

# Purification and Characterization of Anti-complementary Polysaccharides from *Teucrium viscidum*

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**Abstract** The anti-complementary activities of aqueous polysaccharides from *Teucrium viscidum* and their inhibitory effects on the complement system were examined. TM4a, a polysaccharide purified by Sepharose CL-6B, showed significant anti-complementary activities on the classical, alternative and lectin pathways. The predominant linkages of TM4a consisted of 3.1 mol of 4-O-linked galactose, 2.8 mol of 3-O-linked galactose and 2.1 mol of 4-O-linked glucose; the branched polysaccharide was also indicated by the presence of 3,6-O-linked and 2,6-O-linked glucose. The inhibitory effects of TM4a and heparin showed similar results with regard to all complement pathways, except at the concentration of 100 µg/mL. After blocking the complement components with TM4a (620 µg/mL), C3 component, C3-deficient serum, or C3-deficient serum with a C3 component were added to the mixture. Hemolysis of the mixture with the C3 component, or C3-deficient serum showed about 18% or 21% hemolysis of EA cell, while the mixture with the C3-deficient serum containing C3 showed an increase in the hemolysis of EA cells of 68% with regard to the classical pathway. Regarding the alternative pathway, C3 component with TM4a was not cleaved into C3b and C3a during electrophoresis.

**Keywords:** complement, anti-complementary, polysaccharide

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## 1. Introduction

The complement system is composed of more than 35 proteins in blood plasma, which can be activated by foreign pathogens, inflammatory agents and by innate immunity [1]. The complement system is activated by a three-route pathway. The differentiation between the classical and alternative pathway is fixed, depending on how C3 is activated. By involving mannose associated serine proteases, the lectin pathway goes through a more difficult process.

The activation of the complement system generates a non-enzymatic assembly of the membrane attack complex (C5b-8(9)n), which penetrate into the target cell and eliminate pathogens. The complement system also involves the opsonization of pathogens that phagocytes recognize using complement receptors [2,3]. Therefore, the deficiency of any complement component can lead to multiple recurring infections [4]. The uncontrolled activation of the complement system can lead to autoimmune diseases [5]. Accumulating evidence indicates that the activation of the complement system is involved in inflammatory disease by releasing the anaphylatoxin, C3a and C5a [1,6,7,8].

Several polysaccharides extracted from herbal drugs have been reported to inhibit complement system

activation; for example, the root of *Angelica acutiloba* contains six types of anti-complementary polysaccharides [9], the leaf of *Artemisia princeps* contains two types of anti-complementary acidic heteroglycans [10], and an anti-complementary polysaccharide isolated from the root of *Bupleurum smithii* forms a homogeneous acidic polysaccharide [11].

Previously, we confirmed that the hot-water extracts of *Teucrium viscidum* inhibit complement system activation [12]. *Teucrium viscidum* has been used for this beneficial function and is given orally to patients for the treatment of diseases for its detoxifying and anti-inflammatory effects in Korea. In this paper, the structural characterization of an anti-complementary polysaccharide isolated from *Teucrium viscidum* and its inhibitory effects on the complement system are described.

## 2. Materials and Methods

### 2.1. Materials

*Teucrium viscidum* was purchased for experimental use at Busan-Jin market in Busan, Korea. DEAE-sepharose Fast Flow and rabbit anti-human C3 serum were obtained from Sigma Co., LTD. Sepharose CL-6B for gel chromatography was purchased from Amersham Pharmacia Biotech. The normal human serum used for the

anti-complementary assay was supplied fresh from healthy adults. IgM hemolysin-sensitized sheep erythrocytes were purchased from Biotest Co.

## 2.2. General Methods

The total carbohydrate and uronic acid contents were determined using the phenol-sulfuric acid method [13] and the m-hydroxydiphenyl method [14], respectively, using glucose and galacturonic acid as the respective standards.

## 2.3. Extraction and Fractionation of Anti-complementary Polysaccharides

The dried leaves of *Teucrium viscidum* (600 g) were extracted with hot water for 4 h and lyophilized. The MeOH-insoluble precipitate from the lyophilized extract was dissolved in distilled water. The precipitate obtained by the addition of ethanol (5 volumes) was collected by centrifugation and redissolved in distilled water, and the crude polysaccharide (TM-1) was obtained as the lyophilisate. TM-1 (15.8 g) was dissolved in distilled water (790 mL) and treated with an equal volume of an 8% solution of cetyltrimethylammonium bromide (cetavlon) using the method of Yamada et al [15]. After standing at 20°C for 20h., the precipitate was dissolved in a 10% NaCl solution. Potassium acetate and 2 volumes of ethanol were added to the solution, and the resulting precipitate, the acidic polysaccharide fraction, was redissolved in distilled water (TM-2). After the addition of 1% H<sub>3</sub>BO<sub>3</sub> to the supernatant, the solution was adjusted a pH of 8.8 using 2 M NaOH. The resulting precipitate was acidified with 2% acetic acid and dissolved in a 10% NaCl solution. Ethanol (3 volumes) was added to the solution together with potassium acetate, and the precipitate was dissolved in distilled water (TM-3). The final supernatant of the cetavlon fraction was acidified to a pH of 4.4 with acetic acid. Three volumes of ethanol were then added to the solution together with potassium acetate, and the precipitate was dissolved in distilled water (TM-4).

## 2.4. Ion-exchange and Gel Chromatography of Anti-complementary Polysaccharides

TM-4 was applied to a DEAE-Sepharose Fast Flow column (3.2×24 cm) with chloride as a counter-ion. The column was first eluted with water at 1 mL/min, followed by an NaCl gradient (0-1 M). TM4- II a showed the most potent anti-complementary activity when eluted with an NaCl concentration between 0.1 and 0.15 M NaCl. TM4- II a was further purified by gel column chromatography on a Sepharose CL-6B column, which produced broad carbohydrate peaks. The carbohydrate profile was determined using phenol-sulfuric acid.

## 2.5. Analysis of Methanolysis

The polysaccharide samples (100µg), standards (100 nmol) and myo-inositol were vacuum dried over P<sub>2</sub>O<sub>5</sub> for 24 h, and then subjected to methanolysis with methanolic HCl for 16 h at 70°C [16]. The samples were dried with nitrogen at room temperature, 2-methyl-2-propanol was

added and the samples were dried again. Prior to trimethylsilylation the samples were vacuum dried over P<sub>2</sub>O<sub>5</sub> for 4 h. The samples were then subjected to gas chromatography.

## 2.6. Methylation Analysis

Methylation analysis was performed according to the Hakomori method [17]. A dry polysaccharide sample (5 mg) was dissolved in dry dimethyl sulfoxide (DMSO) under nitrogen at room temperature for 20 min. After the addition of methyl sulfinyl methyl sodium to produce alkoxide, which was permethylated by treatment with methyl iodide (0.3 mL), the permethylated polysaccharide was hydrolyzed with 2 M TFA (1 mL) at 121°C for 1.5 h. The permethylated monosaccharides released upon hydrolysis were reduced by the addition of a sodium borodeuteride solution, followed by acetylation with acetic anhydride (1 mL). The alditol acetates were analyzed by gas chromatography-mass spectrometry (GS/MS) on a Varian Saturn 2000 instrument.

## 2.7. Anti-complementary Activity Assay via the Classical, Alternative and Lectin Pathways

The anti-complementary activity assay using the classical pathway was performed with gelatin-veronal-buffered saline containing Mg<sup>++</sup> and Ca<sup>++</sup> (GVB<sup>++</sup>). Dilutions of various polysaccharide fractions in distilled water and heparin (used as a positive control), were incubated with 1:10 dilutions of NHS and GVB<sup>++</sup>. The mixtures were pre-incubated at 37°C for 30 min, followed by a second incubation with IgM hemolysin-sensitized sheep erythrocytes (EA, 4×10<sup>8</sup> cells) at 37°C for 1 h, according to the method of Kabat and Mayer [18]. The anti-complementary activity assays using the alternative and lectin pathways were performed using the procedure described above, except for the use of rabbit erythrocytes (ER, 3×10<sup>8</sup> cells, EGTA-GVB<sup>++</sup>) instead of EA for the alternative pathway and sheep erythrocytes (EA, 5×10<sup>8</sup> cells) coated with mannan instead of EA for the lectin pathway. The anti-complementary activities of the polysaccharide fractions were expressed as the percent inhibition relative to the TCH<sub>50</sub> of the control.

## 2.8. Periodate Oxidation and Controlled Smith Degradation

The polysaccharide sample was oxidized with 20 mM sodium metaperiodate at 4°C for 56 hr, and reduced with sodium borohydrate at room temperature for 4 h. The reduced polyalcohols were hydrolyzed with 0.1M sulfuric acid for 24 h and neutralized with BaCO<sub>3</sub>. The neutralized sample was purified on a Sephadex G-25 column and converted to partially methylated alditol acetate.

## 2.9. Inhibition of the C3-component by the Anti-complementary Polysaccharide

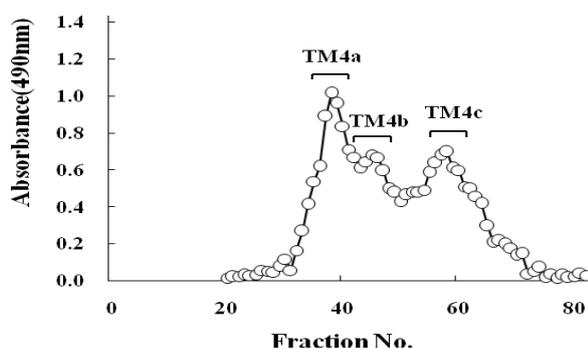
The inhibition effects of the C3 component by anti-complementary polysaccharides were assessed in a

C3-deficient serum (Sigma) via the classical pathway [11]. The minimum concentration of polysaccharides required for the complete loss of hemolytic activity in a 1:10 dilution of NHS (100 $\mu$ l) was 620  $\mu$ g/mL. A 1:10 NHS solution (100 $\mu$ l) with an equal volume of polysaccharide (620  $\mu$ g/mL) was incubated at 37°C for 30 min. The mixtures were then incubated at 37°C for 15 min after the addition of a C3-deficient serum (1:5 dilution, 50 $\mu$ l), and a C3 component (10  $\mu$ g) in GVB<sup>++</sup> (1:5 dilution, 50 $\mu$ l). After incubation at 37°C for 15 min, the mixtures were incubated again with IgM hemolysin-sensitized sheep erythrocytes at 37°C for 20 min. The percent hemolysis was determined as the absorbance of the supernatant after centrifugation. Additionally, we confirmed C3-inhibition using the alternative pathway by electrophoresis. C3 was incubated in with or without polysaccharides for 30 min at 37°C, and then incubated with the alternative complement components (Factor B, Factor D, and Properdin) for 20 min at 37°C. SDS-PAGE was performed under reducing conditions on 10% acrylamide gels.

### 3. Results

#### 3.1. Purification of the Anti-complement Polysaccharides

TM4, the most active fraction as determined using cetavlon, was further separated into one unabsorbed fraction (TM4-□) and four absorbed fractions (TM4-□ a, -□ b, -□ c, and -□ d) by elution with a linear gradient of NaCl (0~1 M) on the DEAE-Sepharose Fast Flow column. TM4- II a, which was eluted between NaCl concentration of 0.1 M and 0.15 M, showed the most potent anti-complementary activity (Min et al., 2001). We further purified TM4- II a by gel column chromatography on a Sepharose CL-6B column. As a result, three polysaccharide fractions (TM4a, TM4b and TM4c) were obtained (Figure 1). To determine the molecular weights of TM4a, TM4b and TM4c, each fraction was applied to a Sepharose CL-6B gel column and eluted with 150 mM NaCl. The molecular weights of TM4a, TM4b and TM4c were 387,000, 260,000 and 52,000 Da, respectively (data not shown), and the fraction showing the highest anti-complementary activity in the classical pathway was TM4a (Figure 3), with an approximate yield of 0.15%. TM4b showed the second highest activity, followed by TM4c.



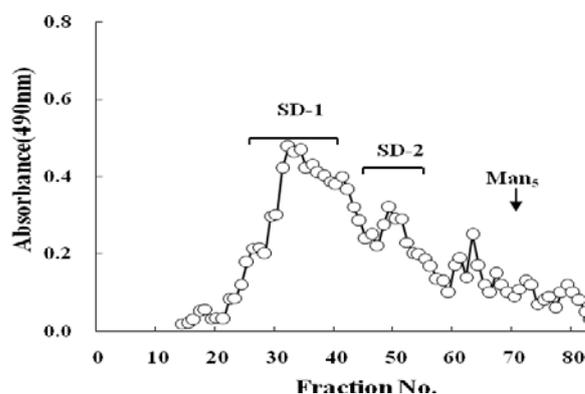
**Figure 1.** Gel column chromatography of TM4-□ a on Sepharose CL-6B. TM4-□ a was chromatography by a column of Sepharose CL-6B eluted with 150mM NaCl

#### 3.2. Structural Characterization of TM4a

TM4a, the most active polysaccharide fraction, was examined to investigate the types of glycosidic linkages by methylation, and periodate oxidation and controlled Smith degradation.

Based on the analysis of the methylated products, the predominant linkages of TM4a were found to be 4-O-linked galactose (3.1 mol), 3-O-linked galactose (2.8 mol) and 4-O-linked glucose (2.1 mol). The 3,6-O-linked and 2,6-O-linked glucose indicating the branched polysaccharide contained 1.8 and 0.8 mol, respectively.

To further structurally characterize TM4a, this fraction was examined during the periodate oxidation of sugars, followed by reduction with sodium borohydrate; then, the reduced polyalcohols were subjected to mild acid hydrolysis of the oligo- or polysaccharides. After the mild acidic hydrolysis of TM4a, two fractions (SD-1 and SD-2) were separated by gel column chromatography on a Sephadex G-25 column (Figure 2). SD-1 was mainly composed of arabinose, galactose and glucose, as well as small amounts of galacturonic acid and glucuronic acid and erythritol. SD-2 contained mostly arabinose and galactose. Using methylation analysis, SD-1 was found to contain mainly the 3-O-linked and the branched 3,6-O-linked galactose. SD-2 mainly contained glycosidic linkages in the forms of 3-O-linked galactose and 3,6-O-linked galactose.



**Figure 2.** Gel column chromatography of the periodate oxidation and controlled Smith degraded TM4a on Sephadex G-25. TM4a was subjected to the periodate oxidation and controlled Smith degradation and then applied to the column with 150mM NaCl

#### 3.3. Inhibition of the Classical, Alternative and Lectin Pathway of the Complement System

TM4a, TM4b, TM4c, and heparin inhibited the hemolysis of the EA and ER cells in a dose-dependent manner in the classical, alternative and lectin pathways. The anti-complementary system activated the classical, alternative and lectin pathways simultaneously when both TM4a and heparin (positive control) were used. TM4a exerted a slightly weaker effect than heparin with regard to inhibiting the activation of all pathways at low concentrations; however, when the concentration was more than 500  $\mu$ g/mL, TM4a exhibited the same inhibitory effect as heparin. The anti-complementary activities of the polysaccharides (TM4a, TM4b, and TM4c) and heparin on

the classical pathway are shown in Figure 3. The percent inhibition caused by TM4a in the classical pathway was approximately 98% when 620 µg/mL of polysaccharides were added, although TM4a was incubated with NHS (1:100 dilution) and EA cells ( $4 \times 10^8$  cells). The inhibitory effects of TM4b, TM4c, and heparin at a concentration of 620 µg/mL were 72%, 45%, and 97%, respectively. The anti-complementary activities of TM4a and heparin valuated using the alternative pathway were lower than those determined using the classical pathway at a concentration of 100 µg/mL. Thus, higher concentrations of TM4a and heparin were required to obtain the same inhibitory effect as observed with the classical pathway (Figure 4). The no difference in the inhibitory effects of TM4a and heparin were observed when the alternative pathway was used, except at a concentration of 100µg/mL. The anti-complementary activities of TM4b and TM4c had the highest inhibitory effects at concentrations of 1 mg/mL of TM4b (69%), and 1.5 mg/mL of TM4c (46%). The inhibitory activities of the complement system evaluated using the lectin pathway are shown in Figure 5. At a concentration of 500µg/mL, TM4a and heparin, caused more than 95%-inhibition of the lectin complement system. Likewise, for the classical and alternative pathways, the inhibitory effects of TM4a and heparin were similar, except at a concentration of µg/mL. Conversely, TM4b showed weak inhibitory effects on the classical and alternative pathways, but had a potent inhibitory effect on the lectin pathway. At a concentration of 1000 µg/mL, TM4b exhibited 90% inhibition of the lectin complement system.

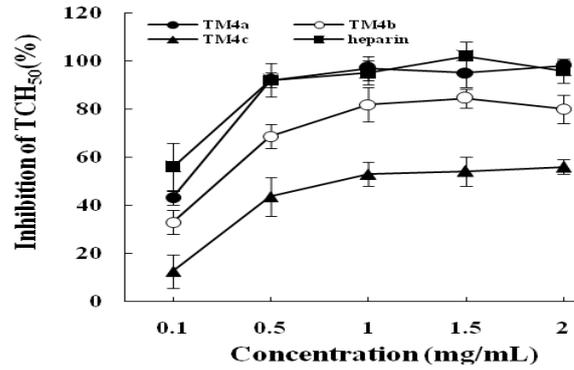


Figure 3. Inhibition effects on the classical pathway of heparin, and polysaccharides obtained by Sepharose CL-6B

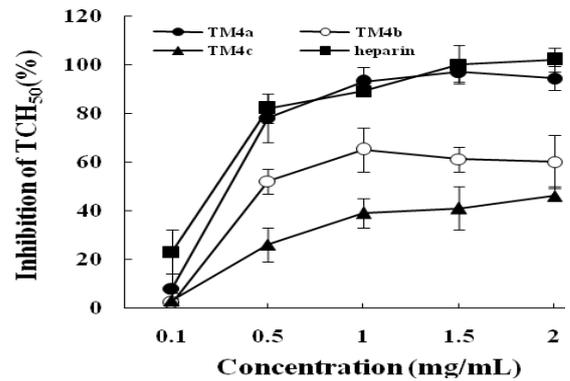


Figure 4. Inhibition effects on the alternative pathway of heparin, and polysaccharides obtained by sepharose CL-6B

Table 1. Methylation analysis of TM4a, SD-1 and SD-2

Glycose	Position of O-Methyl groups	Deduced glycosidic linkages	Molar ratio		
			TM4a	SD-1	SD-2
Rhamnose	2,3,4	Terminal	0.3	-	-
	3	2	0.7	-	-
Arabinose	2,3,4	Terminal(p)	1.6	-	-
	2,4	3(p)	2.2	1.1	0.7
	2,5	3(f)	0.9	1.9	2.1
Galactose	2,3,4,6	Terminal	0.6	trace	-
	2,3,6	4	3.1	0.2	-
	2,4,6	3	2.8	2.0	4.8
	2,4	3,6	1.8	2.2	2.4
Glucose	2,3,4,6	Terminal	0.5	-	-
	2,4,6	3	1.0	1.0	1.0
	2,3,6	4	2.1	trace	-
	3,4	2,6	0.8	0.5	trace

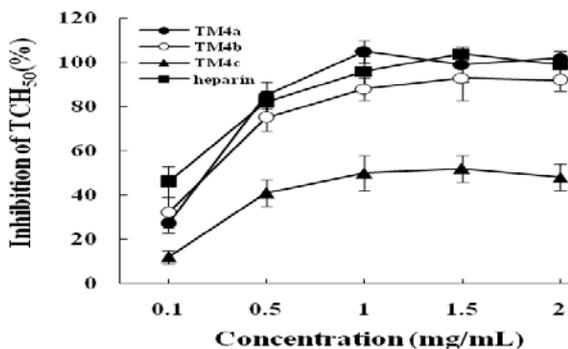


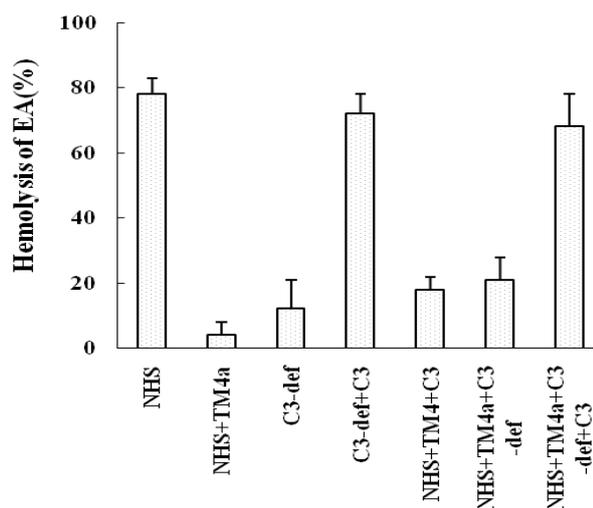
Figure 5. Inhibition effects on the lectin pathway of heparin, and polysaccharides obtained by sepharose CL-6B

Table 2. Chemical composition of the SD-1 and SD-2 fractions

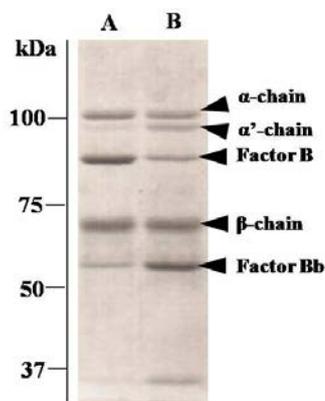
Glycose	SD-1	SD-2
Arabinose	20.1	17.9
Galactose	46.2	68.3
Glucose	16.0	5.6
Galacturonic acid	8.4	3.3
Glucuronic acid	3.6	1.6
Erythritol	4.7	trace

### 3.4. Inhibition of the Complement Components by TM4a

We measured the hemolysis of EA cells in normal human serum (approximately 1:10 dilution) with 620  $\mu\text{g/mL}$  of TM4a, C3-deficient serum, and C3-deficient serum with C3 component on the classical pathway. As shown in Figure 6, the NHS control hemolyzed approximately 78% of EA cells ( $4 \times 10^8$  cell). NHS with TM4a (620  $\mu\text{g/mL}$ ) showed the inhibition effect of approximately 96% of the EA cells, although there was a small discrepancy when the classical pathway was used. The only 14% of the EA cells were hemolyzed when they were incubated with C3-deficient serum, but 72% of the EA cells were hemolyzed when the C3 component was added. After blocking the complement components with TM4a (620  $\mu\text{g/mL}$ ), C3 component, C3-deficient serum, or C3-deficient serum with C3 component were added to the mixture. Hemolysis of the mixture with the C3 component, or C3-deficient serum showed about 18% or 21% hemolysis of EA cell, while the mixture with the C3-deficient serum added C3 component showed an increase in the hemolysis of EA cells of 68% with regard to the classical pathway.



**Figure 6.** The inhibition of classical pathway by blocking the complement components of TM4a. TM4a-treated normal human serum (NHS) to restore hemolytic capacity was incubated with C3-deficient serum (C3-def) or C3 component



**Figure 7.** The inhibition of the alternative pathway by blocking C3 component with TM4a as determined using electrophoresis. A: TM4a-treated with NHS; B: TM4a-not treated with NHS

We also investigated whether TM4a inhibits the C3 component in the alternative pathway (Figure 6). C3 with TM4a were not cleaved into C3b and C3a, as determined by electrophoresis. The C3 mixtures that contained other alternative components (Factor B and D, and Properdin) cleaved into C3b, but the C3 with TM4a mixture showed only a partial release of C3b from the original C3.

## 4. Discussion

Inflammation by several plasma enzymes, including complement, involves a variety of different mechanisms that are activated in the body in response to tissue injury. Inflammatory mediators are also released by resident cells in tissues, such as mast cells, and by cells recruited from the blood stream, such as activated monocytes and neutrophils. The role of complement in inflammation is to release small fragments of the anaphylatoxins C3a and C5a [19,20,21], these fragments are 77 and 74 amino acids in size and are cleaved from the N-terminal of  $\alpha$ -chains of C3 and C5, respectively. Both C3a and C5a peptides are converted to C3a des-Arg and C5a des-Arg, but do not exhibit the same activities as anaphylatoxin, due to the removal of the C-terminal arginine by endogenous serum carboxypeptidase N [22]. C3a and C5a express a wide variety of biological activities, including smooth muscle contraction, platelet and neutrophil activation, and immunoregulatory reactions [23,24,25]. The mode for release of C3a differs on how it is cleaved in from an activation pathway. C3a is cleaved from the C4bC2a complex that is formed by the C1qrs complex in the classical pathway, and by mannose-associated serine protease-2 (MASP-2) in the lectin pathway [26,27]. In the alternative pathway, C3a is cleaved by spontaneous low-rate hydrolysis of the thioester of C3; this process generates C3(H<sub>2</sub>O), which is a molecule that functions in a manner similar to that of C3b [28]. The mode of generating C5a utilizes C5 convertase, which interacts with the C3b in each activation pathway and controls its formation. It is clear that complement plays an important role in the innate immune system. However, it may also cause inflammation and autoimmune diseases if the complement cascade is inappropriately activated or insufficiently regulated. Therefore, the inhibition and modulation of complement activity has been recognized as a promising therapeutic strategy for many years. Several polysaccharides isolated from Chinese herbs have been reported to reduce the inflammation response due to complement activation.

In this study, we isolated a polysaccharide, TM4a, which contains a high amount of arabinose and galactose, and inhibits complement activation. This polysaccharide was extracted from the *Teucrium viscidum* leaf using hot-water. TM4a mainly consisted of 4-O-linked galactose, 3-O-linked galactose and 4-O-linked glucose with branched 3,6-O-linked galactose. We also investigated a partial structure and interconnection of the backbone of TM4a using the periodate oxidation and Smith degradation techniques. Periodate oxidation and Smith degradation of polysaccharides are valuable analytical techniques that have been used in structural characterization

and sequence determination; TM4a residues containing hydroxyl residues on adjacent carbon atoms are oxidized to dialdehydes by cleavage between their hydroxyl residues. The oxidized TM4a residues were reduced with sodium borohydrate; the TM4a was then subject to acid hydrolysis and separated into two fractions (SD-1, SD-2) using a Sepadex G-25 column. The detection of erythritol in SD-1 indicates that the 4-position of galactose and(or) glucose at the reducing terminal in SD-1, a sub-fraction of TM4a, was attached to SD-2, which contains a long chain (1→3)-galactan.

It has previously been reported that a branched structure and an abundance of arabinose and galactose in the pectic and neutral polysaccharides is important for anti-complementary activity [9,10,11]. However, the complicated three-dimensional structures of anti-complementary polysaccharides continue to hinder the elucidation of the relationship between the structure of polysaccharides and their anti-complementary activity. Heparin containing carbohydrates are known to be complement inhibitors [29]. Heparin is a sulfated copolymer of uronic acid and glucosamine, which blocks the interaction between C1q and complement activators, such as C1s, and inhibits the assembly of C3 convertases in the classical and alternative pathways [30,31]. The complement inhibition of TM4a with regard to all activation pathways was similar to that of heparin. NHS (1:10 dilution) with a TM4a concentration chosen to achieve the complete eradication of hemolytic activity didn't exhibit restored hemolytic behavior with regard to EA cells when the C3-deficient serum was added. If TM4a did not block C3 in 1:10 diluted NHS, the addition of the C3-deficient serum would attenuate the hemolysis of EA cells in the classical pathway. Therefore, the results of the hemolysis experiments with C3-deficient serum indicated that TM4a might selectively block either C3-cleavage or the assembly of C3 convertase in the classical pathway. In the alternative pathway, TM4a, did not cleave C3 into C3b and C3a, even though C3 was incubated with other alternative components. These results suggest that TM4a, a polysaccharide isolated from the leaves of *Teucrium viscidum*, is able to effectively control the inflammation response through the classical, lectin and alternative pathways. The utilization of TM4a may be a novel non-toxic and low-cost complement-targeted therapeutics strategy because of the abundance of this natural resource.

## 5. Conclusions

The present study demonstrated that Polysaccharides were separated from the hot-water extracts of *Teucrium viscidum* using the gel filtration and among these TM4a exhibited the highest anti-complementary activity. TM4a mainly consisted of 4-O-linked galactose, 3-O-linked galactose and 4-O-linked glucose with branched 3,6-O-linked galactose, and contained a long chain (1→3)-galactan. TM4a has an anti-complementary activation in the classical, alternative, and lectin pathway and an inhibitory effect in inflammatory reaction by releasing the anaphylatoxin, C3a and C5a.

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## References

- [1] Walport, M.J., Complement First of two parts. *N Engl. J. Med.*, 344, 1058-1066, 2001.
- [2] Carroll, M.C., "The complement system in regulation of adaptive immunity," *Nature Immunology*, 5, 981-986, 2004.
- [3] Muller-Eberhard, H.J., "The membrane attack complex of complement," *Annu Rev Immunol.*, 5, 503-506, 1986.
- [4] Morgan, B.P. and Walport, M.J., "Complement deficiency and disease," *Immunol. Today*, 12, 301-306, 1991.
- [5] Kozlov, L.V., Burdelev, O.O., Bureeva, S.V. and Kaplun, A.P., "Artificial inhibition of the complement system," *Russian journal of bioorganic chemistry*, 33, 449-473, 2007.
- [6] Volanakis, J. E. and Arlaud, G. J., "in *The Human Complement System in Health and Disease* (Frank, M., and Volanakis, J. E., eds)," Marcel-Dekker, New York, 49-81, 1998.
- [7] Sahu, A., Morikis, D. and Lambris, J.D., "Complement inhibitors targeting C3, C4, and C5; in Therapeutic interventions in the complement system (eds)," 75-112, 2000.
- [8] Markiewski, M.M. and Lambris, J.D., "The role of complement in inflammatory diseases from behind the scenes into the spotlight," *Am. J. Pathol.*, 171, 715-727, 2007.
- [9] Kiyohara, H. and Yamada, H., "Structure of an anti-complementary arabinogalactan from the root of *Angelica acutiloba* Kitagawa," *Carbohydrate Res.*, 193, 193-200, 1989.
- [10] Yamada, H., Otsuka, Y. and Omura, S., "Structural characterization of anti-complementary polysaccharides from the leaves of *Artemisia princeps*," *Planta medica*, 52, 311-320, 1986.
- [11] Han, X., Zhang, Y., Zhang, J. and Chen, D., "Isolation and characterization of anti-complementary polysaccharide D3-S1 from the roots of *Bupleurum smithii*," *Int. J. Immunopharmacol.*, 7, 175-182, 2007.
- [12] Min, J.G., Kim, T.J., Lee, D.S., Cho, S.W., Yoon, H.D. and Park, J.H., "Characterization of anti-complementary polysaccharide from *Teucrium viscidum*," *J. Food Sci. Nutr.*, 6, 137-141, 2001.
- [13] Dubios, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A. and Smith, F., "Colorimetric method for determination of sugars and related substances," *Analyt. Chem.*, 28, 250-256, 1956.
- [14] Blumenkrantz, N. and Asboe-Hansen, G., "New method for quantitative determination of uronic acids," *Analyt. Biochem.*, 54, 484-489, 1973.
- [15] Yamada, H., Ra, K.S., Kiyohara, H., Cyong, J.C., Yang, H.C. and Otuka, Y., "Characterization of anti-complementary neutral polysaccharides from the roots of *Bupleurum facatum*," *Phytochemistry*, 27, 3163-3169, 1988.
- [16] Chaplin, M.F., "A rapid and sensitive method for the analysis of carbohydrate components in glycoproteins using gas-liquid chromatography," *Analyt. Biochem.*, 123, 336-340, 1982.
- [17] Hakomori, S.I., "A rapid permethylation of glycolipid, and polysaccharide catalyzed by methylsulfinylcarbanion in dimethylsulfoxide," *J. Biochem. (Tokyo)*, 55, 205-208, 1964.
- [18] Kaba, E.A. and Mayer, M.M., "Complement and complement fixation, *Experimental Immuno-chemistry*." Charles C Thomas publisher, Illinois, 133, 1964.
- [19] Law, S.K. and Reid, K.B.M., "Complement, in *Focus Series(2ed)*," D. Male, Ed. IRL Press, Oxford, UK, 66-71, 1995.
- [20] Makrides, S.C., "Therapeutic inhibition of the complement system," *Pharmacol. Rev.*, 50, 59-87, 1988.
- [21] Pugsley, M.K., Abramova, M., Cole, T., Yang, X. and Ammons W.S., "Inhibitors of the complement system currently in development for cardiovascular disease," *Cardiovascular Toxicology*, 3, 43-69, 2003.
- [22] Wetsel, R.A., Kildsgaard, J. and Haviland, D.L., "Complement anaphylatoxins (C3a, C4a, C5a) and their receptors (C3aR, C5aR/CD88) as therapeutic targets in inflammation," Humana Press, pp 113-153, 2000.

- [23] Ehrenguber, M.U., Geiser, T. and Deranleau, D.A., "Activation of human neutrophils by C3a and C5A: comparison of the effects on shape changes, chemotaxis, secretion, and respiratory burst," *FEBS Lett.* 346,181-184, 1994.
- [24] Fukuoka, Y. and Hugli, T.E., "Demonstration of a specific C3a receptor on guinea pig Platelets," *J. Immunol.*, 140, 3496-3501, 1988.
- [25] Haviland, D.L., McCoy, R.L., Whitehead, W.T., Akama, H., Molmenti, E.P., Brown, A., Haviland, J.C., Parks, W.C., Perlmutter, D.H. and Wetsel, R.A., "Cellular expression of the C5a anaphylatoxin receptor (C5aR): demonstration of C5aR on nonmyeloid cells of the liver and lung," *J. Immunol.*, 154, 1861-1869, 1995.
- [26] Schwaeble, W.J., Dahl, M.R., Thiel, S., Stover, C.M. and Jensenius, J.C., "The mannan-binding lectin-associated serine proteases (MASPs) and Map19: four components of the lectin pathway activation complex encoded by two genes," *Immunobiology*, 205, 455-466, 2002.
- [27] Thiel, S., Vorup-Jensen, T., Stover, C.M., Schwaeble, W., Laursen, S.B., Poulsen, K., Willis, A.C., Eggleton, P., Hansen, S., Holmskov, U., Reid, K.B.M. and Jensenius, J.C., "A second serine protease associated with mannan-binding lectin that activates complement," *Nature*, 386, 506-509, 1997.
- [28] Pangburn, M.K. and Müller-Eberhard, H.J., "Initiation of the alternative complement pathway due to spontaneous hydrolysis of the thioester of C3," *Ann. N.Y. Acad. Sci.*, 421, 291-298, 1983.
- [29] Ecker, E.E. and Gross, P., "Anticomplementary power of heparin," *J. Infect. Dis.*, 44, 250-253, 1929.
- [30] Wuillemin, W.A., Velthuis, H., Lubbers, Y.T.P., de Ruig, C.P., Eldering, E. and Hack, C.E., "Potentiation of C1 inhibitor by glycosaminoglycans: Dextran sulfate species are effective inhibitors of in vitro complement activation in plasma," *J. Immunol.*, 159, 1953-1960, 1997.
- [31] Kirschfink, M., Blasé, L., Engelmann, S. and Schwartz-Albiez, R., "Secreted chondroitin sulfate proteoglycan of human B cell lines binds to the complement protein C1q and inhibits complex formation of C1," *J. Immunol.*, 158, 1324-1331, 1997.