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Immunopotentiating and Antitumor Activities of Purified Lectins and Polysaccharides from Trichosanthes Rhizoma and Taraxii Herba*

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Abstract—Water-soluble lectins isolated from Trichosanthes Rhizoma and Taraxii Herba and their deproteinized polysaccharides were purified through DEAE cellulose column and were applied to immunopotentiating and antitumor studies to clarify the nature of their activities of our previous reports. As the results of works, the lectins and deproteinized polysaccharides increased the number of circulating leukocytes and total peritoneal cells. And they markedly elevated the lowered production of antibody and reactivity of T lymphocyte in tumor-bearing mice, which were rapidly recovered by discontinuance of sample treatments. They also decreased the growth of Sarcoma 180 solid tumor in mice.

Keywords □ Trichosanthes Rhizoma, Taraxii Herba, polysaccharide, immunopotentiation, antitumor activities.

Various kinds of polysaccharides from plants were reported to exhibit diverse biological activities, especially including the activities on immune system and tumor. Our previous reports dealt with the immunopotentiating and antitumor activities of polysaccharides from Trichosanthes Rhizoma and Taraxii Herba (Chung, *et al.*, 1990; Jeong, *et al*, 1991). As the continuation of the works to clarify such activities, chemical and biological studies were attempted with further purified lectins or deproteinized (protein-unbound) polysaccharide obtained from the herbs.

Materials and Methods

General

Plant materials were purchased at local market and identified. The E.P. grade solvents were used for extraction and G.R. grade chemicals were used all throughout the experiments. Animals were obtained from private experimental animals suppliers and were adapted to laboratory conditions for enough periods.

Isolation and purification of lectin

Partially purified polysaccharides obtained by usual procedure described as previous reports (Chung, 1990 and Jeong, 1991) were identified protein-bound polysaccharide and were further purified by following procedures.

Precipitation by Salt

Obtained polysaccharide was dissolved in small amount of water and was precipitated by the addition of ammonium sulfate to make the solution up to 70%. Precipitated polysaccharide was dissolved in water again and the procedure was repeated to get crude lectin.

Ion exchange column chromatography

Obtained crude lectin was dissolved into 25 mM Tris-HCl buffer solution (pH 7.2) and applied to DEAE Sephadex A-50 ion exchange column (5×70 cm, elution solvent: Tris buffer) which were washed enough with elution solvent and stood free for one day before application. Then the obtained elutes using saline solution (0.0~1.0 M) by linear gradient method were collected by monitoring the agglutinating activities of human blood cells. The active elutes were collected and concentrated by PEG 6000 and dialyzed with 10 mM-phosphate buffer (pH 6.8).

Hydroxyapatite column chromatography

The obtained lectin solution was applied to hydroxyapatite column (elution solvent: 10 mM phosphate buf-

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fer) and washed enough. Then the elutes of consequent elution with 50~200 mM of phosphate buffer (pH 6.8) by linear gradient method were lyophilized, then solubilized with small amount of 25 mM Tris-HCl buffer solution and dialyzed.

Gel filtration

The dialyzed production was applied to Sephacryl S-200 column (3×70 cm, elution solvent: 50 mM Tris-HCl buffer, pH 7.2, containing with 0.5 M NaCl) then the active elutes of lectin were collected, dialyzed with distilled water and lyophilized to produce purified lectin.

Determination of Protein

The protein contents bound to polysaccharide was determined by the method of Lowry (Lowry, *et al.*, 1951) with bovine serum albumin (Sigma Fr. IV) as a standard.

Determination of Purity

Determination of the purities of lectins of each isolation and purification step was carried out through calculation based on hemagglutination units and amount of protein those of crude lectin and of next step, respectively.

Preparation and Purification of Deproteinized Polysaccharides

One part of crude whole polysaccharide was deproteinized by digestion, dialysis and purified to produce protein-free polysaccharide (Scheme 1). The deproteinized polysaccharide was purified using DEAE cellulose column (chloride form, 3×30 cm). The positive elutes by anthrone test (Herbert, 1971) were collected, concentrated and precipitated with 95% ethanol. The final deproteinized polysaccharides were whitish or pale brownish powder.

Determination of Hemagglutinating Activities

Prepared lectin solution (20 μl) was placed in a well of microtiter plate and diluted with saline solution in double-folded gradient serially. A 20 l of 3%-red blood cells solution was added into each well and stood free for one hour. Unagglutinated red blood cells in each well were washed with saline solution for four times and the 1% solution of trypsin was treated for one hour at 37℃. Then the remaining trypsin was washed away with saline solution (Chung, *et al.*, 1980). The haemagglutinating activity of lectin was calculated from the reciprocal of final dilution fold which caused agglutination.

Activities on Immune Function

To prove the immunopotentiating activities of purified polysaccharides prepared by step-wise purification during the studies, measurements of the numbers of

Whole Polysaccharide
| alkalified with 0.1N NaOH to pH 7.5
| digested with protease (37℃, 24 hrs)
Digested Polysaccharide
| concentr'n by boiling to 1/2 vol.
| deproteinized with TCA
| centrifugation (8,000 rpm, 30 min)
Polysaccharide Supernatant
| dialysis (2 days)
| add 3 vol. 95% ethanol
| centrifugation (3,000 rpm, 10 min)
Deproteinized Polysaccharide

Scheme 1. Deproteinization Procedure of protein-bound Polysaccharide.

circulating leukocytes (Mitruka, 1981), total peritoneal cells (Weir, 1973), the effects on antibody-mediated and delayed-type hypersensitivities in tumor-bearing mice were accomplished following the same methods (Katsura *et al.*, 1977; Ruddle, 1981; Titus and Chiller, 1981; Henningsen, 1984) described in previous reports.

Activities on Solid Tumor

Measurements for antitumor activities were accomplished by the same methods (Goldin, *et al.*, 1980) as described previously. Each 10 ICR mice were implanted with 0.1 ml of Sarcoma 180 tumor cell suspension (1×10^6 cells) in left groin, then 24 hours later, samples were administered intraperitoneally once a day for ten consecutive days. In day 30 of inoculation of tumor, animals were sacrificed and the changes in weight of solid tumor by sample treatments were measured (Goldin, *et al.*, 1980).

Results and Discussion

Purification of Isolated Lectins

The productions of purified lectins (identified by the agglutination of human red blood cells) and deproteinized polysaccharides are shown in Table I. Considerable amounts of lectin and pure polysaccharides could be yielded from the crude polysaccharide fractions. It is questionable, by the way, that the polysaccharide content is only about 70% in deproteinized polysaccharide.

The purity of polysaccharides in each purification step is summarized in Table II. Direct comparison of final purification folds revealed more successful results in Trichosanthes Rhizoma than in Taraxii Herba. It might be considered that smaller amount of purer poly-

saccharide was extracted from Taraxii Herba, because the amounts of Tarax-WP, Tarax-PL and Tarax-DP are not deviated greatly from the values in case of Trichosanthes Rhizoma as shown in Table I. On the other hand, the non-linear increase of purification folds in each purification step of Trichosanthes Rhizoma seemed to be caused by inaccurate identification in each purification step or determinations of protein amount or hemagglutination units. So it is needed to adapt the electrophoresis in each identification step and more accurate determination of protein amount and hemagglutinating activity.

Effects on Immune Function

Polysaccharides isolated from the herbs were subjected to the effects on immune system using the methods of comparing the changes in numbers of blood leukocytes and peritoneal exudate cells. All the polysaccharides markedly increased number of cells which are important marks of immune system and such increments were greater along the repeated treatments of sample within one week (Table III). The numbers of cells were recovered after seventh day of final treatments.

The effects of polysaccharides on hypersensitivity are summarized in Table IV. Implantation of tumor cells greatly decreased the productivity of antibody (antibody-mediated) and T lymphocyte reactivity (delayed-type) as 5.71 from 7.01 and 5.14 from 6.54,

Table I. Amounts and ratios of polysaccharides in each purification step

Plant materials	Whole Polysaccharide	Purified Lectin[a]	Deproteinized[a]
Trichosanthes 1.2 kg	Trich-WP 32 g (57.2)[b]	Trich-PL 27.4 g (57.2)	Trich-DP 16.8 g (71.6)
Taraxii 1.2 kg	Tarax-WP 28 g (43.2)	Tarax-PL 21.3 g (55.6)	Tarax-DP 14.6 g (69.1)

[a]Converted values to the amount of whole polysaccharides. Actually obtained amounts were half of the values.
[b]Numbers in parentheses mean the polysaccharide contents in percents obtained from anthrone method (620 nm).

Table II. Purification of lectins from Trichosanthes Rhizoma (Trich) and Taraxii Herba (Tarax) in each step

Purification Step	Total protein (mg)		Hemagglutination Activity[a] ($\times 10^{-2}$)		Specific Activity[b]		Purification Folds[c]	
	Trich	Tarax	Trich	Tarax	Trich	Tarax	Trich	Tarax
Crude[d]	713.78	345.21	2500.0	2500.0	3.50	7.24	1.00	1.00
Salt fraction	98.60	83.77	312.5	625.0	3.17	7.46	0.90	1.03
DEAE Sephadex	28.93	23.27	78.1	156.3	2.70	6.72	0.77	0.93
Hydroxyapatite	1.39	10.36	19.5	78.1	14.03	7.54	4.01	1.04
Sephacryl S-200	0.82	4.52	9.8	19.5	11.95	4.31	3.41	0.60

[a]The unit of haemagglutinating activity is defined as the reciprocal of final dilution fold which caused agglutination of human erythrocytes.
[b]Specific activity was calculated from division of hemagglutination units by amounts of total protein.
[c]Purification folds were calculated from division of hemagglutination units of each step by that of crude (whole polysaccharide) respectively.
[d]Whole polysaccharide.

Table III. Effects of prepared polysaccharides on the number of leukocytes in blood and peritoneal exudate cells.

Treatments[a]	Cell Number (mean± S.E.)			
	Leukocytes (10^3 cells/mm^3)		Exudate Cells (10^5 cells/ml)	
	Day 1[b]	Day 7	Day 1	Day 7
Control	7.02± 1.31	7.23± 1.34	9.3± 2.3	13.2± 2.7
Trich-PL	9.52± 1.48	10.13± 1.51	19.7± 3.0	14.0± 3.2
Tarax-PL	10.01± 1.43	9.34± 1.44	27.2± 6.5	14.2± 2.1
Trich-DP	7.84± 1.51	10.13± 1.25	17.3± 4.5	15.2± 2.7
Tarax-DP	9.34± 1.27	10.32± 1.31	27.5± 7.4	13.5± 4.0

[a]Samples were treated 10 mg/kg, *i.p.*, once a day for seven consecutive days to each group which consisted of ten (for leukocytes) or seven (for exudate cells) ICR mice. Control group (ten mice) were treated with saline only.
[b]Days after the final treatment of sample.

Table IV. Effects of prepared polysaccharides on the antibody-mediated hypersensitivity (AMH) and delayed-type hypersensitivity (DTH) in tumor bearing mice represented by the increase of footpad thickness

Treatments[a]	Increased footpad thickness[b]	
	AMH	DTH
Normal mice	7.01	6.54
S-180 bearing	5.71	5.14
Trich-PL	7.11	6.65
Tarax-PL	6.35	6.27
Trich-DP	4.24	3.17
Tarax-DP	6.32	5.01

[a]Samples were injected 5 mg/kg, *i.p.*, once a day for seven consecutive days to each group which consists of six ICR mice. Each ten of normal and tumor-bearing mice were treated with saline only.
[b]Value in 10^{-1} mm.

Table V. Antitumor activities of prepared polysaccharides against Sarcoma-180 solid tumor type in mice.

Treatments*	Tumor weight (g) (mean± S.E.)	Inhibition Ratio (%)
Control	4.36± 0.34	–
Trich-WP	3.74± 0.29	14.2
Tarax-WP	4.02± 0.37	7.8
Trich-PL	2.50± 0.14	42.7
Tarax-PL	2.45± 0.22	43.8
Trich-DP	2.69± 0.27	38.3
Tarax-DP	3.12± 0.33	28.4

*Samples were injected 5 mg/kg, *i.p.*, once a day for seven consecutive days to each group which consists of seven ICR mice.

represented by the increase of footpad thickness, respectively. In such conditions, lectin from Trichosanthes Rhizoma (Trich-PL) markedly recovered the decreased sensitivity. On the other hand, deproteinized polysaccharide from the herb (Trich-DP) lowered the suppressed sensitivity, which is to be elucidated later.

For the more detailed elucidation of the mode of action, it should be accomplished the experiments for immune system, for examples, effects on macrophage and complements with purified polysaccharides as already reported with crude polysaccharides in our previous reports.

Antitumor Activities

To clarify the mode of action in antitumor activities of crude polysaccharide as shown in previous reports, purified polysaccharides and lectins were applied to solid tumor in mice. Considerable lowered dose (5 mg/kg, *i.p.*) of each sample inhibited the growth of solid tumor. As shown in Table V, lectins from each herb (Trich-PL and Tarax-PL) exhibited stronger potencies than deproteinized polysaccharides (Trich-DP and Tarax-DP). Such results imply the nature of antitumor activities are in lectins from the herbs, as the earlier report (Shim, 1980) and our previous reports. But other reports (Lee, *et al.*, 1986) revealed that the immunopotentiating and antitumor activities of the herbs were shown at the dose levels of 15~20 mg/kg, dose levels of 5 mg/kg of lectin or deproteinized polysaccharide could be emphasized as more concentrated activities, which need further studies for the mechanism of such actions.

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