BIOCHEMICAL AND PHYSICOCHEMICAL CHARACTERIZATION OF A MITOGEN OBTAINED FROM AN ORIENTAL CRUDE DRUG, TOHKI (ANGELICA ACTILOBA KITAGAWA)

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Chemical composition and physicochemical properties of an immunomodulator, which is a non-dialyzable and acetone precipitable material(s) extracted with hot water from Angelica actiloba KITAGAWA (Yamato Tohki) (AIP), were investigated. AIP was composed of about 90% sugar and 10% protein. The major polysaccharide was identified as pectic substance(s) because its main component sugars were found to be arabinose, glucose, and galacturonic acid by gas liquid chromatographic analysis. The pectic substances(s) was not concerned with the mitogenicity of AIP since the activity was similar before and after pectinase(endo-polygalacturonase) treatment. More than half of the mitogenicity was destroyed by acid or alkali treatment. With pronase treatment, the activity was not affected, but the molecular weight of the mitogen was lowered. In addition, the mitogenic substance was partially purified from AIP by pectinase treatment and Westphal's phenol/water fractionation. The partially purified mitogenic substance(s) was rich in protein. These facts suggest that the mitogenicity of AIP was carried by a heat stable and protease resistant protein.

Keywords—B cell mitogen; immunomodulator; Angelica actiloba; Tohki; oriental crude drug; hot water extract; pectic substance; Wakanyaku

INTRODUCTION

Immunomodulators are found in various sources including bacteria, fungi, plants, synthetic compounds, and animals.1–4 These are known to act to various points in immune systems. Lectins, such as phytohemagglutinin (PHA) and concanavalin A (Con A) (T cell mitogen), have been shown to stimulate T cells. Bacterial endotoxin (LPS) and polysaccharides (B cell mitogen) have been shown to stimulate B cells. In addition, B cells are specifically activated by thymus independent antigens such as pneumococcal polysaccharide SIII, polymerized flagellin (POL), polyvinylpyrrolidone (PVP) and dextran. Several immunomodulators such as Propionibacterium acnes cells and LPS have been shown to stimulate macrophages. Except for lectins, the structure required for lymphocyte stimulation is still unknown.5 In the case of B cell mitogen, it has been speculated that the mitogens require a polymerized structure, but detailed chemical characterizations have been elucidated for several of the mitogens described above.

Recently, examination of Sino-Japanese herbal medicines (Oriental crude drugs, Wakanyaku) based on contemporary medical philosophy and approach has attracted attention. Kojima et al. have screened 71 materials extracted with hot water from Sino-Japanese herbal medicines to find interferon-inducers and mitogens in vitro.6,7 Twenty-four herbs were found to have significant interferon inducing activity, and some of them were useful interferon-inducers. Eight herbs, seven of which were interferon-inducers, showed mitogenic activity with stimulation indices exceeding 5. Kumazawa et al. have been studying these materials immunologically, and reported the immunomodulating activity of an acetone-
precipitable, non-dialyizable fraction of hot water extract of *A. actiloba* KITAGAWA (Yamato Tohki)(AIP). AIP was mainly composed of carbohydrates, and showed potent immunomodulating activity on the murine immune system as adjuvant, polyclonal B cell activator, interferon inducer, and mitogen, in addition to antitumor activity against Ehrlich ascites tumor cells. These immunomodulating properties of AIP were thought to be due to the carbohydrate portions because the major component of AIP was carbohydrate and the activity disappeared after periodate oxidation and borohydride reduction. However, purification and structural outline of these carbohydrate portions, and what constituents showed mitogenicity were not yet known. In this paper, we reported the chemical composition and physicochemical properties of the mitogenic substances in AIP and a partial characterization of the substance.

**MATERIALS AND METHODS**

*Preparation of AIP* — One kilogram of the minced root of *A. actiloba* KITAGAWA (Yamato Tohki), purchased from Uchida Wakanyaku Co., Ltd., was mixed with 10 l of water and heated in a boiling water bath for 1 h. The root was separated from the extract by filtration with filter paper (Toyo Roshi). One volume of acetone was added to the filtrate and it was stored at 4°C for 24 h. The precipitate, produced by the above procedure, was separated by centrifugation (27000 \( \times g \), 5 min), dissolved in aliquots of water and dialyzed against water. The non-dialyzable fraction was lyophilized (yield 26 g)(AIP).

*Analytical Methods* — Contents of protein, carbohydrate, hexosamine, and uronic acid were determined by literature cited with bovine serum albumin, glucose, glucosamine and galacturonic acid as references, respectively. Before analysis of hexosamine, fractions were hydrolyzed with 4 M HCl for 2 h at 110°C, and then N-acetylated. Phosphate ester was quantified as described in literature cited.

*Mitogen Assay* — Mice(C3H/HeN) were purchased from Shizuoka Agricultural Cooperative Association for Laboratory Animals, Hamamatsu, Japan. Lymphocyte suspensions were prepared from the spleens by tearing the organs in the cold medium. The cell suspensions were passed through a 200 gauge stainless steel sieve and then were allowed to stand to remove tissue fragments. The supernatant containing single cells were separated. The cell suspensions were centrifuged (600 \( \times g \) for 10 min), and washed with fresh medium. RPMI 1640 medium (Nissui, Tokyo, Japan) containing N-2-hydroxyethyl-piperezine-N’-2-ethanesulfonic acid (HEPES) (5 mM), penicillin G(100 U/ml) and streptomycin (100 U/ml) was used in these treatments. After the cells were washed with the medium for three times, the cells were suspended to \( 5 \times 10^6 \) viable cells per ml by trypan blue exclusion method in the above medium containing 7.5% heat inactivated foetal calf serum (FCS, Gibco, Grand Island, New York, U.S.A.).

Each sample solution(25 \( \mu l \)) was placed in a flat bottomed microtiter plates(Nunclon 163320, Nunc, Denmark) and 2 fold enriched medium(25 \( \mu l \)) was added for osmotic stabilization. And then the cell suspension(100 \( \mu l \)) prepared above was added to each well. The final assay condition described above was \( 5 \times 10^5 \) cells in 150 \( \mu l \) RPMI 1640 medium containing HEPES, penicillin G, streptomycin and 5% FCS per well. Each culture plate was incubated at 37°C for 48 h in a humidified atmosphere of 5% CO\(_2\)-95% air. Before harvesting, cultures were pulsed for 20 h by 0.5 \( \mu Ci \) of tritiated thymidine (20 \( \mu l \))(NET-355, Thymidine, (6-\(^3\)H), specific activity: 225 Ci/mmol; New England Nuclear, Massachusetts, U.S.A.) dissolved in the above medium. The cultures were harvested with a Labo Mash multiple cell harvester(Labo Science Co., Ltd. Tokyo, Japan) on glass fiber filters. Incorporated thymidine into the cells was determined by a liquid scintillation counter(Arora 903, Tokyo, Japan) with omnifluor/toluene scintillator. Results were expressed as a arithmetic mean cpm±SD of dupli or triplicate cultures or as stimulation indices(SI)(mean cpm in mitogen-treated cultures per mean cpm in control.
Gas Liquid Chromatographic Analysis of Sugar Components — Each fraction (1 mg) was hydrolyzed with 1 M trifluoro acetic acid (TFA) for 5 h. After the acids were distilled off, the hydrolyzate, dissolved in an aliquots of water, was heated at 60°C for 10 min with BaCO₃. After filtration through membrane filter (0.45 μm), the filtrate was passed through Dowex 1 (Ac⁻) column equilibrated with water. The unabsorbed fraction (neutral sugar fraction) was reduced with excess sodium borohydride for 2 h at room temperature. After the excess reagent was decomposed by the addition of Dowex 50 WX8 (50—100 mesh, H⁺) resin, boric acid was removed by repeated evaporations with methanol in the usual way. The resulting mixture of alditols was acetylated with pyridine and acetic anhydride (1:1) for 1 h at 100°C, and analyzed by gas liquid chromatography (GLC). The absorbed fraction (uronic acid fraction) was eluted from the resin with 4 M acetic acid (3 volumes) and evaporated to dryness. The fraction was reduced with sodium borohydride and then derivatized to the methyl ester by heating with methanol containing Dowex 50 WX4 (H⁺) resin (20—50 mesh) at 100°C for 1 h, in a sealed vial. After the reaction was completed, the resin was removed, and the solution containing methyl ester of aldonic acids was concentrated to dryness. The resulting reaction mixture was derivatized to alditol acetates and analyzed by GLC. GLC was performed on a Shimadzu GC-6A instrument (Shimadzu, Tokyo, Japan) with a flame ionization detector equipped with a glass column (0.3 × 200 cm) containing 3% silicone OV-225 on Gas Chrome Q.

Pectinase Treatment of AIP — Pectinase (Sigma, St. Louis, MO, U.S.A., 1.5 mg) was added to AIP (3.0 mg), dissolved in 0.1 M NaCl (pH 6.0) and incubated at 37°C for 9 h. The reaction mixture was dialyzed against water, and then concentrated. Control experiments were run in parallel. (Preparative scale) Pectinase (Wako, Tokyo, Japan, 6 g) was added to AIP (26 g), dissolved in 50 mM citrate buffer pH 5.0, and incubated at 37°C for 48 h. The incubation mixture was dialyzed against water, and then centrifuged at 27000 × g for 10 min. The precipitate was designated as AIP-I and the non-dialyzable supernatant was as AIP-II.

Acid or Alkali Treatment of AIP — AIP (2 mg) was dissolved in 1.0 ml of H₂O, 10 mM, or 100 mM HCl, 10 mM or 100 mM NaOH, respectively. The reaction mixture was incubated at 100°C for 10 min for HCl and 37°C for 3 h for NaOH. After each reaction was terminated by neutralization, each reaction mixture was dialyzed and concentrated.

Pronase Treatment of AIP — Pronase (Kakenkagaku Co. Ltd., Tokyo, Japan, 20 mg) was added to AIP (3.0 mg), dissolved in 0.1 M Tris-HCl buffer containing 10 mM CaCl₂ (pH 7.8), and incubated at 37°C for 9 h. The reaction mixture was dialyzed against water, and then concentrated. (Preparative scale) Pronase (10 mg) was added to AIP-II (210 mg), dissolved in the above buffer, and incubated at 37°C for 20 h. The reaction mixture was dialyzed and concentrated. The supernatant solution was applied on a Sepharose 2B column.

Phenol-Water Fractionation of AIP and AIP-II — Phenol/water fractionation was performed at 70°C as described by Westphal and Jann for isolation of lipopolysaccharides from gram negative bacteria. The reaction mixture, cooled to room temperature, was centrifuged at 3000 rpm for 20 min. Each of the upper layer (water), middle layer (emulsion), and lower layer (phenol) was dialyzed extensively and lyophilized.

Solubilization of AIP-I by Urea Together with 2-Mercaptoethanol — AIP-I (10 mg) was suspended in 0.1 M Tris-HCl buffer pH 8.5 containing 8 M urea (1 ml), and then 2-mercaptoethanol (100 μl) was added in this reaction mixture. The reaction mixture was stood at room temperature with constant stirring overnight. Then, the reaction mixture was dialyzed against water. The non-dialyzable fraction was centrifuged and the supernatant fraction was applied to Sepharose 2B column.

RESULTS

Chemical Composition of AIP
As shown in Table I, AIP was composed mainly of polysaccharides. The main component sugars of AIP were glucose and arabinose as neutral sugars, and galacturonic acid as the uronic acid.

FIG. 1. Gas Liquid Chromatogram of the Neutral Sugar and Uronic Acid Fractions of AIP
Alditol acetates were derived from acid hydrolyzates of AIP described in “Materials and Methods.” I, neutral sugar fraction; II, uronic acid fraction; 1, fucose; 2, ribose; 3, arabinose; 4, xylose; 5, mannose; 6, galactose; 7, glucose; 8, mannulonic acid; 9, galacturonic acid; 10, glucuronic acid.

TABLE I. Composition of AIP and Fractions Obtained by Several Treatments from AIP

<table>
<thead>
<tr>
<th>Compositions a)</th>
<th>AIP</th>
<th>Fraction names AIP-I</th>
<th>AIP-II</th>
<th>Phenol–water fractionated AIP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Upper</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>32.2</td>
<td>19.8</td>
<td>60.9</td>
<td>37.3</td>
</tr>
<tr>
<td>Uronic acid</td>
<td>41.5</td>
<td>22.7</td>
<td>18.1</td>
<td>60.7</td>
</tr>
<tr>
<td>Protein</td>
<td>9.0</td>
<td>41.6</td>
<td>9.3</td>
<td>1.5</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>2.1</td>
<td>4.5</td>
<td>1.0</td>
<td>n.d.</td>
</tr>
<tr>
<td>Phosphate</td>
<td>4.7</td>
<td>3.5</td>
<td>1.0</td>
<td>2.4</td>
</tr>
<tr>
<td>Yield</td>
<td>100</td>
<td>10</td>
<td>40</td>
<td>31.8</td>
</tr>
</tbody>
</table>

a) Each data was expressed as w/w%.
b) n.d. not determined.
Characterization of Tohki Mitogen

907

acid (Fig. 1). Since the aqueous solution of AIP had high viscosity, gel filtration methods could not be applied to purify the mitogenic substance.

Recently, Tomoda et al. reported that the hot water extract of A. actiloba Kitagawa (Yamato Tohki) contained acidic polysaccharide\(^4\) stained by toluidine blue, which is usually used for detecting acidic substances such as mucopolysaccharides and pectic substances. AIP was also stained by toluidine blue. The chemical composition and physicochemical properties suggest that AIP was composed mainly of "pectic substance."

**Effect of Acid of Alkali Treatment on AIP**

As one of the physicochemical characterizations of AIP, acid or alkali stability was determined. As shown in Fig. 2, about 50% of the mitogenic activity was destroyed by acid or alkali treatment (at a mitogen dose of 5 and 25 \(\mu\)g/culture). Therefore, it appeared that the mitogenic substance was relatively weak to such treatment.

**Effect of Pectinase Treatment on AIP**

To clarify the mitogenic substance in AIP, pectinase (endo-polygalacturonase) treatment on AIP was performed, and the mitogenic activity of the non-dialyzable fraction was compared. As shown in Fig. 2, the activity was not diminished after the treatment, which suggested that the pectic substance which was the main component of AIP did not play a role in mitogenic activity. In addition, the commercially available pectins, which are structurally similar to the pectic substance in AIP, did not show mitogenic activity (data not shown).

Because pectinase treatment of AIP eliminates pectins as low molecular weight dialyzable materials, this treatment could be useful as one step in the purification of the mitogenic substance. During the pectinase treatment of AIP, insoluble materials were produced. After extensive dialysis of the reaction mixture, the insoluble material (AIP-I) was separated by centrifugation. The non-dialyzable soluble fraction was denoted as AIP-II. Yields of AIP-I and -II were about 10 and 40% of AIP, respectively. The mitogenic activity of AIP, AIP-I and AIP-II are shown in Fig. 3. Both AIP-I and AIP-II possessed mitogenic activity, but AIP-I showed higher specific activity than AIP and AIP-II.

Chemical compositions of each fraction are shown in Table I. Since AIP-II showed lower viscosity than AIP, gel chromatography could be applied. When a Sepharose 2B column was used, the majority of the carbohydrate fraction was eluted near the bed volume (Figs. 3 and 4). However, the mitogenic substance was recovered from a higher molecular weight fraction than the

FIG. 2. Effect of Chemical(I) or Enzymic(II) Treatment on the Mitogenicity of AIP

Experimental details were given in "Materials and Methods." (I) Acid or alkali treatment: A, untreated AIP; B, 10 mM HCl; C, 100 mM HCl; D, 10 mM NaOH; E, 100 mM NaOH; (II) Pectinase or pectinase treatment: F, untreated AIP; G, pectinase; H, pectinase treated AIP; I, pectinase treated AIP. 0.5 \(\mu\)g/culture; 5 \(\mu\)g/culture; 25 \(\mu\)g/culture; 50 \(\mu\)g/culture.
main carbohydrate fraction. AIP-I was mainly composed not of carbohydrates but of proteins (Table I). The amino acid compositions of AIP-I and -II were shown in Table II. Both AIP-I and -II showed quite similar compositions, and contained much quantities of aliphatic amino acids. AIP-I was hardly solubilized by water, but part of AIP-I could be solubilized by 8 M urea solution together with 2-mercaptoethanol. The solubilized part of AIP-I was chromatographed on a Sepharose 2B column (Fig. 4(III) ). The mitogenic substance was eluted in similar fractions as in the case of AIP-II. These facts suggest that the mitogenic substance was not composed of carbohydrate.

Effect of Pronase Treatment on AIP and AIP-II

The above results strongly suggest the protein requirement for mitogenic activity. To confirm protein requirement for mitogenic activity, pronase treatment was performed on AIP and AIP-II. As shown in Fig. 2, pronase treatment did not affect the mitogenic activity. On the other hand, when pronase treated AIP-II was chromatographed on a Sepharose 2B column, the mitogenic activity shifted to lower molecular weight fractions than native AIP-II (Fig. 4). These facts suggest that the mitogenic activity was pronase resistant, but possessed pronase sensitive moieties in its molecules.

Phenol-Water Fractionation of AIP and AIP-II

The above results suggest that the mitogenic substance was composed of protein or protein rich macromolecular components. Therefore, we applied the phenol/water fractionation to AIP

![Graphs showing dose response curves of AIP, AIP-I, and AIP-II(I) and 2B-1 and 2B-2(II)]

*FIG. 3. Dose Response Curves of AIP, AIP-I and AIP-II(I), and 2B-1 and 2B-2(II)*

I: AIP (---); AIP-I (-O-); AIP-II (-●-); II: AIP-II (-●-); 2B-1 (-O-); 2B-2 (- -). Mitogenic responses to control cultures were 6425 ± 849 in (I) and 6880 ± 504 in (II).
and AIP-II. Proteins are known to be recovered from the phenol layer by this method.\textsuperscript{13} The dose response curves and chemical compositions of phenol/water fractionated AIP and AIP-II are shown in Table I, and Fig. 5. In each case, the mitogenic substance was mainly fractionated on the phenol and middle layers. The specific activity was 5 to 10 fold higher in the phenol layer than in AIP and AIP-II, and the phenol layer fraction was mainly composed of proteins. This fact also suggests that the mitogenic substance in AIP was not covalently linked with the pectic substance in AIP.

\section*{DISCUSSION}

AIP, a non-dialyzable, acetone precipitable, hot water extract of \textit{A. actiloba} KITAGAWA (Yamato Tohki), was found to be mainly composed of pectic substances. It is suggested that the mitogenic activity of AIP was due to the presence of neither pectic substances nor other polysaccharides for the following reasons. (1) The commercially available pectins did not show mitogenic activity on murine spleen cells. (2) Although about half of the AIP was eliminated by pectinase treatment as low molecular weight dialyzable materials, the mitogenic activity of AIP did not decrease (Fig. 2). (3) AIP-I, which was produced as a precipitate during pectinase treatment and showed 5 to 10 fold higher specific activity than AIP, was composed mainly of proteins (Fig. 3, Table I). (4) When AIP-II was chromatographed on a Sepharose 2B column, the mitogenic substance was recovered from higher

\begin{figure}
\centering
\includegraphics[width=\textwidth]{fig4.png}
\caption{Elution Profiles of AIP-II(I), Pronase Treated AIP-II(II), and Urea/2ME Solubilized AIP-I(III) on a Column of Sepharose 2B}
\textit{The column was equilibrated with saline. Mitogenicity on murine spleen cells (......); carbohydrates (\textbullet --); proteins (\textbullet --). Arrows indicate void and bed volumes.}
\end{figure}
molecular weight fractions than the main carbohydrate fractions (Fig. 4). (5) When phenol/water fractionation was applied to AIP, the mitogenic substance was not recovered from the water layer (Fig. 5).

Although the mitogenic substance in AIP was only partially purified, it appeared to be a protein or a protein rich macromolecular component because AIP-I was rich in protein and the mitogenic substance was not recovered in the water layer by phenol/water fractionation of AIP and AIP-II. Furthermore, the fact that pronase treatment caused partial degradation of the mitogenic substance suggests the possibility described above. Although the mitogenic activity was recovered both in AIP-I and -II by pectinase treatment, both activities should result from a very similar substance because the elution profiles on a Sepharose 2B column were very similar (Fig. 4).

Until recently, almost all of immunomodulators obtained from higher plants have been found to be lectins, which are heat unstable. It is quite interesting and novel that the mitogenic substance obtained from A. actiloba is a heat-stable, protease resistant protein(s).

Molecular weight of the mitogenic substance is about a million. The facts that treatment of the mitogen with 8 M urea/2-mercaptoethanol did not give significant change in molecular weight (Fig. 4) suggest that the mitogen did not form aggregates. Physicochemical and biochemi-

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**FIG. 5. Dose Response Curves of Phenol-Water Fractionated AIP (I) and AIP-II (II)**

Experimental details were described in "Materials and Methods". Water layer (upper) (— ○ —); middle layer (— ● —); phenol layer (lower) (— — —). AIP in (I) and AIP-II in (II) (———). Mitogenic response to control culture was 6880 ± 504 (I and II).
Characterization of Tohki Mitogen

The biopolymer is established in the analysis of the biopolymer is established in the fields of nucleic acid and polysaccharides. However, in the field of protein, nevertheless molecular weight of less than 100000 can be precisely analyzed, proteins of larger molecular weight could be analyzed only in a limited number of materials, such as self aggregatable tubulin (microtubules) and collagenous protein. Considering these facts, it is a limit to obtain partially purified mitogen. Further purification of the tohki mitogen require advanced separation technology.

Previously, Kumazawa et al. reported that the mitogenic activity of AIP was due to the carbohydrate portion because AIP was mainly composed of carbohydrates, and the mitogenic activity disappeared after periodate oxidation followed by borohydride reduction (IO₄⁻/BH₄⁻).⁷ This conversion in the present paper, we showed that the major carbohydrate portion was not concerned with the mitogenicity. And when IO₄⁻/BH₄⁻ treatment was performed on AIP-II, we also observed that the mitogenicity was decreased. However, when the IO₄⁻/BH₄⁻ treated AIP-II was eluted from a Sepharose 2B column, the mitogenic activity was recovered in a similar fraction to that of AIP-II (data not shown). The IO₄⁻/BH₄⁻ treatment was usually used for the characterization of biologically active polysaccharides and complex carbohydrates. However, it was found to be dangerous to determine active sites in vitro only by IO₄⁻/BH₄⁻ treatment because cytotoxic materials for the cells are sometimes produced by such treatment, especially acidic polysaccharides (manuscript in preparation).

It has been reported that the hot water extracts of many Sino-Japanese herbal medicines contain acidic materials which were stained by toluidine-blue and appeared to be pectic substances.¹⁴,¹⁸) Kojima et al. reported that the hot water extracts of many Sino-Japanese herbal medicines show immunomodulating activity, such as mitogenic

| TABLE II. Amino Acids Compositions of AIP-I, -II, and 2B-1 |
|-----------------|-----------------|-----------------|
| Gly             | 1.0             | 1.0             | 1.0             |
| Ala             | 0.62            | 1.3             | 1.7             |
| Val             | 0.47            | 0.55            | 0.80            |
| Ile             | 0.32            | 0.19            | 0.27            |
| Leu             | 0.57            | 0.25            | 0.30            |
| Thr             | 0.49            | 0.74            | 0.74            |
| Ser             | 0.60            | 1.0             | 1.0             |
| Cys             | 0.06            | 0.11            | -               |
| Met             | -               | -               | -               |
| Tyr             | 0.19            | 0.15            | 0.40            |
| Phe             | 0.25            | 0.12            | 0.22            |
| Try             | -               | -               | -               |
| Lys             | 0.50            | 0.44            | 0.31            |
| His             | 0.17            | 0.14            | -               |
| Asp             | 0.81            | 0.65            | 0.64            |
| Glu             | 0.84            | 0.98            | 0.91            |
| Arg             | 0.39            | 0.17            | -               |
| Pro             | 0.32            | 0.35            | -               |

Each fraction was hydrolyzed by 4M HCl at 110°C for 24 h. Amino acids compositions were expressed as ratio to Gly as 1.0 in each fractions. Amino acids were analyzed by Hitachi 835 auto amino acids analyzer.
and interferon inducing activities.6) The present paper suggests the usefulness of pectinase treatment in the purification of the mitogenic substance in AIP. These facts indicate that pectinase treatment would be useful as one of the purification steps for immunomodulators in other Sino-Japanese herbal medicines.

It is known that Sino-Japanese herbal medicines act gradually when compared with contemporary synthetic drugs. The partially purified mitogenic substance, the phenol layer fraction of AIP, showed higher mitogenic potential than AIP (maximum incorporation of 3H-ThdR was about 50000 cpm in the phenol layer fraction of AIP compared with about 30000 cpm in AIP, Fig. 5). This suggests that contaminated pectic substance and other materials decrease the mitogenic activity in vitro. The above results can be considered as a model of such characteristic properties on Sino-Japanese herbal medicines.

Lipopolysaccharide and lipoteichoic acid show various and potent immunomodulating properties. Almost all of these activities of LPS are known to be due to the lipid A part. It must be clarified whether the partially purified mitogenic substance in AIP possesses all of the immunomodulating activity of the starting materials.

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