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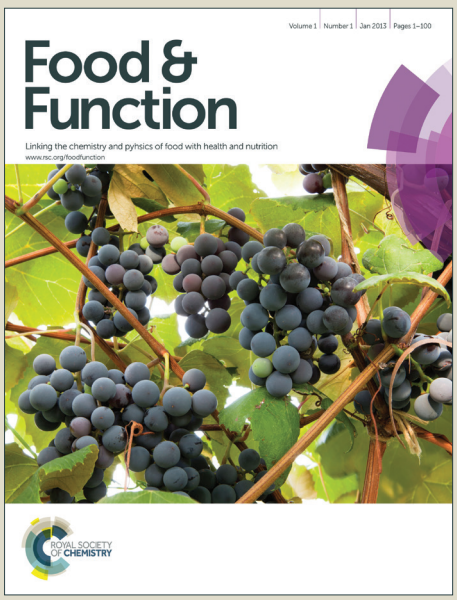

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Immune Activity of Sweet Potato (*Ipomoea batatas* L.) Glycoprotein after Enzymatic and Chemical Modifications

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Abstract: This study aims to investigate the immune activity of sweet potato (*Ipomoea batatas* L.) glycoprotein (SPG-1) before and after enzymatic and chemical modifications. The protein portion of SPG-1 was modified by pepsin, trypsin, and acetylation treatments. The carbohydrate portion was modified by glucoamylase, $NaIO_4$, and sulfation treatments. The carbohydrate chain of SPG-1 (SPG-1-C) was obtained. Immune activity was analyzed by measuring the serum lysozyme activity and T cell immune response. SPG-1 increased immune activity with a dose-response effect. Immune activity was slightly decreased after pepsin and trypsin hydrolysis, while increased after moderate degree (DS = 0.68) of acetylation. Immune activity was partly decreased after glucoamylase hydrolysis, remarkably decreased after $NaIO_4$ oxidation, or even loss after high modification by sulfation. Compared with SPG-1 groups, SPG-1-C groups increase immune activities with insignificant ($P > 0.05$) differences. Hence, appropriate modifications of protein portion could be conduct and high modifications of carbohydrate portion should be avoided to improve or maintain the immune function of SPG-1.

Keywords: Sweet potato glycoprotein; modification; carbohydrate chain; immune activity

Introduction

The industrial production of glycoprotein has developed rapidly owing to its various important bioactivities,[1] such as hypolipidemic activity,[2] hypoglycemic activity,[3] immunity,[4] anticancer,[5] and antioxidant.[6] As potential raw materials of glycoprotein production, sweet potatoes (*Ipomoea batatas* L.) are one of the most important economic crops in many tropical and subtropical countries in Asia, Africa, and Latin America. China shares approximately 90% of sweet potato production worldwide, with an annual production 117 million tons.[7] Therefore, studies on sweet potato glycoprotein (SPG) are important.

Some bioactivities of SPG have being found out. In our previous study,[2] we found that SPG exhibits dose-dependent hypolipidemic effect. Ozaki *et al.*[8] and Oki *et al.*[9] found that an arabinogalactanprotein (WSSP-AGP) from white-skinned sweet potato has hypoglycemic effects. Recent years, several methods, such as genetic, physical, chemical, and enzymatic modifications, have been introduced to improve the functional properties and technological applications of glycoproteins.[10] Thus, it is necessary to study the relationship between the structure and activity of SPG.

This study was conducted to determine the immune activity of SPG-1 after enzymatic and chemical modifications. Considering that functions may be dependent on either the protein or carbohydrate portion or on both.[11] Pepsin, trypsin, and acetylation treatments were conducted to modify the protein portions of SPG-1. Simultaneously, glucoamylase, $NaIO_4$, and sulfation treatments were conducted to modify the carbohydrate portions. Furthermore, its carbohydrate chain (SPG-1-C) was

obtained. Serum lysozyme activity and T cell immune response of SPG-1, modified SPG-1, and SPG-1-C were examined. The study should be able to provide theoretical insights for future improvements in SPG-1 manufacture process.

Materials and methods

Materials and animals

Sweet potato (*I. batatas* L. var. Beijing no. 2) was harvested and identified at the Department of Botany, College of Horticulture, Southwest University, Chongqing, China.

Male and female 8- to 10-week old BALB/c mice (18–20 g) were obtained from the animal center of Chongqing Medical University. All experimental protocols were approved by the Ethics Committee of Chongqing Medical University (Reference number: 24301035). The mice were acclimatized for 2–3 days prior to the experiment. During the experiment, the mice were fed under controlled environmental conditions (25 ± 1 °C temperature; normal day/night cycle; and 55% to 60% humidity). The mice were provided with basal diet and free access to drinking water. All conditions and handling of the animals were conducted according to the International Guiding Principles for Biomedical Research Involving Animals.[12]

Physicochemical Properties of SPG-1

Isolation, purification, total carbohydrate content, protein content, and molecular weight determinations of SPG-1 were conducted in our previous study.[2]

To study amino acid composition of SPG-1, the β-elimination reaction was performed according to the methods described by Chen *et al.*[13] with some modifications. SPG-1 (2 mg) was dissolved in 0.1 mol/L NaOH, 1.0 mol/L $NaBH_4$ solution; the reaction was conducted at 37 °C for 72 h and terminated with addition of 25% (v/v) acetic acid. The amino acid composition and absorbance of the solutions before and after β-elimination were determined with a Hitachi 835 amino acid analyzer (Hitachi Co., Japan) at 240 nm.

Modification of SPG-1

Protease (Pepsin and Trypsin) Treatments

SPG-1 was dissolved in distilled water and hydrolyzed separately by pepsin (P7000, from porcine gastric mucosa, powder, ≥ 250 units/mg solid; Sigma) and trypsin (T4799, from porcine pancreas, powder, 1,000-2,000 BAEE units/mg solid; Sigma), under optimized conditions (pH 1.5/37 °C for pepsin and pH 8.0/37 °C for trypsin).[14] Protease digestions were conducted according to the methods described by Kim *et al.*[15] with modifications. The pH of the solutions was adjusted with 1.0 mol/L NaOH or 1.0 mol/L HCl prior to and during hydrolysis. The digests with different enzyme concentrations (2.0 and 5.0 g/100 mL) were incubated for different duration (1, 2, 3, 4, and 5 h). Afterward, the pH of the solutions were adjusted to 7.0, the solutions were dialyzed against deionized water for 24 h, precipitated, dried, and stored at −20 °C.

Glucoamylase Treatment

SPG-1 hydrolysis with glucoamylase (A7420, from *Aspergillus niger*, lyophilized

powder, 30-60 units/mg protein (biuret), ⩽ 0.02% glucose; Sigma) was conducted according to the method described by Bailey and MacRae[16] with some modifications. SPG-1 was dissolved in sodium acetate-acetic acid buffer (0.2 mol/L, pH 4.5) and hydrolyzed with two concentrations (0.1 and 0.15 g/100 mL) of glucoamylase at 60 °C for 1, 2, 3, 4, and 5 h. Afterward, the pH of the solutions were adjusted to 7.0, and the reaction mixtures were then cooled, dialyzed, precipitated, dried, and stored at −20 °C.

$NaIO_4$ Treatment (Periodate Oxidation)

SPG-1 was also oxidized with $NaIO_4$ solution to release the aldehyde group of the sugar moiety. The $NaIO_4$ oxidation was conducted according to the method described by Wang *et al.*[17] with some modifications. SPG-1 (100 mg) was oxidized with 100 mL of 0.35 mol/L $NaIO_4$ (31148, ACS reagent, ⩾ 99.8%; Sigma) at 18 °C in the dark. The absorbance of the reaction mixture at 223 nm was monitored daily until it stabilized (about 108 h). The reaction was terminated with 2 mL ethylene glycol, and the reaction mixture was then dialyzed against deionized water for 24 h, precipitated, dried, and stored at −20 °C.

Acetylation Treatment

Acetylated SPG-1 was prepared following the methods of Kumar *et al.*[18] with some modifications. A 20% (w/v) glycoprotein suspension was prepared by dissolving 1 g of SPG-1 in distilled water. The pH of the solution was adjusted to 8.0–8.5 with 1.0 mol/L NaOH, and the slurry was stirred magnetically for 30 min at room temperature. Various amounts of acetic anhydride (0.8, 1.6, and 2.4 g) were then added dropwise to the uniformly mixed suspension to obtain three different (low,

medium and high) degrees of substitution. The stirring continued for 180 min, while maintaining the pH between 8.0 and 8.5. Afterward, the pH of the solution was adjusted to 7.0 with 0.5 mol/L HCl, and the reaction mixture was dialyzed against deionized water for 72 h, precipitated, dried, and stored at −20 °C. The degree of substitution (DS) of the acetylated SPG-1 was then determined by the method of Wurzburg.[19]

Sulfation Treatment

Sulfated glycoprotein was prepared according to the method described by Inoue *et al.*[20] with slight modifications. 1.0 g SPG-1 was suspended in 8 mL of formamide, and added with 4 mL of varying ratios of sulfation reagent (chlorosulfonic acid:pyridine solution = 1:2, 1:1, and 2:1, v/v) to obtain three different (low, medium and high) degrees of substitution. Stirred magnetically for 1 h, cooled, and added with 8 mL of distilled water and 15 mL of 2.5 mol/L NaOH. The reaction mixture was then dialyzed against deionized water for 72 h, precipitated, and dried to obtain a white sample. The DS was determined following the method of Doigson and Price.[21]

Isolation and Purification of SPG-1-C

SPG-1-C was isolated after β-elimination reaction of SPG-1. After deproteinating the aqueous solution using the Sevag method,[22] the supernatant was collected and dialyzed against a flowing clean water for 72 h, and then against distilled water for 24 h using a dialysis bag with 1,000 Da molecular-weight cut-off (Sigma). The eluted solution was characterized at 280 nm using T6 UV and visible spectrophotometer (Beijing Persee General Instrument Co., China) to ensure that no protein or amino

153 acid was left.[11] Fehling's solution was used to ensure that no monosaccharide was
154 left.[23] The trapped fluid was mixed with distilled water and purified on a
155 DEAE-cellulose-52 anion-exchange column (2.6 cm × 50 cm; Whatman, England)
156 with a gradient of NaCl solution (0.0–3.9 mol/L) as eluting solvent at 1.6–1.7 mL/min
157 flow rate.[24] The carbohydrate content of every 5 mL of eluted solution was
158 determined at 490 nm according to phenol-sulfuric acid method.[25] After dialysis, the
159 trapped fluid was precipitated with four volumes of absolute ethanol, mixed, kept
160 overnight in a refrigerator at 4 ℃, and centrifuged (5810; Eppendorf, Germany) at
161 5000 *g* for 5 min. The precipitate was washed twice with ethanol and acetone[26] to
162 obtain the crude SPG-1-C prior to vacuum drying at 40 ℃ (Shanghai Yuejin Medical
163 Apparatus Factory, China).

164 The crude sample was dissolved in distilled water and further purified using
165 Sephadex G-100 gel filtration column (1.6 cm × 100 cm; Pharmacia, Sweden) and
166 distilled water as eluting solvent at 0.5 mL/min flow rate.[27] The absorbance of the
167 eluted solution was determined at 490 nm, and then dialyzed, precipitated, and dried.
168 The purity of SPG-1-C was confirmed by a Sepharose CL-6B gel filtration column
169 (1.6 cm × 100 cm; Sigma, America) using 0.02 mol/L Tris-HCl buffer (pH 7.2) as
170 eluting solvent at 0.5 mL/min flow rate.

171 Furthermore, HPLC method was used to reconfirm the purity and determine the
172 molecular weight of SPG-1-C. Agilent 1200 HPLC system (Agilent, USA) with gel
173 permeation chromatography (GPC) on a TSK-G2000SW column (TOSOH
174 Corporation, Japan) was used based on the method developed by Li *et al*.[1] Dextran

standards (T-500 Mw=500,000; T-110 Mw=110,000; T-70 Mw=70,000, T-40 Mw=40,000 and T-10 Mw=10,000; Amersham Pharmcia, Sweden) were used to establish a standard curve (Wang et al., 2013). Finally, a calibration curve was prepared from the known Mw Dextran T system standard: $\lg\overline{Mw} = 8.6099 - 0.2858RT$ (r=0.9736, RT: retention time).

Serum Lysozyme Activity

The BALB/c mice were randomly divided into groups with 10 animals in each group. The experimental groups were treated with following doses: 50 and 80 mg/kg·d of SPG-1; 80 mg/kg·d of modified SPG-1; 50 and 80 mg/kg·d of SPG-1-C. A similar volume of normal saline was administered to the normal control (NC) group. The drugs were given by gavage once a day for 10 days. A day after the last day of drug administration, the mice were lightly anaesthetized under ether, and blood samples were taken from their eye sockets. The serums were separated by centrifugation at 4000 *g* for 5 min and stored at −20 °C for the lysozyme activity measurements.[28]

Lysozyme activity in mice serums was determined by agar diffusion plate method as described by Yin *et al*.[29] A series of lyophilized hen egg-white lysozyme (HEWL, Sigma) standards (0, 5, 25, 100, and 500 mg/L) were prepared in phosphate/citrate buffer (0.067 mol/L, pH 6.4). A suspension of 50 mg to 100 mg of *Micrococcus lysodeikticus* (ATCC 4698, Sigma Chemical, Germany) in 100 mL of buffer was freshly prepared on the assay day. Briefly, 10 × 10 cm 4 mm agar plates with 0.5–1.0 g/L *M. lysodeikticus* were carefully prepared. Circular holes (2 mm) were cut and

197 filled with 25 μL of the samples. After incubation at 25 ℃ for 18 h, the diameters of
198 the zones inhibition (transparent zone) around the holes with sample and HEWL
199 standards were measured.
200
201 **T Cell Immune Response**
202 The standard lymphocyte transformation test (LTT) described by vonBaehr *et al.*[30]
203 was used to determine the T cell immune response of the samples. Peripheral-blood
204 mononuclear cells were isolated from the heparinized venous blood of normal human
205 volunteers by density-gradient centrifugation (Ficoll-Paque, Pharmacia, Sweden). Up
206 to 0.1 mL mononuclear cell fraction was suspended in 1.0 mL RPMI 1640 culture
207 medium with penicillin (100 μg/mL), streptomycin (100 μg/mL), L-glutamine
208 (200 μg/mL), sodium bicarbonate (7.5%, w/v), and calf serum (10%, v/v). Up to
209 0.05 mL of sample or culture medium (control group) was added to the mixture and
210 incubated for 27 ℃ for 48 h. For the final 24 h of culture incubation, 1 mCi/mL ^{3}H
211 thymidine (Amersham, UK) was added to the mixture. The cells were then harvested
212 onto glass-fiber filters (Wallac, Sweden) and counts per minute (cpm) of the
213 incorporated ^{3}H thymidine activity were determined using a beta-counter (Wallac,
214 Sweden).
215
216 **Statistical Analyses**
217 The results were expressed as mean ± standard deviation (SD) of the indicated
218 number of experiments. Statistical significance was estimated using a Student's *t* test.
219 *P*-values < 0.05 and < 0.01 were considered statistically and highly significant,

respectively.

Results and Discussion

Physicochemical Properties and immune activities of SPG-1

1.23 g of SPG-1, in which protein content (21.5 mg/g) was lower than carbohydrate (973.2 mg/g), was yield from 100 g of potato tuber (dry basis). Average molecular weight of SPG-1 was 508.3 kD. The 5 mg of glycoprotein yield per 1 g of fresh tuber that was reported by Kim *et al.*[31] and the 1.73 mg of potato lectin yield from 100 g of potato tuber that was reported by Pramod and Venkatesh[32] are all lower than that of the present study. Nevertheless, the fact that potato lectin is only a portion of glycoproteins and the yield from this study was based on a dry basis should be noted. The average molecular weight of SPG-1 is higher than that of potato lectin (100–110 kD)[32] and white-skinned sweet potato (*Ipomoea batatas* L.) arabinogalactanprotein (13 kD)[9]. This difference may be attributed to that both lectin and arabinogalactanprotein are only portions of glycoprotein.

The SPG-1 solution that was treated with NaOH exhibited more obvious absorbance (OD_{240nm} = 1.340) than the untreated compound (OD_{240nm} = 1.022). SPG-1 was found to contain 15 types of amino acids (date not shown). Compared with the untreated compound, the treated compound showed decreased threonine and serine contents from 2.35 mg/g to 1.90 mg/g, 1.35 mg/g to 0.59 mg/g, respectively. Conversely, alanine increased from 2.30 mg/g to 3.10 mg/g. These results show that SPG-1 consists of O-glycopeptide linkages.[33]

Agar diffusion plate assay and lymphocyte transformation test were performed to detect serum lysozyme activity and T cell immune response (cpm value), respectively.

The serum lysozyme activity and cpm value of SPG-1 group were higher than the NC group (Figure 1). The activities increased as the doses were increased, when the dose was 80 mg/kg·d, the difference between the SPG-1 group and NC group were significantly ($P<0.05$).

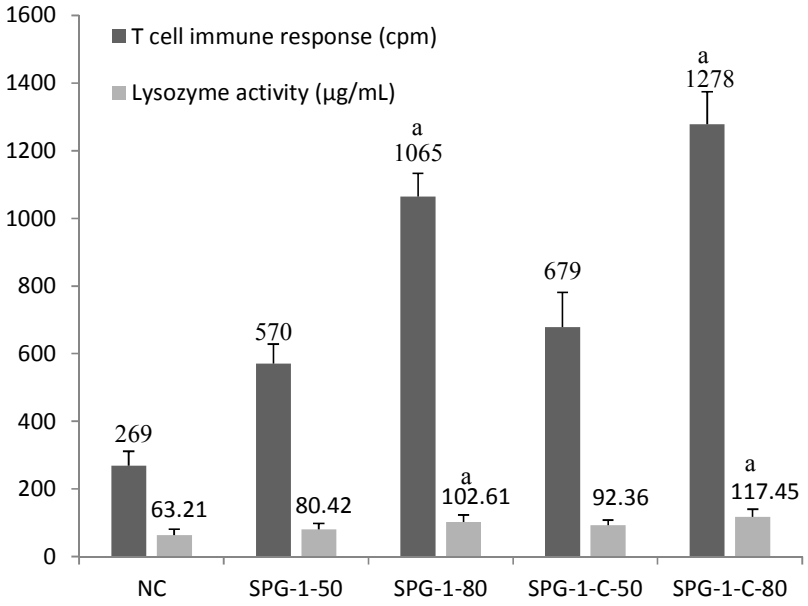

Fig. 1 SPG-1 and SPG-1-C immune activities

Note: NC: normal control group; SPG-1-50: 50 mg/kg·d SPG-1 group; SPG-1-80: 80 mg/kg·d SPG-1 group; SPG-1-C-50: 50 mg/kg·d SPG-1-C group; SPG-1-C-80: 80 mg/kg·d SPG-1-C group; a: $P < 0.05$ compared with NC group.

Effect of Protease Treatments on SPG-1 T Cell Immune Response

SPG-1 was treated with pepsin and trypsin in vitro to determine if T cell immune response of SPG-1 increases or decreases antigenicity after digestion. The changes in cpm values are shown in Table 1. The cpm values of SPG-1 decreased with the

increase in duration and dose of protease treatment. After treatment with 5.0 g/100 mL protease for 5 h, the antigenicity activity significantly ($P < 0.05$) changes with respect to that of the NC and 0 h- treated group. However, both treated groups showed significantly ($P < 0.01$ or $P < 0.05$) higher activities than that of the NC group. Thus, pepsin and trypsin slightly decreased SPG-1 immune activity.

Table 1. Effect of protease (pepsin and trypsin) treatments on T cell immune response (cpm value) of SPG-1.

Enzyme	Content of Enzyme (g/100 mL)	Enzyme-treatment time (h)					
		0	1	2	3	4	5
Pepsin	2.0	1135±65[b]	1090±52[b]	1061±56[b]	903±49[b]	831±43[b]	706±42[ac]
	5.0	1135±65[b]	1060±50[b]	945±51[b]	875±48[b]	770±46[ac]	687±32[ac]
Trypsin	2.0	1135±65[b]	964±48[b]	880±52[b]	784±38[b]	709±29[b]	653±36[bc]
	5.0	1135±65[b]	922±42[b]	837±38[bd]	760±41[ad]	667±33[d]	560±29[ac]

Note: Cpm value of normal control (NC) group is 250 ± 27; a: $P < 0.05$ compared with NC; b: $P < 0.01$ compared with NC; c: $P < 0.05$ compared with 0 h treatment group; d: $P < 0.01$ compared with 0 h- treated group.

Effect of Glucoamylase Treatment on SPG-1 T Cell Immune Response

T cell immune response of SPG-1 decreased with increase in duration and dose of glucoamylase treatment (Fig. 2). After treatment with 0.15 and 0.15 g/100 mL glucoamylase for 5 and 4 h, cpm values of SPG-1 significantly ($P < 0.05$) changed with respect to that of 0 h-treated group. This finding may be attributed to the ability of glucoamylase (1,4-α-D-glucanglucohydrolase) to change carbohydrate structures

by hydrolyzing α-(1→4), and, at lower rate, α-(1→6) glycosidic linkages to remove glucose units from the nonreducing end of substrates.[34] However, the cpm values of the treated groups were still significantly ($P < 0.01$ or $P < 0.05$) higher than that of the NC group. Thus, the glucoamylase treatment partly decreased SPG-1 immune activity.

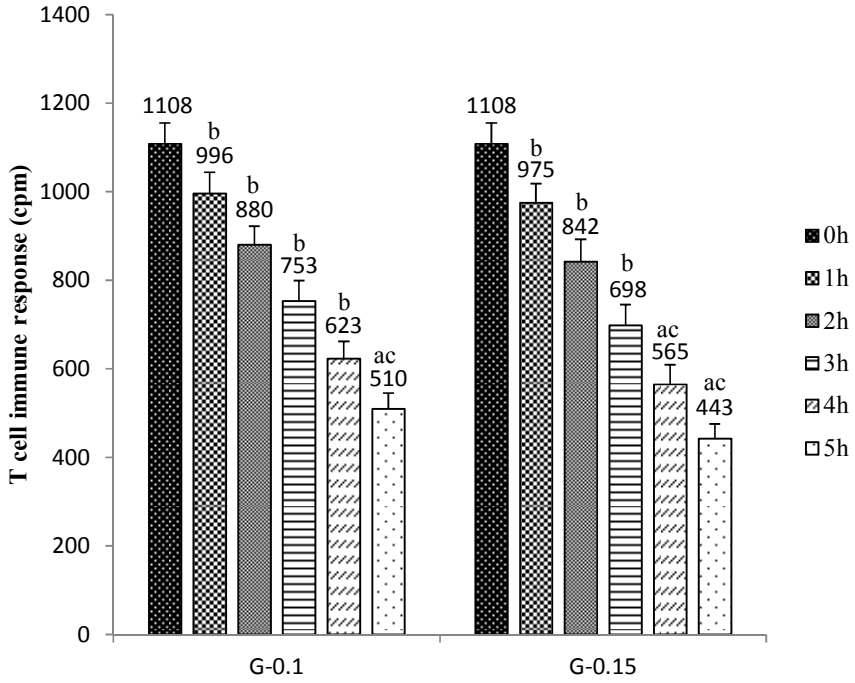

Fig. 2 Effect of glucoamylase treatment on T cell immune response (cpm value) of SPG-1

Note: Cpm value of NC group is 246 ± 25; G-0.1: 0.1 g/100 mL glucoamylase concentration; G-0.15: 0.15 g/100 mL glucoamylase concentration; a: $P < 0.05$ compared with NC; b: $P < 0.01$ compared with NC; c: $P < 0.05$ compared with 0 h-treated group.

Effect of $NaIO_4$ Treatment on SPG-1 T Cell Immune Response

The cpm values of $NaIO_4$ treatment group (293 ± 19) showed no significant difference ($P > 0.05$) with that of the NC group (246 ± 25), while decreased significantly ($P<0.01$) compared with that of the 0 h - treated group (1108 ± 47). Therefore, $NaIO_4$ oxidation remarkably decreased the T cell immune response of SPG-1. Considering that $NaIO_4$ could oxidize the carbohydrate portion of SPG-1,[35] these findings may indicated that the carbohydrate portion of SPG-1 is the major immune active site.

Effect of Acetylation on SPG-1 Immune Activity

The result of the effect of acetylation on SPG-1 immune activity is shown in Table 2. The serum lysozyme activity and T cell immune response of SPG-1 increased when the DS of acetylation increased from 0.28 to 0.67, and subsequently decreased when DS increased from 0.67 to 0.98. Except the high modification group, serum lysozyme activities of modification groups and unmodified control group were significantly ($P<0.01$ or $P<0.05$) higher than NC group. The T cell immune responses of modification groups and unmodified control group were significantly ($P<0.01$) higher than NC group, meanwhile the medium modification group was significantly ($P<0.05$) higher than unmodified control group. Thus, moderate degree of acetylation increased SPG-1 immune activity. This finding is consistent with previous reports,[36] in which biological properties were related to acetylation degree.

Table 2. Effect of acetylation on SPG-1 immune activity.

Group	Modification degree (DS)	Lysozyme activity (μg/mL, n=10)	T cell immune response (cpm)
NC	—	62.56±14.35	234±28
Unmodified control	0.00	103.30±18.22[a]	1060±47[b]
Low modification	0.28	114.52±16.54[b]	1245±68[b]
Medium modification	0.67	135.45±20.82[b]	1897±79[bc]
High modification	0.98	83.61±17.25	742±45[b]

Note: —: not detected; a: $P < 0.05$ compared with NC group; b: $P<0.01$ compared with NC group; c: $P < 0.05$ compared with unmodified control group.

Effect of Sulfation on SPG-1 Immune Activity

As shown in Table 3, the serum lysozyme activity and T cell immune response of the unmodified control group significantly ($P < 0.05$) higher than NC group, and the activities decreased when the DS by sulfation increased. The immune activities of highly modified group (DS = 1.56) decreased significantly ($P < 0.05$) compared with that of the unmodified control group. Furthermore, the high modification group showed no significantly ($P > 0.05$) difference with NC group. These results suggest that high modification by sulfation led to the loss of SPG-1 immune activity, which may be attributed to the fact that sulfation is a functionally significant biological modification of carbohydrates that could alter biological recognition and/or facilitate rapid protein excretion from the body when present in glycoproteins.[37]

Table 3. Effect of sulfation on SPG-1 immune activity

Group	Modification degree (DS)	Lysozyme activity (μg/mL, n=10)	T cell immune response (cpm)
NC	—	62.76±13.48	227±26
Unmodified control	0.00	101.40±17.56[a]	1093±52[b]
Low modification	0.32	91.65±12.78	942±41[b]
Medium modification	0.94	78.49±10.26	709±39[b]
High modification	1.56	65.38±12.76[c]	392±31[c]

Note: —: not detected; a: $P < 0.05$ compared with NC group; b: $P < 0.01$ compared with NC group; c: $P < 0.05$ compared with unmodified control group.

Isolation and Purification of SPG-1-C

After DEAE-cellulose-52 anion-exchange column separation, [Fig. 3(A)], 41.4 g of crude SPG-1-C was yield from 100 g of SPG-1 (dry basis). After further purification using Sephadex G-100 gel filtration column [Fig. 3(B).], 87.5 g of white SPG-1-C was yield from 100 g of crude SPG-1-C (dry basis). These results imply that the yields from SPG-1 and sweet potato to SPG-1-C are 36.2% and 0.45% (w/w, dry basis), respectively. A single peak was observed in the Sepharose CL-6B gel filtration column chromatograph [Fig. 3(C)] and a main component of absorbance at 490nm was obtained by HPLC (RT = 27.52 min, figure not shown), which suggests the homogeneous state of SPG-1-C. The average molecular weight of SPG-1-C is 392.4 kD.

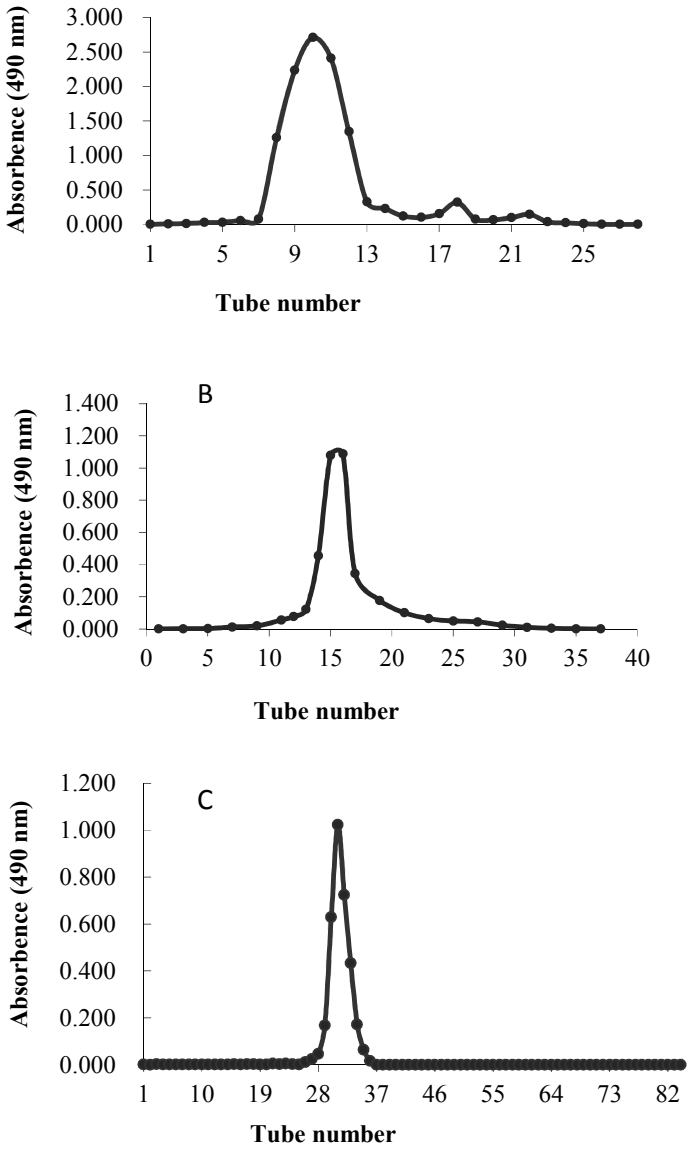

Fig. 3 Chromatographic purification of SPG-1-C

Note: (A) DEAE-cellulose-52 anion-exchange chromatograph of SPG-1-C; (B) Sephadex G-100 gel filtration chromatograph of SPG-1-C; and (C) Sepharose CL-6B gel filtration chromatograph of SPG-1-C.

SPG-1-C Immune Activities

Compared with NC group, SPG-1-C increased serum lysozyme activity and cpm

value; the activities increased as the doses were increased (see also Fig. 1). The immune activities of SPG-1-C group were higher than those of SPG-1 group at similar dose, but the differences insignificant ($P > 0.05$). Hence, the major immune activity of SPG-1 is attributed to its carbohydrate chain (SPG-1-C), which may be due to the fact that the carbohydrate content of SPG-1 is considerably higher than its protein content and the glycoprotein-glycans are valuable tools for regulating protein structure and function.[38]

Conclusions

SPG-1 increased serum lysozyme activity and T cell immune response correlated with dose. After pepsin, trypsin, and acetylation treatments, the protein portion of SPG-1 was changed, resulting to the increased or slight decreased of its immune activities. Furthermore, SPG-1 immune activities decreased or were loss after its carbohydrate portion was modified by glucoamylase, $NaIO_4$, and sulfation treatments. SPG-1-C increased immune activities with insignificant difference from SPG-1. Hence, the carbohydrate portion of SPG-1 was found to be its major immune active site. $NaIO_4$ and sulfation treatments should be avoided to maintain the immune function of SPG-1; however, moderate acetylation could be conducted. The results from this study would provide theoretical insights for future improvements on the manufacture of SPG-1. More treatments and immune tests should be conducted in further studies, and the structure of SPG-1 after treatments should be determined to completely explain its immune function.

Conflict of interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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