BIOCHEMICAL AND IMMUNOLOGICAL STUDIES ON ASPERGILLUS

II. IMMUNOLOGICAL PROPERTIES OF PROTEIN AND POLYSACCHARIDE FRACTIONS OBTAINED FROM ASPERGILLUS FUMIGATUS

by

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(with 7 figs.)

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INTRODUCTION

The clinical feature of bronchopulmonary aspergillosis varies depending upon the site and mode of the *Aspergillus* infection in the respiratory tracts. The most distinguishable type of bronchopulmonary aspergillosis is aspergilloma of fungus ball which shows the typical radiographic appearance of a round mass with air-containing halo. The definite diagnosis of aspergillosis is made by means of the mycological procedure. However, the detection of this fungus from the specimens was in some cases difficult. Therefore, it was necessary to establish the immunological diagnostic procedure by skin testing or serological technique.

Recently many investigators (10, 11, 12, 13, 17, 18, 21) have suggested that immunological techniques were available for the clinical diagnosis of aspergillosis. As an antigen of skin and precipitation tests, LONGBOTTOM et al. (17) reported the isolation of "C substance" from the mycelia of *Aspergillus fumigatus*. BIGUET et al. (10) described that "C substance" was present in other *Aspergillus* species and also suggested the presence of another antigen which was designated "X substance" in *Aspergillus* species. However, the detail of chemical properties and immunological activities of these antigens were not investigated.

In previous papers (4—6) the authors have reported the isolation and purification of protein and polysaccharide fractions from *Aspergillus fumigatus*, and the chemical nature of these fractions

was investigated in detail. It was shown that protein and polysaccharide, which were designated as AAP and APS-66, respectively, were useful for the antigens of skin and precipitation tests in rabbits or guinea pigs which were immunized with *A. fumigatus* and in patients with aspergillosis.

In the present experiments, the authors will describe the immunological properties of protein and polysaccharides obtained from *A. fumigatus* in immunized guinea pigs or rabbits and in patients with aspergillosis.

Materials and Methods

Organisms *Aspergillus fumigatus* (IFO No. 5840) was supplied by Research Institute for Microbial Diseases, Osaka University. *A. fumigatus* Itono strain was isolated from the patient with pulmonary aspergillosis. *Aspergillus nidulans* (IFO No. 5719), *Aspergillus niger* (IFO No. 4414), *Aspergillus terreus* (IFO No. 6123) and *Aspergillus flavus* (IFO No. 4053) were supplied by the Fermentation Institute of Takeda Pharmaceutical Co. Ltd., Osaka. The fungi were cultured in Czapek Dox synthetic medium at 30 °C for 71 hours and were lyophilized.

Protein fraction Protein (AAP) fraction used for skin reaction antigen was extracted from the mycelia and spore of *A. fumigatus* (No. 5840), *A. niger*, *Penicillium* DC 11, *Streptomyces griseus*, *Cryptococcus neoformans*, acetone-dried *Mycobacterium tuberculosis* (Aoyama B strain), *Mycobacterium smegmatis*, *Mycobacterium fortuitum*, a typical mycobacteria P1 strain and *Nocardia asteroides*. The detail of the extraction and purification procedures was described in a previous paper (5).

Polysaccharides Two kinds of polysaccharides, glucan and galactomannan, were extracted and purified from the mycelia and spores of *A. fumigatus* (No. 5840) by the method described previously (5).

Proteolytic enzymes All proteinase, trypsin (EC 3.4.4.4.), papain (EC 3.4.4.1.) and "pronase" were commercial products. "Pronase" is a peptidohydrolase obtained from *Streptomyces griseus* by Kaken Co. Ltd., Tokyo.

Analytical methods The protein was determined by the method of Lowry (19). The determination of amino residue was carried out by ninhydrin method at 570 mμ (15).

Treatment of protein (AAP) fraction with proteolytic enzymes Twelve mg of the AAP fractions were dissolved in 36 ml of 0.1 M phosphate buffer (pH 7.0), and 3 ml of pronase or trypsin solution (60 μg of enzyme/ml of buffer solution) were added to 27 ml of antigen solution. When papain was used as an enzyme, 3 ml of enzyme solution (30 μg of papain/ml of buffer solution) containing 0.01 M mercaptoethanol and 0.002 M ethylenediaminetetraacetic acid (EDTA) were added to the 27 ml of antigen

solution. The reaction mixture was incubated at 37 °C and at various intervals, 2 ml of solution were removed and heated at 100 °C for 5 minutes. These solutions diluted with saline were used for skin reaction and for the determination of amino residue of antigen.

Periodate oxidation of polysaccharide To the 6 mg of antigen dissolved in 3 ml of water, 3 ml of 0.1 M $NaIO_4$ solution were added and the reaction mixture was kept for 5 days at 5 °C. The reaction mixture was dialyzed against a running water, and was reduced by the addition of 35 mg of $NaBH_4$ at room temperature. After the addition of 6 N acetic acid, the reaction mixture was dialyzed against running water for 2 days. The inner solution was diluted to a solution containing 10 μg of antigen/ml and was used for the skin reaction.

Immunization of rabbits and guinea pigs with A. fumigatus Schedule A: Rabbits were immunized by the subcutaneous injection of 1 ml of conidia suspension (1×10^4 of conidia/ml saline) and further immunization was performed by the intravenous injection of 0.5 ml of conidia suspension (1×10^4/ml) for 2 times, at 7 days intervals. At 7 days after the third injection, the rabbits were infected by the infusion of 0.5 ml of conidia suspension (1×10^8/ml) of *A. fumigatus* Itono strain into the right lower lobe of the lung by bronchoscopic technique.

Schedule B: Rabbits were immunized by the intramuscular injection of 2 mg of acetone-dried *A. fumigatus* (No. 5840) in 1 ml of Freund's complete adjuvant for 3 times at 2 weeks intervals. Rabbits were further immunized by the intravenous injection of 2 ml of saline extract (1 g of acetone-dried *A. fumigatus*/25 ml of saline) twice at 3 days intervals.

Guinea pigs were immunized by the intramuscular injection of 2 mg of acetone-dried *A. fumigatus* (No. 5840) in 1 ml of Freund's complete adjuvant for 3 times at 2 weeks' intervals. The immunization of rabbits with *A. flavus, A. niger, A. nidulans, A. terreus* was carried out by schedule B.

Skin test The material to be tested was dissolved in saline and passed through the millipore filter to remove the organisms and cell debris. Each 0.1 ml of the antigen solution was injected intracutaneously and saline was used as a control.

Precipitation reaction Precipitation reaction was carried out by the ring test. The quantitative precipitation test was performed with 0.5 ml of antigen solution and 0.5 ml of rabbit antiserum which was treated previously for 30 minutes at 56 °C. The amount of precipitate was determined by the method of LOWRY (19).

Complement fixation test Complement fixation test was carried out by the method of MAYER (15). The reaction mixture containing 0.4 ml of diluted rabbit antiserum, 0.5 ml of guinea pig serum containing 5 fifty % hemolytic units of complement (5 $C'H_{50}$) and 0.4 ml of antigen solution, was kept for 20 hours at 2° to 4 °C.

At the end of the incubation period, 0.2 ml of sensitized sheep erythrocyte suspension were added and tubes were incubated for 60 minutes at 37 °C in water bath with continuous agitation. The degree of lysis in the test and in the control tubes was estimated visually. The titer was expressed as the highest dilution of antigen and antiserum which indicated approximately 50 % lysis in the system.

Passive hemagglutination test Passive hemagglutination test was performed by STAVITSKY's method (22). Sheep erythrocytes were treated with 1 : 20,000 dilution tannic acid solution at 37 °C for 10 minutes. One-tenth ml of tanned-cells which were suspended in 1 ml of saline were sensitized with 0.5 mg of polysaccharides in 5 ml of phosphate buffer-saline solution (pH 6.4) at room temperature for 30 minutes. To 0.5 ml of rabbit antiserum, 0.05 ml of sensitied tanned-cells suspension were added and then kept at room temperature for 12 hours. As a diluent, 1 : 100 normal rabbit serum in phosphate buffer-saline (pH 7.2) was used. The titer was expressed as the highest dilution of an antiserum which gave positive grade 2+ as described by STAVITSKY (22).

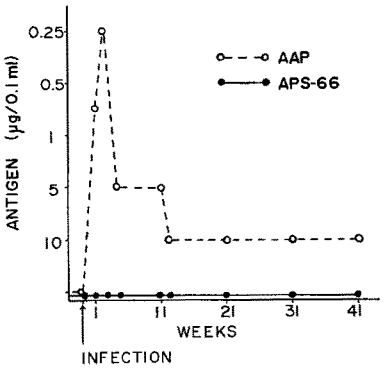
Passive cutaneous anaphylaxis test The test was carried cut by the following procedure. Normal guinea pigs were injected intracutaneously with 0.1 ml of diluted guinea pig antiserum. Twenty hours later, they were injected intracutaneously with 0.025 mg of antigen dissolved in 0.025 ml of saline at the same sites. Immediately after antigen injection, 1 ml of 1 % Evans Blue solution was injected intravenously. The reaction, a marked bluing of the injected area, was measured at 30 minutes after the Evans Blue injection.

RESULTS

Skin Reaction

The progress of delayed type skin reaction with protein (AAP) and galactomannan (APS-66) in rabbit which was immunized with *A. fumigatus* by Schedule A is shown in fig. 1. The maximal reaction was observed at two weeks after the infection with *A. fumigatus*. As shown in fig. 2, similar progress was observed by the precipitation reaction in rabbit antiserum against *A. fumigatus* by Schedule A. The rabbit and rabbit antiserum obtained by the immunization with *A. fumigatus* by Schedule B showed more potential antibody titer by the skin and precipitation reactions in comparison with the case of Schedule A. The protein (AAP) showed the Arthus type and delayed type skin reactions in immunized rabbits and guinea pigs. On the other hand, the galactomannan (APS-66) which is pure polysaccharide showed the Arthus type skin reaction as shown in fig. 3.

From the results of chemical analysis, it was shown that the protein (AAP) fraction contained about 15 % of carbohydrate



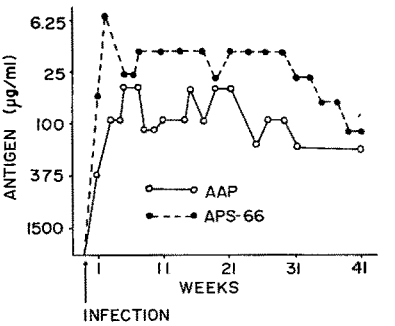
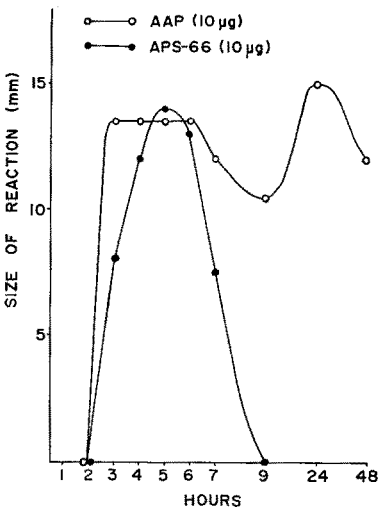


Fig. 1. The progress of delayed type skin reaction with the protein (AAP) and galactomannan (APS-66) in the rabbit immunized with *A. fumigatus* by Schedule A. The reaction was measured at 24 hours after the intracutaneous injection of antigen and the curves show the minimal concentration of antigens which produce redness more than 10 by 10 mm in diameter.

Fig. 2. The progress of precipitation reaction with the protein (AAP) and galactomannan (APS-66) in the rabbit antiserum obtained by the immunization with *A. fumigatus* by Schedule A. The curves show the minimal concentration of antigens which produce the precipitin with undiluted rabbit antiserum.



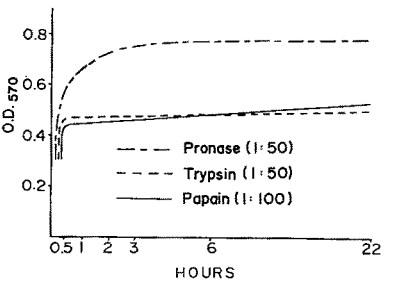


Fig. 3. Skin reaction of the protein (AAP) and galactomannan (APS-66) in the rabbit immunized with *A. fumigatus* by Schedule B.

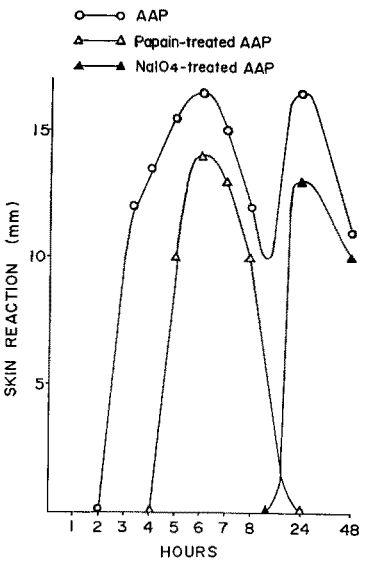
Fig. 4. The digestion of the protein (AAP) fraction with proteolytic enzymes. The degradation-ratio of the protein (AAP) was determined by ninhydrin metnod at 570 mμ.

TABLE I

Delayed-type skin reaction with the protein (AAP) and protease-treated AAP fractions in the rabbits immunized by Schedule B.

Rabbits	Original AAP (1 μg)	Skin Reaction (mm by mm)						
		Pronase-treated [a] AAP (1 μg)		Papain-treated [a] AAP (1 μg)		Trypsin-treated [a] AAP (1 μg)		Control (Saline)
		15 min	22 hrs	15 min	22 hrs	15 min	22 hrs	
1	10×10	8×8	8×8	0×0	0×0	7×7	7×7	0×0
2	17×16	11×10	9×9	9×9	0×0	8×8	6×6	0×0
3	18×18	10×10	11×10	6×7	0×0	9×9	9×9	0×0

[a]) The protein (AAP) fraction was treated with pronase, papain or trypsin for 15 minutes or 22 hours. One microgram of each preparation was injected intracutaneously and the reaction was measured at 24 hours after the injection.



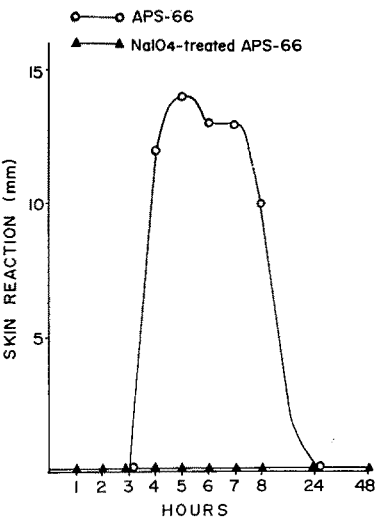


Fig. 5. Skin reaction with the protein (AAP), papain-treated AAP and $NaIO_4$-treated AAP fractions, in 10 μg doses, in the rabbit immunized with *A. fumigatus* by Schedule B.

Fig. 6. Skin reaction with galactomannan (APS-66) and $NaIO_4$-treated APS-66, in 10 μg doses, in the rabbit immunized with *A. fumigatus* by Schedule B.

moiety. The protein (AAP) fraction was digested with proteolytic enzymes, pronase, trypsin or papain. The rate of degradation of the protein (AAP) fraction was determined by the ninhydrin method. The treatment of pronase was most effective on the digestion of the protein (AAP) fraction as shown in fig. 4.

The skin reactivity of protease-treated AAP fraction was examined and the results are shown in Table I and fig. 5.

The delayed type skin reactivity of the protein (AAP) fraction was reduced by the treatment with pronase, trypsin or papain. By the treatment with papain for 22 hours, the delayed type skin reactivity was almost completely lost as shown in Table I and fig. 5. However, the Arthus type skin reactivity of the protein (AAP) fraction was not affected by the treatment of papain (fig. 5).

On the other hand, the Arthus type skin reactivity of protein (AAP) and galactomannan (APS-66) was destroyed by the periodate oxidation as shown in figs. 5 and 6.

The delayed type skin reactivity of the protein (AAP) fraction was not affected by the periodate oxidation procedure.

Serological Reactions

The precipitation, complement fixation, passive hemagglutination and passive cutaneous anaphylaxis reactions were examined in rabbit and guinea pig antisera obtained by the immunization with *A. fumigatus*. The quantitative precipitation curve of galactomannan (APS-66) with rabbit antiserum is shown in fig. 7.

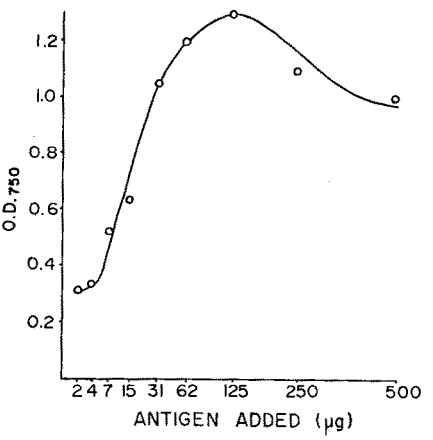


Fig. 7. Quantitative precipitation curve of galactomannan (APS-66) with rabbit antiserum.

At equivalence, galactomannan (APS-66) precipitated 1,320 μg of antibody per ml of antiserum. The results of precipitation, complement fixation and passive hemagglutination reactions with galactomannan (APS-66) are shown in Table II.

Passive cutaneous anaphylactic test with the protein (AAP) and galactomannan (APS-66) using guinea pig antiserum was carried out in normal guinea pigs. The bluing sizes of injected area are shown in Table III.

TABLE II

Chemical and serological properties of polysaccharides obtained from Aspergillus fumigatus.

Polysaccharides	Sugar composition [a]	Precipitation reaction [b]	Complement fixation test [c]	Passive hemagglutination test [d]
Galactomannan (APS-66)	Galactose 1 Mannose 3.1	Antigen titer 1 : 256,000	Ag. 0.097 μg Ab. 1 : 160	1 : 20,480
Glucan (APS-33)	Glucose	Negative	Not tested	Not tested

[a]) Sugar composition was determined by the method of SWEELEY et al. (23).
[b]) Antigen titer was determined by the "ring test".
[c]) The titer was expressed as micrograms of antigen and antiserum dilution necessary for 50 % lysis of sensitized sheep erythrocytes by the method of MAYER (15).
[d]) The titer was expressed as the highest dilution of antiserum which gave positive grade 2+ by STAVITSKY's method (22).

TABLE III

Passive cutaneous anaphylaxis test with the protein (AAP) and galactomannan (APS-66) in guinea pigs.

Dilution of antiserum (times) [a]	Skin reaction (mm by mm)			
	AAP (25 μg)		APS-66 (25 μg)	
	Antiserum	Normal serum	Antiserum	Normal serum
10	16×18	3×4	15×15	3×3
10	15×15	2×2	19×18	3×4
20	14×13	3×3	12×14	3×5
20	13×12	3×3	15×15	3×3
50	15×15	2×3	15×13	3×4
50	15×16	3×2	14×14	3×3

[a]) Antiserum was obtained from the guinea pigs which were immunized with *A. fumigatus* in FREUND's complete adjuvant.

Cross Reaction in Skin and Serological Reactions

In rabbits immunized with *A. fumigatus, A. flavus, A. niger* or *A. nidulans* in Freund's complete adjuvant, the delayed type skin reaction was provoked by the injection of the protein (AAP) from *A. fumigatus* in 10 μg doses, however, skin reaction could not be detected in the rabbits immunized with *A. terreus* as shown in Table IV.

By the injection of 1 μg of the protein (AAP), no skin reaction was elicited in the rabbits immunized with *A. flavus, A. niger, A. nidulans* or *A. terreus*. The galactomannan (APS-66) gave

precipitation reaction with all rabbit antisera. No cross reaction was observed by the serological tests in rabbits antisera against heat-killed *Nocardia asteroides, M. tuberculosis, M. fortuitum, M. smegmatis* or atypical mycobacteria P1 strain. On the other hand, no delayed type skin reaction was elicited by the injection of protein fractions which were obtained by the same method with the AAP from *Cryptococcus neoformans, Streptomyces griseus, Penicillium* DC11 strain, *A. niger, Nocardia asteroides, M. tuberculosis, M. fortuitum, M. smegmatis* and atypical mycobacteria P_1 strain in the rabbits immunized with *A. fumigatus*. The polysaccharides of *Candida albicans*, glucan and mannan, did not show any cross reaction by immunological examination in rabbit immunized with *A. fumigatus*. Trichophytin which was purified from culture filtrate or mycelia of *Trichophyton rubrum* did not provoke delayed type skin reaction, but gave precipitation reaction with rabbit antiserum against *A. fumigatus* (antigen titer 1 : 64,000).

TABLE IV

Immunological cross-reactivities of various Aspergillus species with the protein (AAP) and galactomannan (APS-66) obtained from A. fumigatus.

Rabbits were immunized with:	Skin reaction (mm by mm) [a]			Precipitation [b] reaction with APS-66 (antigen titer)
	Hours	AAP (10 μg)	AAP (1 μg)	
A. fumigatus	24	14×15	15×12	1 : 256,000
	48	12×12	13×11	
A. flavus	24	13×14	8×9	1 : 256,000
	48	13×13	Negative	
A. niger	24	13×14	Negative	1 : 256,000
	48	12×11	Negative	
A. nidulans	24	10×10	Negative	1 : 64,000
	48	Negative	Negative	
A. terreus	24	Negative	Negative	1 : 256,000
	48	Negative	Negative	

[a] Skin reaction was measured at 24 and 48 hours after the injection.
[b] Galactomannan (APS-66) which was isolated from *A. fumigatus* was used as an antigen, and antigen titer was determined by "ring test".

Application for Clinical Diagnosis

On the basis described above, the protein (AAP) and galactomannan (APS-66) fractions were applied for the clinical diagnosis of human aspergilosis. The skin test with the protein (AAP) fraction was performed on 600 patients with pulmonary tuberculosis and 50 healthy hospital nurses. Detailed clinical information on the results of positive cases will be reported in a successive paper. We describe here 4 cases which indicated different type skin

TABLE V.

Skin reaction with the protein (AAP) and galactomannan (APS-66) in patients with aspergillosis.

Patients	Type of aspergillosis	Bacteriological and mycological examination		Skin reaction (mm by mm) [a]			
				AAP (1 μg)		APS-66 (10 μg)	
		M. tuberculosis	*A. fumigatus*	Reaginic	Delayed	Reaginic	Delayed
1. H. S. (20 years)	Aspergilloma	Positive	Negative	26×30	Negative	27×28	Negative
2. K. T. (40)	Aspergilloma	Negative	Positive	45×42	Negative	24×24	Negative
3. K. M. (66)	Bronchopulmonary aspergillosis	Positive	Positive	54×40	87×54	11×11	Negative
4. S. I. (30)	Bronchopulmonary aspergillosis	Positive	Positive	Negative	14×13	Negative	Negative

[a] Reaginic type skin reaction was measured at 30 minutes and delayed type at 48 hours after the injections. The results show the diameter of redness.

reaction. As shown in Table V, 2 patients, case 1 and 2, showed wheal-erythema skin reaction by the injection of the protein (AAP) and galactomannan (APS-66) fractions.

It seems that the development of wheal-erythema reaction with the protein (AAP) fraction is due to the carbohydrate fraction which is contained in the AAP. A typical fungusball shadow was observed roentgenographically in the right upper lobe in both patients. In the third case which was complicated with pulmonary tuberculosis, *A. fumigatus* was isolated from the sputum and the purulent discharge in the left thorax. Wheal-erythema and delayed type skin reactions were provoked by the injection of 1 or 5 μg of the protein (AAP) and whealerythema reaction with galactomannan (APS-66) in the same dose. In the fourth case which was also complicated with pulmonary tuberculosis, delayed type skin reaction was provoked by the injection of the protein (AAP). Wheal-erythema skin reaction was not detected with the protein (AAP) and galactomannan (APS-66). The Arthus type skin reaction could not be elicited with both test antigens. Delayed type skin reaction was not detected with galactomannan (APS-66) in all cases. In addition to the negative result of skin test in the patients with pulmonary tuberculosis, the results were also negative in patients with lung cancer, lung sarcoidosis, lung abscess and vesicular eczema. Further investigations are being undertaken on the application of the protein (AAP) and galactomannan (APS-66) to the clinical diagnosis in man.

Discussion

Many efforts have been done on the isolation of the skin and serological test antigen and tried to apply for the diagnosis of aspergillosis. However, these antigens used in previous experiments were crude extract or culture filtrate of *A. fumigatus*. In the previous papers (4—6), the authors described the purification of protein and polysaccharide from *A. fumigatus*. The chemical nature of both fractions were investigated in detail. It was shown that polysaccharide fraction contained two kinds of polysaccharides, glucan and galactomannan. Galactomannan which was designated as APS-66 showed the skin and serological activities.

In the present experiments, the immunological properties of protein and polysaccharides were investigated in the rabbits and guinea pigs which were immunized with *A. fumigatus*. Fukui (14) and Wada (24, 25) have reported that polysaccharide obtained from *A. fumigatus* could elicit delayed type skin reaction in sensitized rabbits or guinea pigs. Similar results were observed by Knight (16) and Bare (8) on histoplasmin or tuberculin, respectively. However, the Arthus type skin reaction was elicited by the injection of galactomannan (APS-66) in the sensitized rabbits or guinea pigs in the present experiments. Delayed type skin reaction

was provoked by the injection of protein (AAP), but not by polysaccharide. These results seem to indicate that polysaccharide could not elicit delayed type skin reaction but elicit Arthus type skin reaction in rabbits or guinea pigs immunized with *A. fumigatus*. This conclusion was supported by the fact that delayed type skin reactivity of the AAP was almost completely lost by the treatment of proteolytic enzyme, especially with papain. On the other hand, the Arthus type skin reactivity of polysaccharide was destroyed by periodate oxidation. Similar supports were obtained by the results of BARKER et al. (9) on trichophytin, AOKI (1—3) on histoplasmin and coccidioidin and AZUMA et al. (7) on tuberculin. OKADA et al. (20) isolated the tuberculin active peptide from the defatted cells of human tubercle bacilli. The tuberculin active peptide (TAP) thus obtained showed considerably high specificity with the skin reaction in sensitized animals. They have also described that their method for preparation of tuberculin active peptide was very useful and applicable for the extraction of specific skin test antigen from other allergens. In the present experiments, the AAP was extracted and purified by the same method with tuberculin active peptide described by OKADA et al. (20). From the results of cross reaction, it was shown that the AAP showed marked specificity with skin test in rabbits which were immunized with *Aspergillus* species and other fungal or bacterial species.

As shown in Fig. 2, the AAP showed precipitation reaction with rabbit antiserum, however, the AAP did not give precipit in with antiserum which was absorbed with galactomannan (APS-66). On the other hand, galactomannan did not give precipitin with rabbit antiserum absorbed with the AAP. Above results suggest that the precipitation activity of the AAP was due to the carbohydrate which was contained in the AAP fraction. It was shown that galactomannan (APS-66) was useful for the antigen to the serological reaction. Similarly, it is considered that the Arthus type and wheal-erythema skin reactivities of the AAP is due to the carbohydrate fraction of the AAP. In the patients with aspergillosis, the galactomannan (APS-66) showed wheal-erythema skin reaction. By the injection of the AAP, delayed and wheal-erythema type skin reactions were provoked in patients with aspergillosis. The relationships between the type of aspergillosis and skin reaction are now being examined in our clinic.

Above results showed that the protein (AAP) and galactomannan (APS-66) were useful for skin and serological test antigen to clinical diagnosis of patients with aspergillosis in man.

Summary

The immunological activity of protein and polysaccharides which were isolated and purified from *Aspergillus fumigatus* was investigated. The protein fraction which was designated AAP

contained about 15 % of carbohydrate. By the intracutaneous injection of the AAP into rabbits or guinea pigs which were immunized with *A. fumigates*, Arthus and delayed type skin reactions were provoked. The delayed type skin reactivity of the AAP was almost completely lost by the treatment with proteolytic enzymes. The AAP showed high specificity by the skin reaction in the rabbits which were immunized with *Aspergillus* and other fungi.

Polysaccharide fraction was further fractionated into 2 components, glucan and galactomannan. Galactomannan which was designated as APS-66 was active by the skin and serological reactions in the immunized rabbits, guinea pigs or patients with aspergillosis. Galactomannan (APS-66) was shown to be available as the antigen of the precipitation, complement fixation and passive hemagglutination tests with the rabbit antiserum. By the intracutaneous injection of galactomannan (APS-66) in immunized rabbits or guinea pigs, the Arthus type skin reaction was elicited. In the cases of aspergillosis, wheal-erythema skin reaction was elicited by the intracutaneous injection of 1 or 5 μg of galactomannan (APS-66), whereas, delayed and wheal-erythema skin reactions were observed by the injection of protein (AAP) fraction in 1 or 5 μg doses. Glucan (APS-33) was inactive with the immunological test when it was tested in the immunized animals or the patients with aspergillosis.

The results lead tentatively to the conclusion that protein and polysacharide (galactomannan) which were purified from *A. fumigatus* were useful for the antigens of the skin and serological reactions in the human cases of aspergillosis as well as in the experimental aspergillosis in the rabbits and guinea pigs.

Acknowledgements

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