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Purification and Chemical Properties of Anti-complementary Polysaccharide from Capsici Fructus

Kyung-Soo Ra, Haruki Yamada*, Ha-Jin Sung, Jong-Chol Cyong* and Han-Chul Yang**

Department of Food Technology, College of Agriculture, Korea Univ., Seoul 136-701 Korea
*Oriental Medicine Research Center of the Kitasato Institute, Minato-ku, Tokyo 108, Japan
**Department of Genetic Engineering, College of Agriculture Univ., Seoul 136-701 Korea

고추의 항보체 다당의 정제와 특성

나경수 · 山田陽城* · 성하진** · 정종철* · 양한철

고려대학교 농과대학 식품공학과, *일본 북리연구소 부속 동양의학 종합연구소,
**고려대학교 농과대학 유전공학과

초 록

고추에서 추출한 수용성 조다당(CAP-0)에 높은 항보체 활성이 존재함을 발견하였으며, 활성은 pronase 처리에 의해 변하지 않았으나 periodate 산화에 의해 활성이 현저히 감소하였다. CAP-0는 cetyltrimethylammonium bromide 처리에 의해 4획분(CAP-1,2,3,4)으로 분리되었으며, CAP-1에서 가장 높은 항보체 활성이 나타났다. CAP-1은 음이온 교환 chromatography에 의해 활성과 수율이 높은 CAP-1-Ⅳ와 Ⅴ로 분리되었으며, Ⅲ은 수율은 높았으나 활성이 매우 낮았다. CAP-1-Ⅲ과 Ⅳ는 gel 여과에 의해 고분자량 획분(M.W. $Ⅲ_a$ 70,000, $Ⅳ_a$ 195,000, Ⅴ 140,000)과 저분자량 획분($Ⅲ_b$, $Ⅳ_b$)으로 나뉘어졌다. 이 4획분과 Ⅴ는 gel 여과와 전기영동상에서 순수하였으며 고분자량 획분에서 항보체 활성이 높았다.

Introduction

It is known that complement system plays an important role in the host defense, inflammations and allergic reactions, and its activation occurs *via* both the classical and alternative pathways. Several anti-complementary polysaccharides have been isolated from bacteria, fungi and plants including Chinese herbs; for example, lipopolysaccharides[1~3], water-insoluble glucans from *Lentinas edodes*[4,5] and *Poria cocos*[4,5], and AR-arabinogalactan $Ⅱ_a$[6,7] and $Ⅱ_b$-1[8] from *Angelica acutiloba*.

Recently, we have found the existence of a potent anti-complementary activity in the extracts from raw foods such as Capsici Fructus (the fruits of *Capsicum annuum* L.) without seeds, which is used as an important spice in Korea. The objective of this study is to purify and identify the chemical properties of the major anti-complementary polysaccharides from Capsici Fructus.

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Corresponding author : K.S. Ra

Materials and Methods

Materials

Powder of Capsici Fructus (the fruits of *Capsicum annuum* L.) without seeds was purchased at Kyung-dong market in Korea. Sephadex G-100 and Sepharose CL-4B (Pharmacia Co. Ltd.) were used for gel filtration, and DEAE-Toyo Pearl 650C was obtained from Toyo Soda Co.

Ltd. (Japan). Pronase was purchased from Kaken Kagaku Co. Ltd. (Japan).

General methods

The total carbohydrate and uronic acid contents were determined by the phenol-H_2SO_4 [9] and m-hydroxybiphenyl methods[10] using arabinose and galacturonic acid as the respective standards. Protein was determined by the method of Lowry et al.[11] with bovine serum albumin as a standard.

Polysaccharides were hydrolyzed with 2 M trifluoroacetic acid at 121°C for 1.5 hr. The hydrolyzates were analyzed by TLC on cellulose-coated plastic sheets(Merck, 5577) with ethyl acetate-pyridine-acetic acid-water (5 : 5 : 1 : 3) as the solvent system. Reducing sugars were detected with alkaline silver nitrate[12], and uronic acid with p-anisidine hydrochloride[13]. FID/GC (flame ionization detector/gas chromatography, Shimadzu GC-6A) analysis was performed on a glass column(0.3×200cm) packed with 1% silicon OV-225 on Uniport HP at 190°C. Nitrogen gas was used as carrier at a flow rate of 60ml/min. Molar ratios of sugars were calculated from peak areas and molecular weights of the corresponding alditol acetates.

Gel filtration was carried out on Sephadex G-100 (2.6×90cm) eluted with 0.2 M NaCl. Electrophoresis (cellulose acetate membrane, Toyo Roshi Co. Ltd.) was performed in 0.08 M pyridine-0.04 M acetate buffer (pH 5.4) at 70 eV for 30 min, and toluidine blue was used for detection. Molecular weights of polysaccharides were estimated on Sepharose CL-4B(1.3×95cm) using standard dextrans(T-2000, 500, 70 and 40).

Isolation of water-soluble polysaccharides

Powder of Capsici Fructus(4kg) without seeds was extracted with methanol (4 vols, 5 times) and centrifuged (7,500 rpm, 30 min). The superantant was collected and concentrated. The cold water(16*l*) was added to the residual material and stirred in the cold room(4°C) for 24hr. The extract was centrifuged (7,500 rpm, 30 min) to remove insoluble material. The supernatant was concentrated to about 2*l* and 5 vols. of ethanol were added. The precipitate was redissolved in H_2O and dialyzed against running H_2O for 3 days. The non-dialyzable portion was centrifuged to remove H_2O-insoluble material and the supernatant (crude polysaccharide) was lyophilized (CAP-0, yield 69.9g).

Fractionation of crude polysaccharide(CAP-0)

Crude polysaccharide (CAP-0, 15.8g) was dissolved in H_2O (790ml) and treated with an equal volume of an 8% solution of cetyltrimethylammonium bromide(cetavlon) by the method of Yamada et al[14]. After standing at 20°C for 20 hr, the resulting precipitate was collected by centrifugation and redissolved in 10% NaCl. Five volumes of ethanol were added to the solution, and the resulting precipitate was dissolved in H_2O, followed by dialysis against running H_2O. The acidic polysaccharide fraction (CAP-1) was then obtained as the lyophilizate of the non-dialyzable fraction (yield, 2.3g).

The supernatant was added to an equal volume (790ml) the pH was adjusted 8.8 by the addition of 2 M NaOH followed by stirring for 24 hr. The resulting precipitate was washed with 0.5% Na-broate buffer (pH 8.8), and dissolved in 2% acetic acid (300ml). Five volumes of ethanol were added to the solution, and the resulting precipitate was dissolved in H_2O, and then dialyzed. The non-dialyzable fraction (CAP-2) was obtained as the lyophilizate (yield, 2.6g).

The pH of the supernatant was readjusted to 9.5 by the addition of 2 M NaOH. The resulting precipitate was collected as the non-dialyzable lyophilizate (CAP-3, yield 0.3g) by the same procedure used to obtain CAP-2. The final supernatant of the cetavlon fractionation was acidified with 2% acetic acid, and 5 volumes of ethanol was added. The precipitate was then washed with 2% acetic acid in ethanol and dissolved in H_2O, followed by dialysis. The non-

dialyzable portion was lyophilized to obtain CAP-4 (yield, 1.0g).

Ion-exchange chromatography

CAP-1 (500mg) was applied to a column(3.2 ×33cm) of DEAE-Toyo Pearl 650C (Cl^-) equilibrated with H_2O. The column was eluted first with H_2O(500ml) until no sugar was detected, and then the absorbed polysaccharide fractions were eluted with 0.1M~2M NaCl (step-wise). The unabsorbed fraction (CAP-1-I) and seven absorbed fractions after dialysis(CAP-1-II-VII) were obtained as lyophilizate.

Pronase digestion of the crude polysaccharide (CAP-0)

CAP-0 (50mg) was dissolved in 50ml of 50 mM Tris-HCl, pH7.9, containing 10mM $CaCl_2$, and then 50mg of pronase was added. The reaction mixture was incubated at 37°C for 48 hr with one drop of toluene. The reaction was stopped by boiling for 5min. The mixture was then dialyzed against H_2O for 2 days, and the non-dialyzable portion was lyophilized to obtain the CAP-0 pronase digest.

Periodate oxidation of the crude polysaccharide (CAP-0)

CAP-0(50mg) was dissolved in 30ml of 50mM acetate buffer, pH 4.5, and then 50mM $NaIO_4$ was added. The mixture was reacted at 4°C in the dark for 3days. Ethylene glycol (5ml) was added to remove the excess periodate, and the reaction mixture dialyzed against H_2O for 2 days. The non-dialyzable solution was concentrated to about 20ml, and then 20mg of $NaBH_4$ was added to the concentrate with continuous stirring for 12 hr at room temperature. After the neutralization of the reaction mixture with acetic acid, the oxidized CAP-0 was obtained as lyophilizate after dialysis.

Anti-complementary activity

Gelatin veronal-buffered saline (pH 7.4) containing 500μg Mg^{++} and 150μg Ca^{++} (GVB^{++}) was prepared by the method of Kabat and Mayer[15], and normal human serum(NHS) was obtained from a healthy adult. Various dilutions of polysaccharides in water (50μl) were mixed with 50μl of NHS and 50μl of GVB^{++}. The mixtures were pre-incubated at 37°C for 30min and 350μl of GVB^{++} was added. IgM-hemolysin-sensitized sheep erythrocytes (EA, 250μl) at 1 $\times 10^8$ cells/ml was added to the mixtures diluted serially and then incubated at 37°C for 1 hr. After addition of phosphate-buffered saline (PBS, pH 7.2) and centrifugation, the absorbance of the supernatants were detected at 412 nm. NHS was incubated with water and GVB^{++} as a control. The anti-complementary activity was expressed as the percentage inhibition of the total complement hemolysis (TCH_{50}) of the control.

Results

Extraction of crude polysaccharide (CAP-0) from Capsici Fructus

The crude polysaccharide (CAP-0) from Capsici Fructus(the fruits of *Capsicum annuum* L.) without seeds was prepared as non-dialyzable fraction after methanol-exrtaction and ethanol-precipitation of cold water-extracts. CAP-0 had

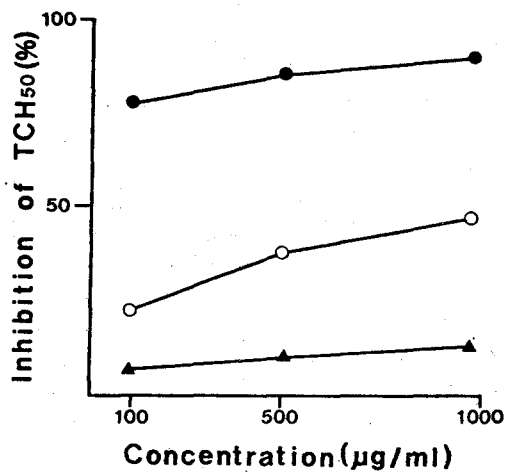

Fig. 1. Anti-complementary activities of extracts from Capsici Fructus
● : CAP-0, ○ : Methanol-soluble fraction and
▲ : Ethanol-soluble fraction

higher potent anti-complementary activity than methanol-soluble and ethanol-soluble fraction (Fig. 1). Pronase treatment on CAP-0(protein, 14.8%) had little effect on anti-complementary, activity, however, the activity of periodate oxidation product of CAP-0 (total sugar, 85.2%) was shaply decreased (Fig. 2). These results indicate that the carbohydrate moiety may contribute to that activity.

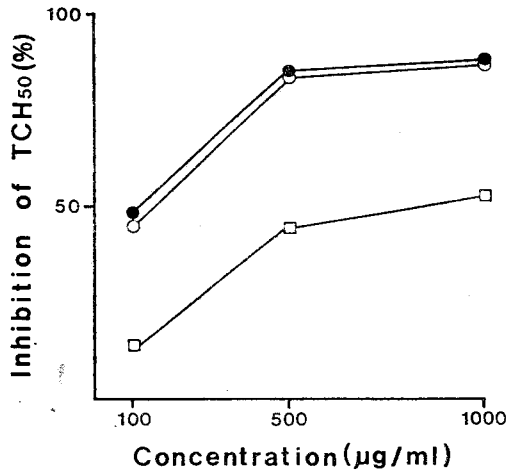

Fig. 2. Lability of anti-complementary activity to periodate and pronase treatment of crude polysaccharide(CAP-0).
● : CAP-0, ○ : CAP-0 pronase digest and
□ : $NaIO_4$ oxidized CAP-0

Fractionation of crude polysaccharide(CAP-0)

CAP-1 was fractionated to give four fractions, CAP-1,2,3 and 4 by the addition of cetyltrimethylammonium bromide. The chemical properties of these fractions are summarized in Table 1. CAP-1 contained rhamnose, arabinose, galactose and galacturonic acid as major sugars (molar ratio, 2.5 : 1.0 : 1.7 : 6.5). Whereas CAP-2,3 and 4 had mainly arabinose, galactose and galacturonic acid, but protein content of CAP-4 was the highest among the polysaccharide fraction.

Fig. 3 shows the anti-complementary activity after the incubaton of the different concentrations of polysaccharide fractions with NHS. The anti-complementary activities are shown to be dose dependent. When 1,000μg/ml of CAP-1 was incubated with an equal volume of NHS, the amount of TCH_{50} was reduced by about 95 %. The order of the activities of these fractions was CAP-1>CAP-4>CAP-2>CAP-3.

Table 1. Chemical properties of polysaccharide fractions on cetavlon treatment of CAP-0

	CAP -0	CAP -1	CAP -2	CAP -3	CAP -4
			(%)		
Neutral sugar (as Ara)	18.9	43.5	39.9	54.0	39.7
Uronic acid (as GalA)	66.3	54.5	50.2	31.6	26.6
Protein(as BSA)	14.8	2.0	9.9	14.4	33.7
Component sugars (Molar ratio)					
Rhamnose	0.7	2.5	0.6	trace	0.2
Arabinose	1.0	1.0	1.0	1.0	1.0
Xylose	0.2	0.4	0.2	trace	trace
Mannose	0.2	0.1	trace	0.2	0.1
Galactose	1.2	1.7	1.6	1.1	0.5
Glucose	0.1	1.0	trace	trace	0.1
Galacturonic acid	10.9	6.5	3.9	1.2	1.1

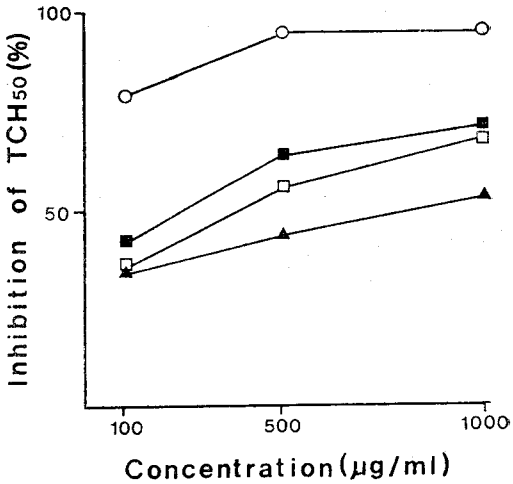

Fig. 3. Anti-complementary activities of polysaccharide fractions on cetavlon treatment of CAP-0
○ : CAP-1, □ : CAP-2, ▲ : CAP-3 and ■ : CAP-4

Fractionation of CAP-1 on ion-exchange chromatography

The most active polysaccharide fraction, CAP-1, was further separated on the column of DEAE-Toyo Pearl 650C (Cl^-) into an unab-

sorbed (CAP-1-I) and seven absorbed fractions by the step-wise elution of NaCl. The anti-complementary activities of three major fractions in yield, CAP-1-III, IV and V(eluted with 0.1 M, 0.15M and 0.2M NaCl, respectively), and two minor fractions, CAP-1-VI and VII (eluted with 0.3 and 0.4M NaCl, respectively) are shown in Fig. 4. The activities of CAP-1-VI and VII had very high activity, but were very low in yield. The activities of CAP-1-V and IV

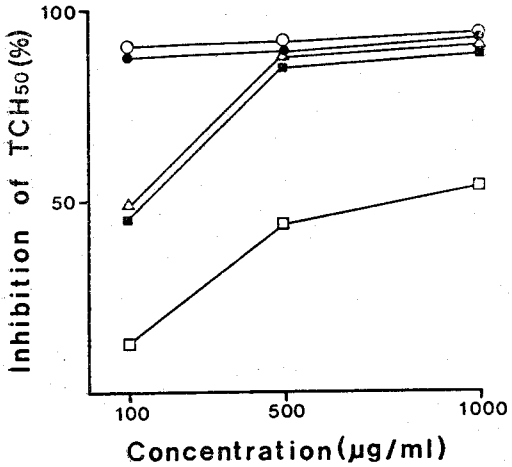

Fig. 4. Anti-complementary activities of polysaccharide fractions on DEAE-Toyo Pearl 650C (Cl^-, 3.2×33cm) of CAP-1
□ : CAP-1-III, ■ : CAP-1-IV, △ : CAP-1-V, ● : CAP-1-VI and ○ : CAP-1-VII

Table 2. Chemical properties of polysaccharide fractions on DEAE-Toyo Pearl 650C (Cl^- form) of CAP-1

	CAP1-III	CAP1-IV	CAP1-V
Neutral sugar(as Ara)	35.2	40.8	58.3
Uronic acid(as GalA)	62.8	57.8	39.6
Protein(as BSA)	2.0	1.4	2.1
Component sugars(Molar ratio)			
Rhamnose	0.6	1.8	3.8
Arabinose	1.0	1.0	1.0
Xylose	0.3	0.3	0.7
Mannose	0.1	0.1	0.1
Galactose	1.1	1.9	1.8
Glucose	trace	0.5	0.2
Galacturonic acid	5.5	7.9	2.7

were higher than that of III. The chemical properties of these fractions are illustrated in Table 2, CAP-1-III, IV and V were consisted of rhamnose, arabinose, galactose and galacturonic acid as major component sugars. But molar ratio of rhamnose and galactose in CAP-1-IV and V was higher than CAP-1-III.

Purification of the anti-complementary polysaccharide

CAP-1-III and IV were further purified by gel filtration on Sephadex G-100 (Fig. 5), and CAP-1-IIIa and b, and CAP-1-IVa and b, respectively. These four subfractions and CAP-1-V gave a single spot on cellulose-acetate membrane electrophoresis and was eluted as a single peak as shown by gel filtration of Sepharose CL-4B(data not shown). These results suggested that these five fractions were homogeneous and pure enough for structural studies.

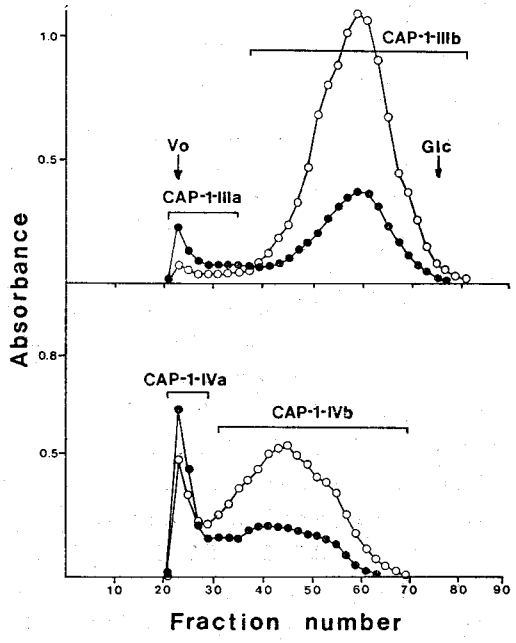

Fig. 5. Gel filtration of CAP-1-III & IV on Sephadex G-100. The column (2.6×90cm) was 0.2M NaCl and elution was performed with 0.2M NaCl.
● : Carbohydrate(490nm), ○ : Uronic acid(520nm), Vo : Void volume and Glc : Glucose

The molecular weights of CAP-1-IIIa, IVa

and V were estimated to be 70,000, 195,000 and 40,000, respectively (Fig. 6). It was observed that the fractions, CAP-1-IIIa and IVa, showed higher anti-complementary activity that those of CAP-1-IIIb and IVb (Fig. 7).

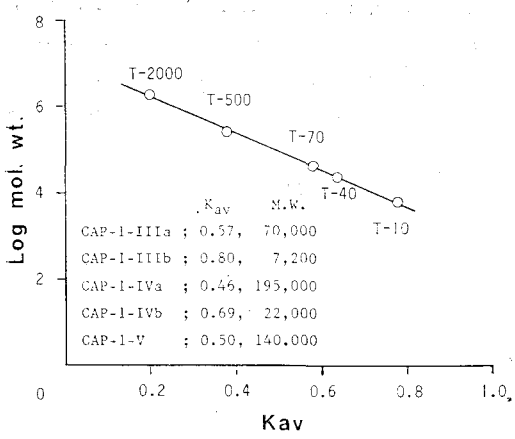

Fig. 6. Determination of molecular weights of CAP-1-IIIa, IVa and V by gel filtration on Sepharose CL-4B. The column (1.3×95cm) was equilibrated with 0.2M NaCl and elution was performed with 0.2M NaCl.

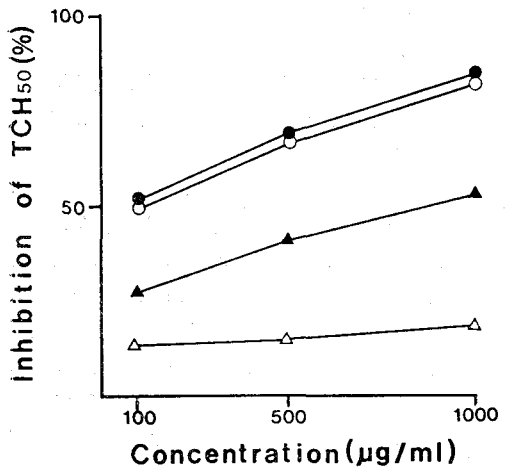

Fig. 7. Anti-complementary activities of polysaccharide fractions on Sephadex G-100 of CAP -1-III IV.
● : CAP-1-IIIa, △ : CAP-1-IIIb,
○ : CAP-1-IVa and ▲ : CAP-1-IVb

But the yield for the former fractions were very poor. The chemical properties of purified fractions on Sephadex G-100 were summarized in Table 3. The most active fractions, IIIa and IVa, contained 74.0% and 78.8% of neutral sugar, respectively. Both fractions were mainly consisted of arabinose, galactose and galacturonic acic, in a molor ratio of 1.0 : 1.3 : 0.9 in IIIa and 1.0 : 1.9 : 0.9 in IVa. Whereas CAP-1-IIIb and IVb contained a large amount of galacturonic acid, and these fractions were mainly consisted of rhamnose, arabinose and galactose as the neutral sugars.

Table 3. Chemical properties of polysaccharide fractions on Sephadex G-100 of CAP-1-III & IV

	CAP 1-IIIa	CAP 1-IIIb	CAP 1-IVa	CAP 1-IVb
Neutral sugar(as Ara)	74.0	12.9	78.8	41.0
Uronic acid(as GalA)	21.8	86.3	18.4	56.2
Protein(as BAS)	4.2	1.1	2.8	2.1
Component sugars (Molar ratio)				
Rhamnose	0.2	1.9	0.6	3.0
Arabinose	1.0	1.0	1.0	1.0
Xylose	0.2	0.5	0.2	0.4
Mannose	0.3	0.4	0.1	0.2
Galactose	1.3	0.8	1.9	1.0
Glucose	0.1	trace	0.1	trace
Galacturonic acid	0.9	30.8	0.9	7.7

Discussion

It has been reported for some anti-complementary polysaccharide from bacteria, fungi and plants, but there is little information on other types of anti-complementary polysaccharides. Recently we have found that the several anti-complementary polysaccharides exist in raw food. Capsici Fructus (the fruits of *Capsicum annuum* L.) is one of them. The cold-water extract from C. fructus without seeds contains a potent anti-complementary activity. The purification of anti-complementary polysaccharide from C. fructus and its chemical characterization were observed in this study. It has been observed that anti-complementary activity was not present in the methanol-soluble and ethanol-soluble fraction but in the crude polys-

accharide fraction. Also the activity did not change by pronase treatment, but decreased greatly by pericdate-oxidationof CAP-0. These indicate that carbohydrate moiety may be related to the anti-complementary activity.

Among the four fractions obtained by cetavlon-treatment, the fraction which contained lower molar ratio of rhamnose and galacturonic acid such as CAP-1 (Rha : GalA, 1.0 : 2.6), showed the higher anti-complementary activity. This fact coincided with GC data of fractions obtained by ion-exchange chromatography(Rha: GalA, CAP-1-III 1.0 : 9.2, IV 1.0 : 4.4, V 1.0 : 0.7). Finally, the fractions of void volume separated by Sephadex G-100, CAP-1-IIIa and IVa, showed higher anti-complementary than IIIb and IVb did. The latter fractions had high molar ratio of Rha:GalA (IIIb 1.0 : 16.2, IVb 1.0 : 2.6) and low molecular weight. It seems that the content and composition of neutral sugar in polysaccharide containing high content of rhamnogalacturonan core may have main effect on the anti-complementary activity.

Continuous researches are now going on for the identfication of chemical structure of these polysaccharides.

Abstract

Water-soluble crude polysaccharide (CAP-0) obtained from the Capsici Fructus(the fruits of *Capsium annuum* L.) showed a potent anti-complementary activity. The anti-complementary activity did not change by pronase digestion of CAP-0, but decreased by the periodate oxidation. CAP-0 was fractionated into four polysaccharide fractions, CAP-1,2,3 and 4, by the addition of cetyltrimethylammonium bromide. CAP-1, showing the highest anti-complementary activity, was refractioned by anion-exchange chromatography to give three major fractions(CAP-1-III, IV and V). CAP-1-III was shown to have low anti-complementary activity, but CAP-1-IV and V had high activity. CAP-1-III and IV were purified on Sephadex G-100 to give each two fractions(CAP-1-IIIa and IIIb, CAP-1-IVa and IVb), respectively. From the results of gel filtration and electrophoresis, these four fractions and CAP-1-V were found to be homogeneous polysaccharides. High molecular polysaccharies(M.W. CAP-1-IIIa 70,000, IVa 195,000, V 140,000) showed relatively higher anti-complementary activity than low molecular polysaccharides(CAP-1-IIIb and IVb).

사 의

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References

1. Muschel, L.H., Schmoker, K. and Webb, P. M.: Proc. Soc. Exp. Biol. Med., 117 : 639 (1964)
2. Gewurz, H., Shin H.S. and Mergenhagen, S. E.: J. Exp. Med., 128 : 1049(1968)
3. Marcus, R.L. and Mayer, M.M.: Proc. Natl. Acad. Sci U.S.A., 68 : 1351(1971)
4. Okuda, T., Yoshioka, Y., Ikekawa, T. and Nishioka, K.: Nature (London), New Biol., 238 : 59(1972)
5. Hamuro, J., Jadding, U. and Bitter, D. Suermann: Immunology, 34 : 695(1978)
6. Yamada, H., Kiyohara, H., Cyong, J.C., Kojima, Y., Kumazawa, Y. and Otsuka, Y.: Planta Med., 50 : 163(1984)
7. Yamada, H., Kiyohara, H., Cyong, J.C. and Otsuka, Y.: Mol. Immunol., 22 : 295(1985)
8. Kiyohara, H., Yamada, H., Cyong, J.C. and Otsuka, Y.: J. Pharmacobio-Dyn., 9 : 339 (1986)
9. Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A. and Smith, F.: Anal. Chem., 28 : 350(1956)
10. Blumenkranz, N. and Asboe-hansen, G.: Anal. Biochem., 54 : 484(1973)
11. Lowry, O.H., Rosebrough, N.J., Farr, I. and Randall, R.J.: J. Biol. Chem., 193 : 265

(1951)
12. Trevelyan, W.E., Procter, D.P. and Harrison, J.S.: Nature (London), 166 : 444(1950)
13. Hough, L., Jones, J.K.N. and Wadman, W. H.: J. Chem. Soc.: 1702(1950)
14. Yamada, H., Ohshima, Y. and Miyazaki, T.: Chem. Pharm. Bull., 30 : 1784(1982)
15. Kabat E.A. and Mayer M.M.: Experimental Immunochemistry, Thomas, Illinois: pp. 133 ~240(1964)