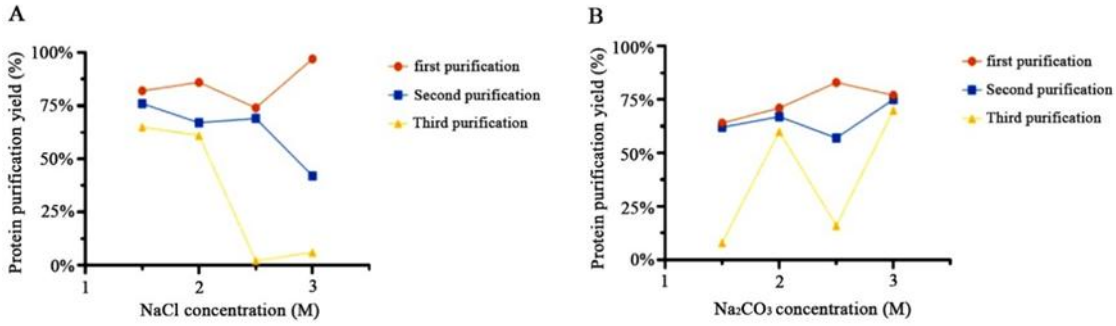

Fig 4. Protein recovery of BglPm-ELPs with different concentrations of NaCl and Na_2CO_3.

A: Protein recovery of BglPm-ELPs purified with different NaCl concentrations. B: Protein recovery of BglPm-ELPs purified with different Na_2CO_3 concentrations

During the purification process, enzyme activity and protein content in each step were measured. The purification times and recoveries were calculated (Fig 4, Table S1-S7). The ITC purification of BglPm-ELPs resulted in the highest protein recovery of 65% under 1.5M NaCl concentration, while the purity recovery and specific activity were the highest (1735.05 U/mg) at 2M. In addition, the highest protein recovery was obtained at 70% under 3 M Na_2CO_3, but the highest specific activity (1384.59U/mg) was obtained under 2M. The results showed that 2M NaCl and 2M Na_2CO_3 were the most suitable purification concentrations when BglPm-ELPs were purified.

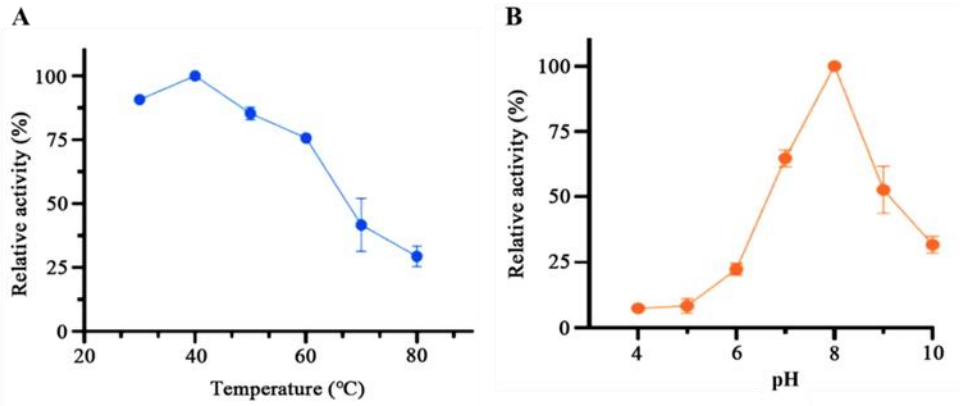

Fig 5. Optimum enzyme reaction temperature (A) and pH (B) of BglPm-ELPs.

3.4. Optimum enzyme reaction temperature and pH of BglPm-ELPs

To determine the optimum enzyme reaction temperature of BglPm-ELPs, the enzyme reaction was carried out in the range of 30-80°C with pNPG (p-nitrophenyl-b-D-glucopyranose) as a substrate, and the OD_{400} was measured to determine the optimum reaction temperature and temperature stability of the enzyme. The measurement results are shown in Fig 5A.

BglPm-ELPs showed the highest enzyme activity at 40°C, maintained more than 50% enzyme activity at 60°C, and decreased enzyme activity by about 30% when the temperature increased to 70°C. To determine the optimum pH of the BglPm-ELPs, the hydrolytic activity of BglPm-ELPs was measured at 40°C and pH 4.0-10.0, as shown in Fig 5B. BglPm-ELPs showed the highest enzyme activity at pH 8.0, after which the enzyme activity decreased rapidly with increasing pH. From the results, the optimum enzyme reaction temperature of BglPm-ELPs was set at 40°C. The optimum enzyme reaction pH was set at 8.0.

3.5. Characterization of ginsenoside hydrolysis of BglPm-ELPs purification enzyme

The purified BglPm-ELPs obtained through ITC purification were subjected to enzymatic reactions using total saponins as substrates. The results of HPLC analysis of the enzyme reaction products after 24h of reaction are shown in Fig 6. As shown in the figure, the enzymatic reaction with supernatant after three purification cycles resulted in the hydrolysis of Rb1 and Rd in ginseng total saponins to produce rare saponin F2. When 1 mg of total ginseng saponin was used as a substrate, the amount of F2 obtained was 207.767μg/mg, with a yield of 20.7%.

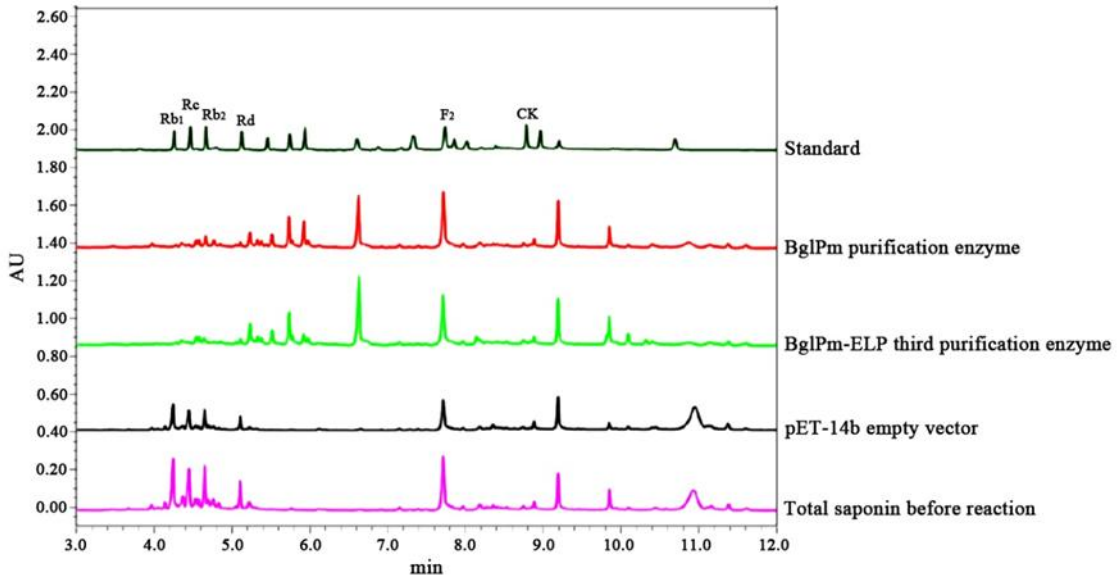

Fig 6. HPLC analysis of the enzymatic reaction products of BglPm-ELPs after three purification cycles

4. DISCUSSION

Purification of recombinant proteins by using the characteristics of ELPs is a new, low-cost and efficient method. The addition of the ELP tag to recombinant proteins does not affect the expression of the target protein. It can also effectively increase the yield. The ELP-tagged protein forms a bilayer-coated protein body after endoplasmic reticulum modification, thus avoiding normal physiological degradation. The expressed ELPs fusion protein gradually accumulates in the cells, resulting in a significant increase in the expression of the target protein. At the same time, ELPs have unique temperature responsiveness, allowing reversible phase-change separation of ELPs fusion proteins by temperature control, thus facilitating rapid and efficient purification of proteins and avoiding the need for complex affinity chromatography purification. It not only reduces the purification cost but also the degradation and inactivation of proteins.

Temperature and pH values are important factors affecting enzyme activity. Too high reaction temperature causes enzyme denaturation, which weakens enzyme activity and slows down the reaction rate. BglPm-ELPs still retain high enzyme activity at 40 °C because ELPs tagging stabilizes the three-dimensional structure of the enzyme at this temperature, reduces thermal denaturation, and enables the enzyme to perform high catalytic functions. The higher activity of BglPm-ELPs in slightly alkaline environments means that

the protein purified under alkaline conditions is not easily deactivated when compared to acidic conditions when purified using ELP-tag.

Different pH values also trigger ELPs to initiate ITC cycling, which reduces the effect of environmental temperature on protein purification. It is advantageous for protein purification that is resistant to high temperatures or is particularly sensitive to temperature changes. However, in different pH environments, proteins can lose their activity by causing physiological dissolution, so using different salts is safe and effective for enzyme separation. Salt can play an important role in the process of protein extraction, separation and purification. Different inorganic salts may cause protein denaturation and precipitation in certain order. Hofmeister Series, an order of effect of certain inorganic salts on protein solubility, stability and activity, initially proposed the sequence of anions, i.e., CO_3^{2-} >SO_4^{2-}>$H_2PO_4^{2-}$>F^->Cl^->NO_3^->I^->ClO_4^- and the sequence of cations, i.e., K^+>Na^+>Mg^{2+}>Ca^{2+}. On the left side of the sequence, precipitation of proteins occurs, and on the right side, increasing protein solubility[34]. In this study, the purification efficiency using sodium chloride was better than that using sodium carbonate, which facilitated protein dissolution because Cl^- was located on the right side of CO_3^{2-} in the Hofmeister series. The pH of the solution increases and the specific activity of the enzyme decreases with the pH increment. Also, high salt concentrations could cause protein inactivation, and when the concentrations reached 2.5 and 3M, the purification amount of enzyme decreased significantly.

5. CONCLUSION

In this study, the purification method of BglPm-ELPs using ITC was optimized. The activity analysis of the purification enzyme confirmed that BglPm-ELPs could hydrolyze the outer glycosyl of the C-3 and C-20 diglucosyl groups to convert ginsenosin Rb1 to the rare ginsenoside F2, but not the inner glycosyl of C-3 and C-20 sites.

Supplementary Tables

Table S1. ITC purification results of BglPm-ELPs using 2M NaCl

BglPm-ELPs	Total enzymic activity(U)	Total protein(mg)	specific activity(U/mg)	Purification ratio	Purification yield (%)
Crude eynzyme	852.47	0.85	1007.18	1.00	100
First supernatant	728.94	0.76	953.26	0.95	86
First precipitate	71.43	0.42	172.11	0.17	8
Second supernatant	568.47	0.44	1287.14	1.28	67
Second precipitate	15.67	0.24	64.35	0.06	2
Third supernatant	522.99	0.30	1735.05	1.72	61
Third precipitate	11.07	0.25	44.10	0.04	1

Table S2. ITC purification results of BglPm-ELPs using 2.5M NaCl

BglPm-ELPs	Total enzymic activity(U)	Total protein(mg)	specific activity(U/mg)	Purification ratio	Purification yield (%)
Crude eynzyme	852.47	0.85	1007.18	1.00	100
First supernatant	632.40	0.83	765.97	0.76	74
First precipitate	176.64	0.48	370.78	0.37	21
Second supernatant	591.22	0.46	1288.43	1.28	69
Second precipitate	121.33	0.32	377.32	0.37	14
Third supernatant	17.13	0.21	81.20	0.08	2
Third precipitate	81.64	0.25	326.35	0.32	10

Table S3. ITC purification results of BglPm-ELPs using 3M NaCl

BglPm-ELPs	Total enzymic activity(U)	Total protein(mg)	specific activity(U/mg)	Purification ratio	Purification yield (%)
Crude eynzyme	852.47	0.85	1007.18	1.00	100
First supernatant	829.16	0.80	1034.06	1.03	97

First precipitate	82.76	0.09	955.84	0.95	10
Second supernatant	356.43	0.43	823.49	0.82	42
Second precipitate	149.52	0.48	313.85	0.31	18
Third supernatant	52.36	0.30	175.28	0.17	6
Third precipitate	74.50	0.29	254.03	0.25	9

Table S4. ITC purification results of BglPm-ELPs using 1.5M Na_2CO_3

BglPm-ELPs	Total enzymic activity(U)	Total protein(mg)	specific activity(U/mg)	Purification ratio	Purification yield (%)
Crude eynzyme	852.47	0.85	1007.18	1.00	100
First supernatant	543.58	0.45	1208.36	1.20	64
First precipitate	83.03	0.42	197.16	0.20	10
Second supernatant	530.76	0.40	1339.71	1.33	62
Second precipitate	15.29	0.29	53.16	0.05	2
Third supernatant	65.03	0.25	255.77	0.25	8
Third precipitate	11.34	0.26	43.05	0.04	1

Table S5. ITC purification results of BglPm-ELPs using 2M Na_2CO_3

BglPm-ELPs	Total enzymic activity(U)	Total protein(mg)	specific activity(U/mg)	Purification ratio	Purification yield (%)
Crude eynzyme	852.47	0.85	1007.18	1.00	100
First supernatant	607.15	0.63	968.45	0.96	71
First precipitate	111.00	0.58	192.47	0.19	13
Second supernatant	602.92	0.52	1163.51	1.16	71
Second precipitate	16.22	0.34	47.66	0.05	2
Third supernatant	544.15	0.39	1384.59	1.37	64
Third precipitate	15.51	0.28	56.00	0.06	2

Table S6. ITC purification results of BglPm-ELPs using 2.5M Na_2CO_3

BglPm-ELPs	Total enzymic activity(U)	Total protein(mg)	specific activity(U/mg)	Purification ratio	Purification yield (%)
Crude eynzyme	852.47	0.85	1007.18	1.00	100
First supernatant	704.26	0.66	1059.65	1.05	83
First precipitate	154.21	0.61	254.38	0.25	18
Second supernatant	704.03	0.62	1134.78	1.13	83
Second precipitate	9.70	0.33	29.35	0.03	1
Third supernatant	133.40	0.34	387.54	0.38	16
Third precipitate	8.07	0.26	31.61	0.03	1

Table S7. ITC purification results of BglPm-ELPs using 3M Na_2CO_3

BglPm-ELPs	Total enzymic activity(U)	Total protein(mg)	specific activity(U/mg)	Purification ratio	Purification yield (%)
Crude eynzyme	852.47	0.85	1007.18	1.00	100
First supernatant	652.43	0.63	1041.73	1.03	77
First precipitate	26.99	0.36	75.23	0.07	3
Second supernatant	640.24	0.53	1208.92	1.20	75
Second precipitate	6.70	0.29	23.23	0.02	1
Third supernatant	599.62	0.44	1369.16	1.36	70

Third precipitate	26.02	0.25	103.62	0.10	3

Supplementary Figures

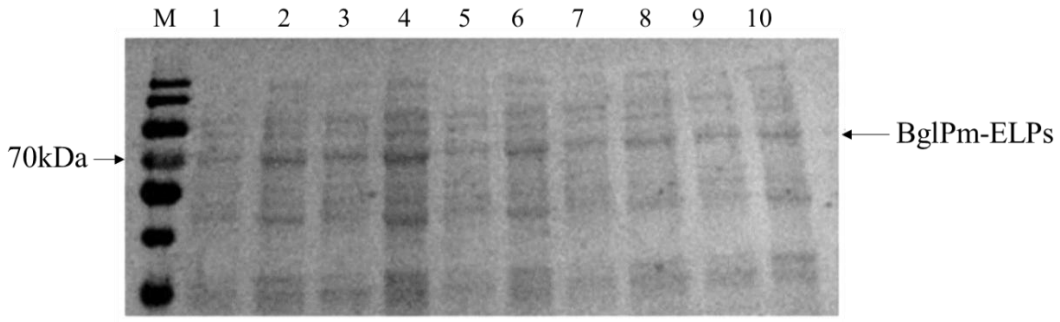

Figure S1. SDS-PAGE analysis of BglPm-ELPs derived expression products according to IPTG concentration

M: Marker, 1: 0.1mM supernatant, 2: 0.1mM precipitation, 3: 0.2mM supernatant, 4: 0.2mM precipitation, 5: 0.3mM supernatant, 6: 0.3mM precipitation, 7: 0.4mM supernatant, 8: 0.4mM precipitation, 9: 0.5mM supernatant, 10: 0.5mM precipitation

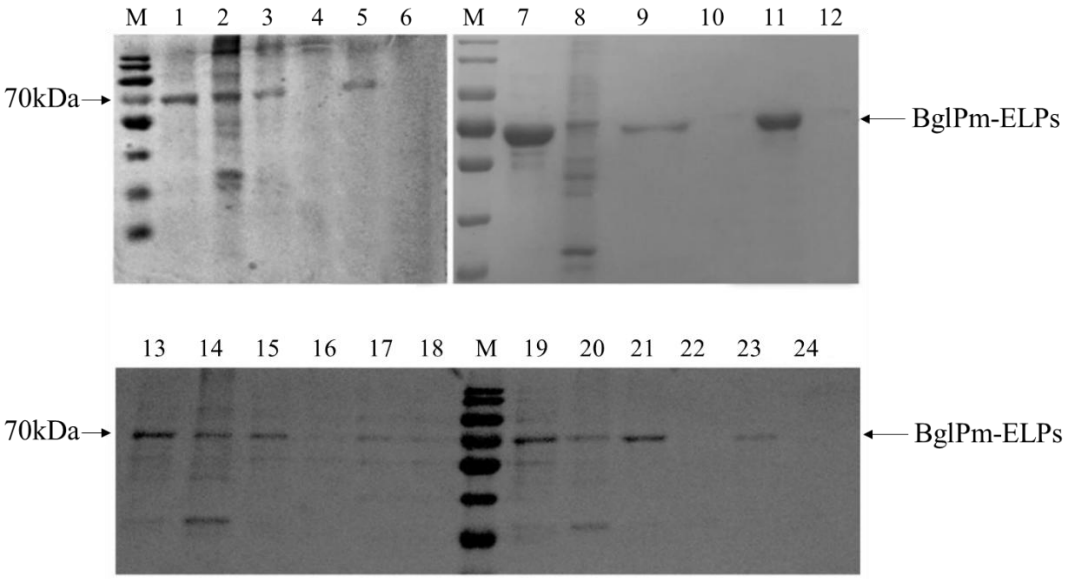

Figure S2. SDS-PAGE analysis of BglPm-ELPs purified protein by different NaCl concentration treatments

M: Marker, 1~6: 1.5M NaCl treatment, 1; first supernatant, 2; first precipitation, 3; second supernatant, 4; second precipitation, 5; third supernatant, 6; third precipitation. 7~12: 2M NaCl treatment, 7; first supernatant, 8; first precipitation, 9; second supernatant, 10; second precipitation, 11; third supernatant, 12; third precipitation. 13~18: 2.5M NaCl treatment, 13; first supernatant, 14; first precipitation, 15; second supernatant, 16; second precipitation, 17; third supernatant, 18; third precipitation. 19~24: 3M NaCl treatment, 19; first supernatant, 20; first precipitation, 21; second supernatant, 22; second precipitation, 23; third supernatant, 24; third precipitation.

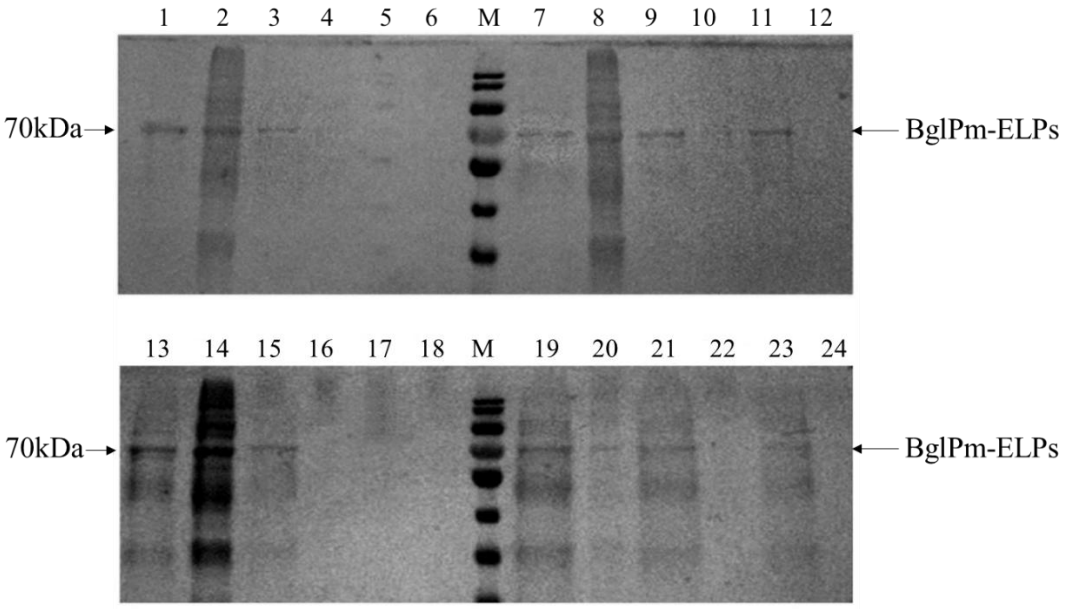

Figure S3. SDS-PAGE analysis of BglPm-ELPs purified protein by different Na_2CO_3 concentration treatments

M: Marker, 1~6: 1.5M Na_2CO_3 treatment, 1; first supernatant, 2; first precipitation, 3; second supernatant, 4; second precipitation, 5; third supernatant, 6; third precipitation. 7~12: 2M Na_2CO_3 treatment, 7; first supernatant, 8; first precipitation, 9; second supernatant, 10; second precipitation, 11; third supernatant, 12; third precipitation. 13~18: 2.5M Na_2CO_3 treatment, 13; first supernatant, 14; first precipitation, 15; second supernatant, 16; second precipitation, 17; third supernatant, 18; third precipitation. 19~24: 3M Na_2CO_3 treatment, 19; first supernatant, 20; first precipitation, 21; second supernatant, 22; second precipitation, 23; third supernatant, 24; third precipitation.

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Author Contributions:

Kwang-Il To: study design. Hyok-Chol Ri: Conceptualization and performing experiment, Shu-Yang Dang: performing experiment and data collection. Jyong-Hyok Kim and Chang-Bong Choe: data analysis and drawing figure, Gyong-Jin Mun and Il-Tae Kim: revision of draft and supervising, Su-Chol Rim: writing and reviewing.

Ethical Approval

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

Informed Consent

Not applicable.

Conflicts of interests

The authors declare that they have no conflicts of interest, competing financial interests or personal relationships that could have influenced the work reported in this paper.

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Data and materials availability

All data associated with this study will be available based on the reasonable request to corresponding author.

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