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Research article

One-step non-chromatography purification of a low abundant fucosylated protein from complex plant crude extract

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Abstract: Effective methods for isolation and purification of glycoproteins and other glycoconjugates are important to biopharmaceutical industry and diagnostic industry. They are also critical to an emerging field of glycoproteomics. In this work, we applied the newly-developed affinity ligand, a fusion protein of elastic like polymer (ELP) and a bacterial lectin, in an affinity precipitation process to purify soybean peroxidase (SBP) based on the presence of fucoseon the protein surface. We addressed, in particular, the challenge of purifying a low abundant protein from a complex dilute crude plant extract. The novel affinity precipitation developed in this work was very promising. One step binding and precipitation resulted in >95% recovery yield directly from crude extract and a 22.7 fold purification, giving a specific activity of 420 U/mg. The SBP isolated using this affinity precipitation meets or exceeds the quality specifications of reagent grade products by Sigma. We showed that the recovery yield had a strong dependence on the molar ratio of ligand to target fucosylated protein, with a ratio of three giving nearly full recovery, which could be predicted based on the total fucose content per protein molecule and the number of binding site per ligand molecule. We additionally developed a method of ligand regeneration and investigated its reuse. A simple wash with pH buffer was shown to be effective to regenerate the binding capacity for the ligand, and the ligand could be used for 10 times, giving an averaged 80% isolation yield based on initial input of soybean peroxidase. Taken together, an effective method of affinity precipitation was developed, which could be used to enrich a low abundant target glycoprotein from a complex mixture with a high recovery yield. The high selectivity for fucosylated protein and its ease of operation make this method particularly useful for purification of low abundant glycoprotein from natural sources. This work establishes a non-chromatography glycoform-specific purification method and extends the useful ELP-based affinity precipitation to glycoproteins.

Keywords: affinity precipitation; bacterial lectins; fucosylated proteins; glycoprotein purification; soybean peroxidase

1. Introduction

Glycosylation is the most prevailing post-translation modification event and impacts on a wide range of biological activities, including cell growth, differentiation, immune response, biological signaling, and fertilization [1–3]. Besides their significance to fundamental research, glycoproteins represent more than 70% of therapeutic drugs on the market, a sector of biopharmaceuticals with growth potentials in many therapeutic areas, including cancer treatment [4]. Animal or plant derived glycoproteins are also widely used as therapeutics, diagnostics, and as research reagents [5,6]. All these applications require efficient and cost effective methods for isolation and purification of glycoproteins. However, obtaining homogenous glycoproteins is particularly challenging due to the high concentrations of other protein contaminants, low abundance of target glycoproteins, and heterogeneity in glycosylation [7–9].

Plant peroxidases are glycoproteins widely used as research reagents. With their activity easily and inexpensively detected, they are essential in numerous commercial diagnostic kits. They are also used widely in food processing and in waste water treatment [10,11]. Horseradish peroxidase (HRP), for example, has been used as a conjugate for CL-ELISA for detection of antibody-mediated binding events, *N*-de-methylation to produce food flavor, and in removal of dyes from polluted water [10,12,13]. Soybean peroxidase (SBP) is functionally equivalent to HRP but more thermal and environmentally stable. As such, it can be used in applications requiring more harsh conditions than HRP can tolerate and can extend shelf life of peroxidase containing diagnostic kits [14–16]. SBP could potentially be derived from soybean hull, a byproduct of the soybean industry [17,18]. However, SBP exists in a complex plant crude extract with a low concentration of 3% of the total hull protein content [18]. It requires an efficient isolation and purification process to achieve a suitable purity for a target application and to meet the cost objectives.

Lectin affinity chromatography has the potential for a one-step purification of glycoproteins. Many lectins have been identified as potential ligands for targeting glycoproteins [19]. However, as a method of glycoprotein purification, it has many challenges. Most frequently cited are: a.) high operation and maintenance costs, including the cost of affinity ligand. b.) ligand binding capacity and flow rate limitations resulting in large dilution of product stream c.) column fouling which requires harsh sanitizing conditions that lead to ligand leaching, product degradation and byproduct formation [20,21]. For these reasons, lectin affinity chromatography for glycoprotein purification has been limited to small scale applications [22,23]. The need to develop more cost-effective methods for protein or glycoprotein purification has motivated researchers to look for non-chromatographic methods for protein purification [23,24,25].

Affinity precipitation is an alternative approach with the potential to overcome the challenges associated with affinity chromatography, as it retains the specific interaction of affinity ligand with the protein of interest while avoiding column operation [26]. Thermo-responsive precipitation is of particular interest as it provides a convenient way to toggle between solution phase where the affinity ligand can interact with the target protein under optimal conditions, and solid phase where protein

complex can be isolated from the precipitate in significantly enriched form. Elastin-like protein (ELP), as a thermal responsive biopolymer, has been used to facilitate precipitation for protein purification [27,28,29]. However, ELP has not been used to purify glycoproteins based on glycoforms present on the protein.

Previously, we reported development of novel thermo-responsive sugar-binding ligands by fusing a small bacterial fucose lectin with an ELP [30]. In this work, we apply the fucose-binding ligand to isolate SBP, a fucosylated protein, from complex plant crude extract. We demonstrate that affinity precipitation is particularly effective in purifying a low abundant protein from a complex mixture, resulting in >95% recovery yield and 22.7 fold purification in one step. We also address the issue of affinity ligand regeneration and its reuse in the purification process.

2. Materials and Methods

2.1. Chemicals

All chemicals were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO) unless otherwise specified and were used without further purification. Soybeans were purchased from a local farmers market.

2.2. Fusion protein expression and fractionation

The lectin from *Ralstonia solanacearum* (RSL) was transcriptionally fused to an ELP peptide. This fusion protein (RSL-ELP40) was expressed in *E. coli* as described previously [30]. A single colony of *E. coli* UT5600 transformant was selected and cultured in 3 mL of LB broth and 100 μg/mL of ampicillin overnight at 37 °C and 250 rpm. This seed culture was used to inoculate LB broth culture containing ampicillin to an OD600 of 0.1. This larger culture was grown at 37 °C and 250 rpm to a cell density of OD600 of about 0.4, at which point isopropyl-β-D-1-thio-galactoside (IPTG) was added to the culture to a final concentration of 1 mM to induce the production of the fusion protein. The cultivation temperature was then reduced to 18 °C and the cells were grown for an additional 48 hours.

Periplasmic and cytoplasmic fractions were prepared according to the method described in the pET system manual (E MD Chemicals, San Diego, CA). A culture was harvested by centrifugation. The pellet was re-suspended in phosphate buffered saline (PBS) solution, sonicated, and centrifuged at 6,000 g at 4 °C for 20 min. The supernatant was saved as a cytoplasmic fraction.

2.3. Soybean crude protein preparation

Soybeans were soaked in 10% (w/v) in 0.1 M potassium phosphate buffer (pH 7.0) at room temperature overnight. The hydrated hull was removed from the bean, and the isolated hulls were again soaked in the same buffer for overnight, and then homogenized by vortexing for 2 minutes. The mixture was centrifuged for 20 minutes at 4 °C at 13,000 g. The supernatant was collected and concentrated about 20 times by acetone precipitation [31].

2.4. Monosaccharide characterization

To analyze the crude carbohydrate composition, the glycans are first hydrolyzed. The hydrolysis is achieved by addition of 6 N HCl to the protein mixture and heating to 121 °C for 45 minutes. After neutralizing with NaOH to a pH of 7, the sample was centrifuged for 30 seconds at 15,000 g to removed precipitates. The sample was diluted for analysis.

The released monosaccharide samples were analyzed by High Performance Anion-Exchange Chromatography (HPAEC) using a DIONEX system equipped with an ED50 electro-chemical detector (Sunnyvale, CA). Monosaccharides were separated on a CarboPac PA-20 column (Dionex). Detection was through pulsed amperometry. (Waveform: t = 0.41 s, p = −2.00 V; t = 0.42 s, p = −2.00 V; t = 0.43 s, p = 0.60 V; t = 0.44 s, p = −0.10 V; t = 0.50 s, p = −0.10 V) The mobile phase consisted of degassed solution A containing 100 mM sodium hydroxide and solution B containing deionized water. The mobile phase was pressurized with inert gas (He) to prevent interference of airborne carbon dioxide. An isocratic method containing 2% solution A and 98% solution B was pumped at a flow rate of 0.5 mL/min.

2.5. Acetone precipitation

Chilled acetone was added in a ratio of 5 to 1 to the crude protein solution. The mixture was vortexed, then refrigerated at −20 °C for an hour. The precipitates were collected by centrifugation at 6,000 g for 15 minutes. The acetone supernatant was removed, and the pellet was resuspended in PBS. An SBP activity assay as performed to ensure no loss of activity.

2.6. Inverse temperature cycling

The transition temperature of the constructs was determined spectrophotometrically in a 96-well microplate reader (Spectramax M5, Molecular Devices, Sunnyvale, California). Each well contained 200 mL of PBS with 0, 0.5, or 1.0 M NaCl with ligand fusion at a concentration of 5 mg/mL. Temperature was increased (in 2 °C increments) from 30 to 50 °C and the absorbance at 310 nm was measured.

2.7. SBP activity assay

For peroxidase assay, a solution of 0.0017 M hydrogen peroxide was prepared by diluting 1mL of 30% hydrogen peroxide to 100mL with DI water. One milliliter of this solution was further diluted into 50 mL of 0.2 M potassium phosphate buffer pH 7.0. A 0.0025 M 4-aminoantipyrine solution was prepared in 0.17 M phenol solution. The hydrogen peroxide solution and aminoantipyrine solution were mixed 1:1 ratio, and diluted horseradish peroxidase was added. Absorbance of the resulting mixture was measured at 510 nm over a period of 5 min. at 25 °C in a microplate reader, Spectramax M5 (Molecular Devices). Specific activity is calculated as follows:

$$\text{Units/mg} = \frac{\Delta Abs_{510}/min}{6.58 \times mg\ enzyme/mL\ reaction\ mixture}$$

2.8. SDS-PAGE

Each sample was combined in a 1:1 ratio with SDS sample buffer (10% SDS, 10% b-mercaptoethanol, 0.3 M Tris-HCl (pH 6.8), 0.05% bromophenol blue, 50% glycerol), boiled for 5 minutes, and resolved by 12.5% (w/v) SDS-PAGE. Each gel was detected by Coomassie blue staining (Bio-Rad).

3. Results

3.1. SBP in Soybean Crude Plant Extract

The simple processing method of soybean hull, as described in Materials and Methods section, resulted in a crude plant extract, in which SBP was a minor component. The total soluble protein content in the soybean crude extract is approximately 3.8 mg/ml. An SDS-PAGE analysis shows that several bands are present (Figure 1). Based on previous studies [18], soybean peroxidase constitutes only 3% of total protein in the crude extract, thus it is not expected to appear as one distinct band. However, the presence of soybean peroxidase was indicated by its activity measured at 18.5 +/− 0.7 U/mg. Crude protein samples were also analyzed for presence of sugars as glycosylation are common in plants. A total sugar concentration was measured at 3.2 +/− 0.5 mg/ml, which is comparable to the total protein concentration (3.8 mg/ml), suggesting significant glycosylation in plant crude sample. The averaged molar ratio of monosaccharide per *N*-Acetylglucosamine (GlcNAc) at the base of each N-glycan was shown in Table 1. Fucose to GlcNAc ratio in the crude was 1.1:2. There were also significant mannosylated proteins in the crude, with the ratio of mannose to GlcNAc 6.5 to 2. In addition, xylosylated proteins were also present. Overall, these results indicate that the targeted fucosylated protein is present at a low concentration and in a rather complex mixture containing other glycosylated proteins. It would be of interest to see how effective the affinity precipitation is in purifying a low abundant fucosylated protein from this complex plant crude extract.

Table 1. Molar ratios of monosaccharides associated with crude and enriched SBP.

	Molar ratios				
	Fucose	GlcNAc	Xylose	Mannose	Reference
Crude	1.1 ± 0.3	2 ± 0.2	1.3 ± 0.4	6.5 ± 0.3	This Study
Purified SBP	1 ± 0.2	2 ± 0.2	0.9 ± 0.04	3.4 ± 0.3	This Study
Literature SBP	0.9	2	0.7	3.3	(Gray et al 1996)

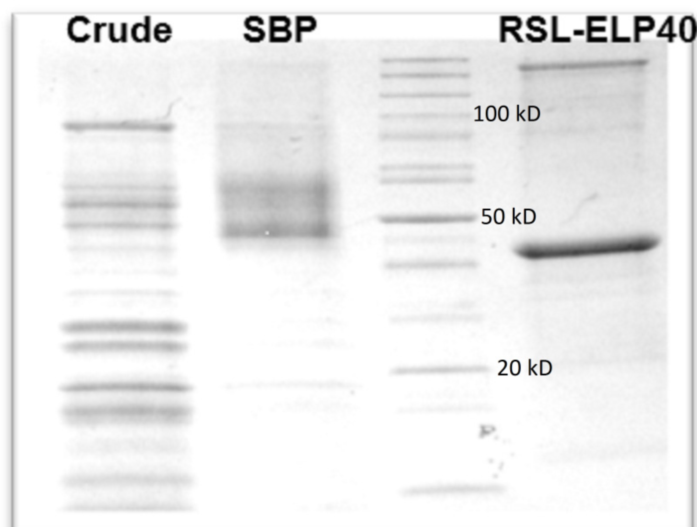

Figure 1. Lane 1. Crude soybean hull protein solution. Lane 2. Enriched soybean peroxidase. Lane 3. Marker. Lane 4. Isolated RSL-ELP40 after SBP purification.

3.2. SBP capture by the affinity ligand and precipitation of SBP-ligand complex

The purification is initiated by addition of the thermo-responsive affinity ligand, RSL-ELP fusion protein, to the crude plant extract. The mixture is incubated at an ambient temperature (~25 ˚C) for 30 minutes to allow the lectin to bind to the fucose on the surface of SBP, resulting in formation of RSL-ELP-SBP complex. Subsequently, the mixture is brought to 50 °C, and the complex precipitates out of the solution. The fraction of SBP activity captured by the ligand is denoted as recovery yield, which represents the activity captured and recovered from the crude after one round of ITC (Inverse Temperature Cycle). The fraction of SBP activity remaining in the soluble crude fraction is denoted as unbound, since the ligand failed to initially capture this fraction. Total activity refers to the absolute activity, in U/mg, of SBP targeted and isolated. As shown in Figure 2, the recovery yield is dependent on the molar ratio of ligand to SBP. Only 20% SBP was captured by the ligand when a molar ratio of ligand to SBP of 0.5 to 1 was used. Increasing the ratio to 3 resulted in a recovery yield of 95%.

The above process was developed using 50 ˚C precipitation, which takes advantage of SBP's thermal stability [12]. For proteins that are less thermally stable, the transition temperature could be lowered by including salt in the precipitation step. The RSL-ELP construct was determined to have a T_t of 45 °C by turbidity studies (Figure 3). Addition of 0.5 M NaCl resulted in a reduction of this temperature to about 35 °C, as seen in Figure 3. Thus the method could be easily adapted to a wide range of proteins with varying stability.

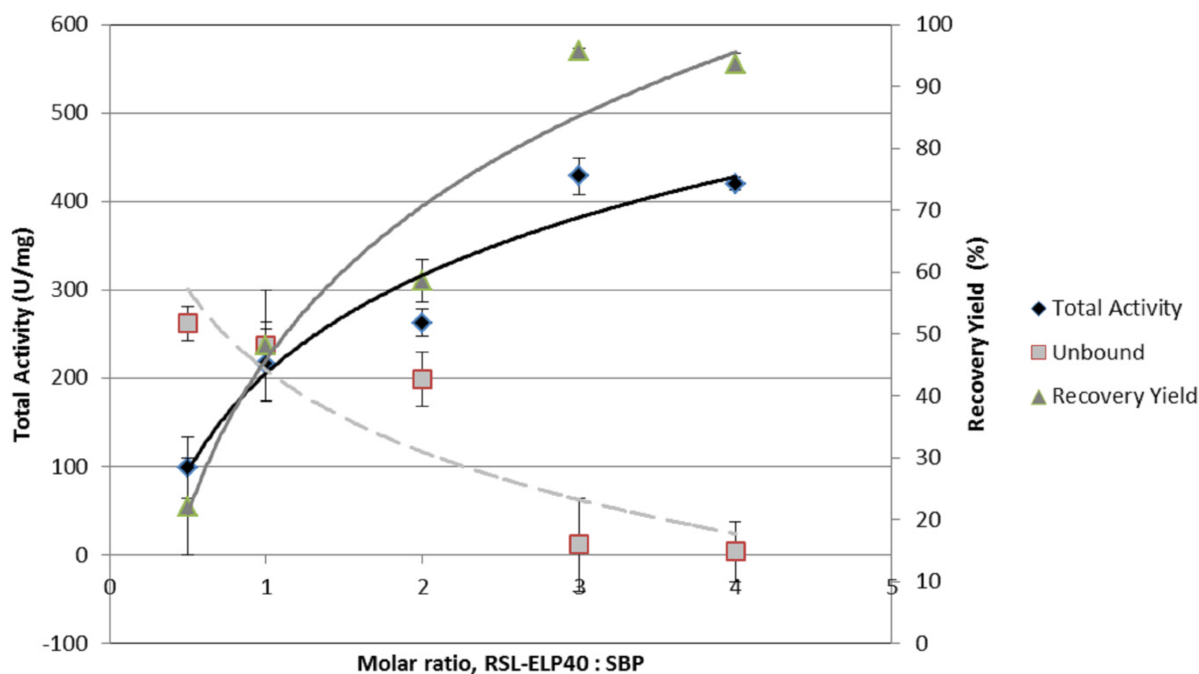

Figure 2. Activity and recovery yield of SBP as a function of the ratio of RSL-ELP to SBP.

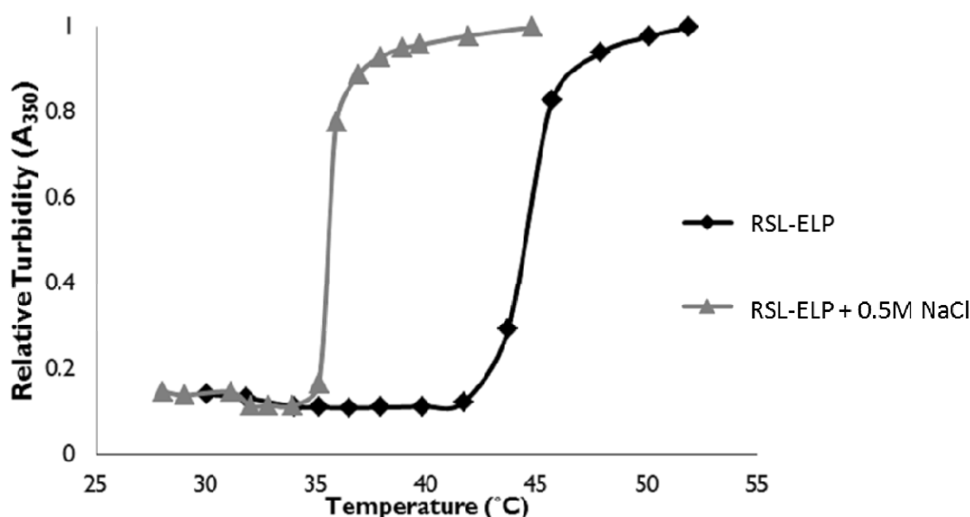

Figure 3. Turbidity of RSL-ELP with increasing temperature.

3.3. Effect of fucose concentration on SBP elution

Typically, release of lectin from binding complex could be achieved by respective cognate sugar. As fucose is relatively expensive, minimizing its use is important for the SBP purification. We investigated the effect of fucose concentration on ligand release. Fucose concentration over a range of 0.001 mM to 10 mM was studied for ligand release. After one temperature cycling to precipitate ligand and bound target (as described in the last section), the complex was resuspended in an elution buffer containing the specified concentration of fucose. SBP released from the ligand was then

separated from the ligand by a temperature induced precipitation. SBP activity present in the soluble portion is denoted as released activity, and was compared to the initial activity of the crude to calculate the relative released activity. SBP activity associated with the ligand is measured as the bound fraction. This bound fraction is again compared to initial activity, allowing for calculation of relative bound activity. Shown in Figure 4 is released relative SBP activity and bound relative SBP activity as a function of fucose concentration. It appears that 1 mM of fucose is optimal, releasing about 80% SBP from protein complex, based on activity measured in the supernatant.

Other methods of elution of SBP such as low pH and solvents were also tested but results were poor due to inactivation of SBP.

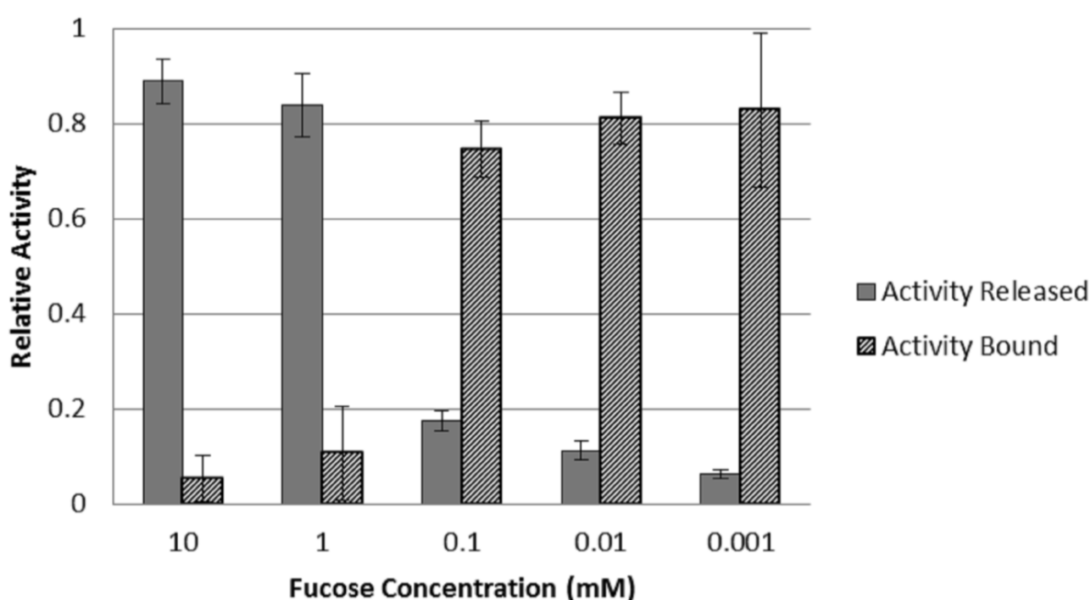

Figure 4. Activity in the bound and unbound fractions when RSL-ELP40 bound SBP complex is suspended in elution buffers of varying fucose concentration.

3.4. Optimal conditions for SBP affinity precipitation and product characterization

Based on the above studies on the capture and elution of SBP, several parameters are critical to the affinity precipitation process. In the capture step, key constraints feature the molar ratio of ligand to target and temperature for precipitation. Using a ratio of 3 (ligand to SBP) and 50 °C for the precipitation resulted in 95% recovery yield. In the elution step, the concentration of fucose is most important with1mM being optimal, which gave 80% release of SBP or isolation yield. A summary of purification and characterization of SBP isolated under the optimal conditions are shown in Table 2. In a typical experiment, after the elution step, a specific activity of soybean peroxidase in the supernatant was measured at 420 U/mg, representing a 22.7 fold purification. The quality of product was indicated by the RZ (Reinheiszahl) value, which is an absorbance ratio of A403/A275, a measure of hemin content. The RZ value was increased from 0.19 for soybean extract to 0.75 after affinity precipitation. Compared to specifications of Sigma product, the SBP from the affinity precipitation has a specific activity up to 7 fold higher and a RZ value higher than the specified

minimum (Table 2). The product stream was further analyzed by SDS-PAGE, which showed significantly reduced number of bands present. While a single homogenous solution was not evident, the target protein was significantly enriched in the product stream (Figure 1), corroborating with the 22.7 fold increase in specific activity. Additionally, total carbohydrate and fucose content of purified SBP were also analyzed. After purification, there was a significant reduction of both mannose and xylose content in the product stream (Table 1). The ratio of mannose to GlcNAc was reduced from 6.5 to 3.4, close to the literature value of 3.3, which was from purified SBP. Similarly, xylose content in isolated SBP is substantially closer to the literature value, suggesting significant enrichment of the target protein as a result of this one-step purification. Notably the fucose content in enriched sample was close to the level reported for purified SBP [32], suggesting that a majority of fucose present in the smaple are associated with SBP (Table 1).Taken together, the affinity precipitation described was effective to enrich the fucose-containing SBP from a mixture containing other glycoproteins. The soybean proteome has not been extensively characterized, so while other fucosylated proteins may be present, primary proteins of higher abundance in the hull are not fucosylated [33].While a homogenous SBP was not evident, most impurity present in plant crude extract was removed and the product stream is significantly enriched with SBP, which should meet most common applications of SBP such as research reagent.

Table 2. Characterization of crude and purified SBP samples.

	Total protein (mg)	Purification Fold	Specific Activity (U/mg)	RZ Value
Crude	3.8	1	18.5	0.19
Purified	0.16	22.7	420	0.75
Sigma	–	–	50–150	≥0.5

3.5. Ligand reuse

As affinity ligand is possibly the most important cost contributor in an affinity precipitation process, we investigated the possibility of its reuse. To this end, after each purification, the ligand was isolated from SBP by temperature-induced precipitation. Before the ligand could be used for the next cycle purification, it is necessary to regenerate ligand by releasing fucose bound to the ligand. Therefore, a wash step was added to the recycle procedure. The pelleted ligand was resuspended in 100 μL PBS solution and resolubilized, releasing residual fucose from the ligand. The wash solution was subject to ITC again to pellet the ligand, which was used in the next cycle of purification with fresh crude. The resulting wash fluid was analyzed for trace activity, designated as “wash” in Figure 5. Thisadditional step restored the ligand function for additional rounds of recycle via dilution and releasing the cognate sugar from the complex. Figure 5A details the purification results of the SBP each time the RSL-ELP was recycled. Recovery yield represents the SBP activity captured by the ligand and present in the complex with the RSL-ELP after precipitation. This yield was decreased gradually from >95% to about 80%. The isolation yield represents the SBP activity after its elution from the complex and removal of ligand from the elution solution via ITC. Similar to the recovery

yield, the isolation yield also showed an appreciable decrease over the 10 recycle of ligand, from initial 95% to 75%. The loss in wash step seemed to relatively minor, <5%. Figure 5B illustrates the distribution of all measured activity throughout the purification process.This unbound activity was measured from the crude after purification with the affinity ligand, and represents the SBP in the crude solution not captured by the ligand. The total combined enzyme activity from three fractionswasclose to 100%, suggesting that no activity is lost due to denaturation or inactiviation. Apparently, activity present in the “unbound” fraction was resonsible to the decreasing isolation yield as the number of ligand reuse increased. Despite noticible decline of ligand efficiency, the isolationyield averages at 81.4% over the course of 10 recycles. These results showed that the affinity ligand can be reused for 10 times as long as a wash step to remove residual fucose is added between two purifications. The ability to reuse ligand is a unique advantage in the design of our affinity precipitation.

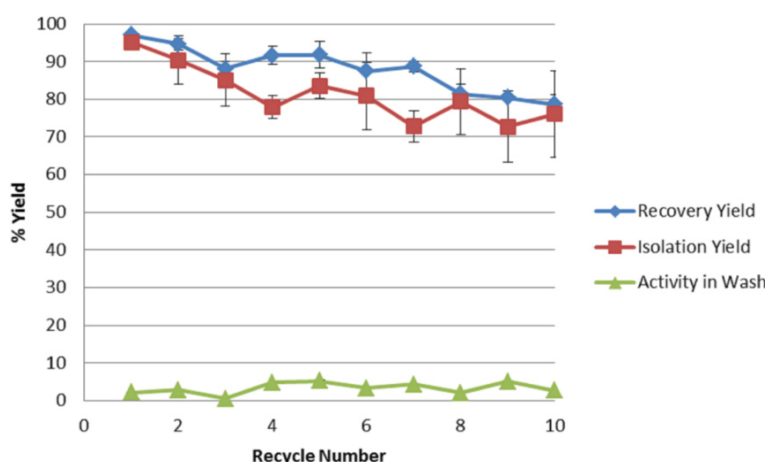

Figure 5A. Recovery activity, isolation activity, and activity left in wash over the course of several recycles.

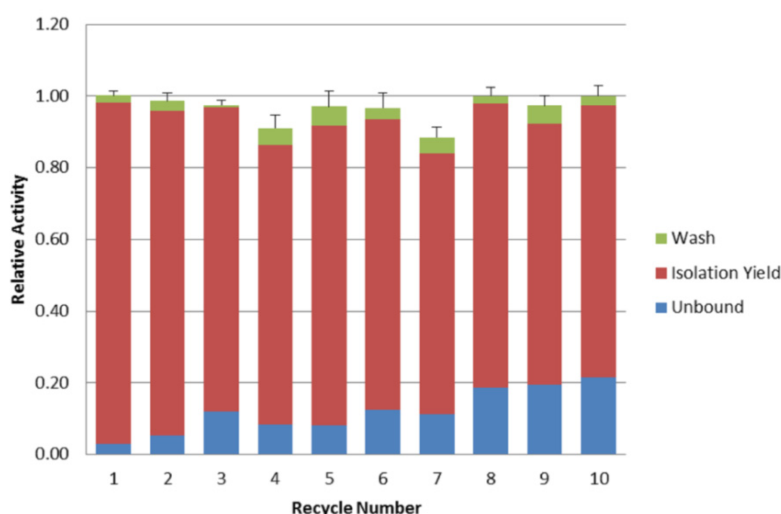

Figure 5B. Distribution of measured activity in each fraction.

4. Discussion

Affinity precipitation is increasingly being embraced as an alternative to affinity chromatography in protein purification. Since the first demonstration by Meyer and Chilkoti (1999) of the use of ELP in affinity precipitation, many researchers have contributed to the development of the method. ELP could be used as a fusion partner to a target protein and produced as a fusion protein. After purification, ELP could be removed by protease cleavage [34,35]. Further development with intein-mediated self-cleavage eliminates the protease use in ELP removal [36–39]. Recently, it was shown that, instead of fusing ELP to a target, it could be fused to an affinity ligand. Recombinant proteins are produced as a fusion with a small affinity tag recognized by the affinity ligand fused with ELP. This approach has the advantage that recombinant protein target could be produced independent of ELP and likely results in higher expression level, as ELP portion would otherwise add 200 amino acids if fused with the target protein [30,40,41]. This approach also addresses misfolding occurring in some large ELP-target protein fusions. Taking advantage of the presence of bacterial lectins and their small size, we recently developed lectin-ELP fusions as affinity ligand [30]. These ligands, as demonstrated in this work, could be used to purify glycoproteins based on the glycoforms present on a protein surface. This sets apart our work from previous other works that are all based on the affinity interaction with polypeptide backbone. This difference is important as glycans attached to a protein are often functionally significant. For example, terminal sialic acid on a therapeutic protein often determines its half-time, as is the case with erythropoietin. Therefore, our glycan-specific approach not only extends useful ELP-based affinity precipitation to a large class of glycoproteins, but also provides a new non-chromatography purification mechanism based on the affinity interaction between carbohydrates and protein ligand, naturally present but not exploited to its fullest for purification. Since there is no tag on a target protein, the glycoprotein isolated from this method is authentic and no post-purification processing is needed. Besides useful in recombinant glycoprotein purification, this approach also opens up vast opportunity to purify glycoproteins from natural plant and animal sources, two major sources of diagnostic enzymes and research regents.

This work serves as the first example that lectin-mediated affinity precipitation could be used to purify a useful and valuable enzyme from a dilute and complex plant crude extract. In this case, the capture of SBP relies on the binding of fucose on SBP to the lectin in the RSL-ELP40 ligand. Not surprisingly, we found that the recovery yield, a measure of capture efficiency, has a strong dependence on the molar ratio of ligand to target protein. An interesting result from our earlier work is that RSL-ELP forms a trimer, just like naked RSL [42]. As such, according to the structural study by Kostlanova et al, there are two fucose binding sites per lectin. Theoretically, only one fucose binding to the ligand is needed for capture of a SBP molecule, which corresponds to a molar ratio of ligand to SBP, 0.5:1. We started our investigation using this ratio. However, this theoretic ratio gave only 20% recovery (Figure 2). This could be explained by steric hindrance that prevents a single lectin molecule from binding two fucose sugars on two separate SBP molecules. Increasing the ratio to 1:1, however, improved the recovery only to 50%, indicating the binding of lectin to target protein was not as simple as one ligand: one protein as the ratio would suggest. About 95.7 +/− 0.5% recovery yield could be achieved using a ratio of 3:1, representing two molar excess for the ligand with respect to target glycoprotein. Since on average there are 5.6 fucose moieties per SBP [32], this ratio corresponds to the saturation state that all fucose present on the SBP are bound to lectin. Increasing the ratio beyond saturation is not expected to increase recovery and our observation is

consistent with this expectation. This study suggests that fucose on the same protein molecule binds preferentially. This notion is supported by studies by other researchers [43,44]. Accordingly, the ratio that gives a near complete recovery is the ratio that gives binding saturation. Therefore, the ratio for full recovery could be predicted by the number of cognate sugar on a protein. This understanding is useful as it greatly simplifies optimizations of an affinity precipitation.

As two molar excess of ligand is needed for full recovery and ligand is likely the most expensive component in the process, we investigated possibility of ligand reuse as a way to reduce its cost impact. This requires a method to regenerate the ligand after each cycle. This was achieved by a wash step using a pH buffer containing no fucose. This simple wash process allowed the ligand to regain much of the binding ability, and very easy to implement, compared to other regeneration methods [45]. However, a gradual decline of isolation yield was observed as number of reuse increases. Despite the decline, we demonstrated that the ligand could be used for 10 times, giving an averaged 80% isolation yield based on initial input of soybean peroxidase. This ligand recyclability is one feature of our approach, significantly different from other approaches that require direct association of ELP and the target [28,36]. Further analysis showed that the decrease of isolation yield was due to decline of binding capacity of the ligand as the number of recycle increases. Repetitive temperature fluctations may have caused denaturation of the lectin after multiple recycles, which could be mitigated by using a low temperature for precipitation by including salt (Figure 3). An alternative explanation for the decline of binding capacity is the incomplete dissociation of fucose from ligand in the wash step. This could be further investigated in the future by increasing the volume of wash buffer or adding a second wash step.

5. Conclusion

In summary, we developed an affinity precipitation process and enriched a low abundant SBP from a complex plant crude extract. Under optimal conditions, one step binding and precipitation resulted in >95% recovery yield directly from crude extract and a 22.7 fold purification. The product isolated using this affinity precipitation meets or exceeds the quality specifications of comparable products by Sigma. We showed that the recovery yield had a strong dependence on the molar ratio of ligand to the target fucosylated protein, with a ratio of three being optimal, which could be predicted based on the total fucose content per protein molecule and the number of binding site per ligand molecule. The optimal condition for elution of target protein was determined and 1 mM fucose resulted in an isolation yield (≥80%). The demonstrated recyclability of ligands provides opportunities to reduce the cost of affinity ligand to further ensure the cost-effectiveness of the bioseparation process.

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Conflict of Interest

All authors declare no conflicts of interest in this paper.

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