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EFFECT OF LIGNOPHENOL ON ALLERGEN MITIGATION

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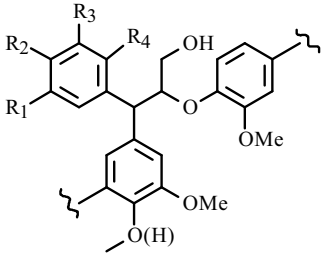
The deactivating of mite allergens was investigated using lignophenols, which are polymers with a light-beige appearance that were synthesized directly from lignocellulose with a phase-separation system. Various applications of lignophenols, instead of the photochemical solar cell dye-sensitized with lignophenol, have been reported. In the present study, lignophenols were applied for the deactivation of mite allergens.

Keywords: mite allergen, lignophenol derivatives, tannic acid, poly-4-vinyl phenol.

Nowadays, indoor allergens have become one of the main causes of atopic dermatitis and bronchitis. The first method of curing this disease is antigen evasion. For example, a patient can move to the highlands above 1,500 meters where mites do not live due to the low humidity [1], or a patient enters a sickroom where *Derp1* (*Dermatophagoides pteronyssinus*) is controlled in the range of less than 0.24 μg/g of dust [2]. The interaction of polyphenol with protein has been investigated as a mechanism to reduce mite allergens [3–6]. Now, commercial tannic acid is on the market for mite allergen reduction in Europe and America [7, 8]. Other polyphenols are also likely to become a promising material for mite mitigation. Moreover, it is very advantageous if the polyphenol is more hydrophobic than water-soluble tannic acid. Hence, in the present study, the effect of lignophenol on allergen mitigation was investigated. Lignophenol can be readily obtained from lignocellulose, and it is insoluble in water.

Lignin is a three-dimensional polymer consisting of phenylpropanoid interunits bound mainly by aryl and alkyl ethers. It is very difficult to process or modify due to its complex nature. Lignophenols, which are light-beige polymers, have been synthesized directly from lignocellulose with a phase-separation system. This system consists of grafting phenols into native lignin using the surface reaction of concentrated acid and phenols at ambient temperature and pressure [9]. Lignophenols are more stable than native lignin because the benzyl positions, which are the active sites, have been blocked by phenols. Moreover, lignophenols can be chemically designed because they have more simple structures than industrial lignins. Hence, these materials are useful in industry instead of petroleum, fossil carbon resources, and coal. Lignophenols have actually been developed as recyclable materials, for example, composites with pulp, additives for plastics, etc [10]. The proposed major substructure in lignophenols is illustrated above. Recently, various applications of lignophenols, such as the recovery of precious metals using lignophenol compounds and photochemical solar cells sensitized with the lignophenol, have been reported. Therefore, in the present study, lignophenols were applied to the deactivation of mite allergens.



Lignopyrocresol: $R_1 = CH_3$, $R_2 = R_3 = H$, $R_4 = OH$
Lignopyrocatechol: $R_1 = R_4 = H$, $R_2 = R_3 = OH$
Lignopyrogallol: $R_1 = R_2 = R_3 = OH$, $R_4 = H$

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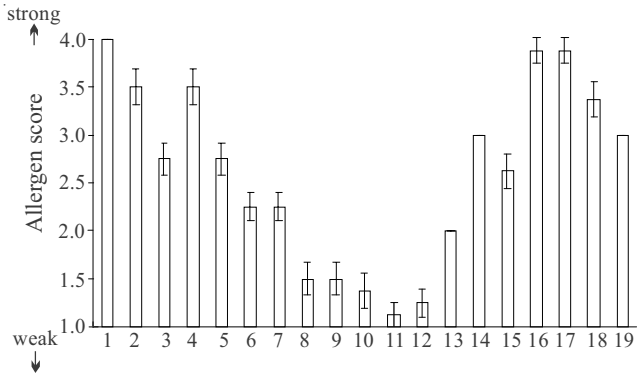

Fig. 1. Deactivation of the mite allergen *Derf2*. Poly-4-vinyl phenol (p4vp) with molecular weights of 8000 and 20000 was used for the treatment: control (1), LC/Ac (2), LC/Me (3), LC/Et (4), LPC/Ac (5), LPC/Me (6), LPC/Et (7), LPG/Ac (8), LPG/Me (9), LPG/Et (10), TA/Ac (11), TA/Me (12), TA/Et (13), p4vp 8k/Ac (14), p4vp 8k/Me (15), p4vp 8k/Et (16), p4vp 20k/Ac (17), p4vp 20k/Me (18), p4vp 20k/Et (19). The abbreviations "Ac," "Me," and "Et" mean acetone, methanol, and ethanol, respectively.

The deactivation of the mite allergen *Derf2* was investigated using the tested materials containing ligno-*p*-cresol, lignopyrocatechol, lignopyrogallol, tannic acid, and poly-4-vinyl phenol. The deactivation of the mite allergen was evaluated using the Asahi Dani Scan checker. The results are illustrated in Fig.1. Although poly-4-vinyl phenol was nearly ineffective at reducing allergens, tannic acid positively affected the deactivation. The lignophenols were effective at deactivating mite allergen, in the sequence lignopyrogallol >lignopyrocatechol >ligno-*p*-cresol. These results mean that the allergen deactivations improve with increase in the number of phenolic hydroxyl groups in the deactivating substances. The deactivating effect of lignopyrogallol was almost the same as that of tannic acid. The organic solvent effect was almost unobservable in deactivating the mite allergen *Derf2*. Therefore, 80 vol.% methanol (in water) was used as the solvent for the deactivation treatment of the mite allergen *Derf1*.

Reduction of the mite allergen *Derf1* was studied using sandwich ELISA analysis. The results are shown in Fig. 2. Although treatment with lignopyrocatechol, lignopyrogallol, tannic acid, and poly-4-vinyl phenol proved their comparatively high inhibition efficiencies, the deactivation efficiency with ligno-*p*-cresol was very low (approximately 5%). Therefore, the deactivations of allergen *Derf1* were in the order lignopyrocatechol = lignopyrogallol >> ligno-*p*-cresol. These results were roughly consistent with the results of the deactivation of the mite allergen *Derf2*.

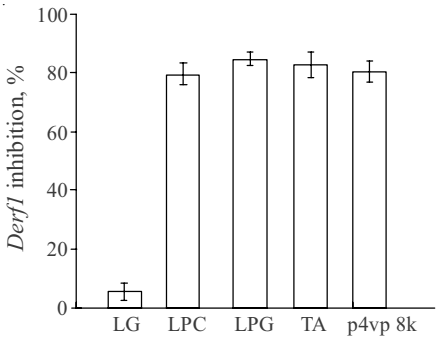

Fig. 2. Deactivation of the mite allergen *Derf1*.

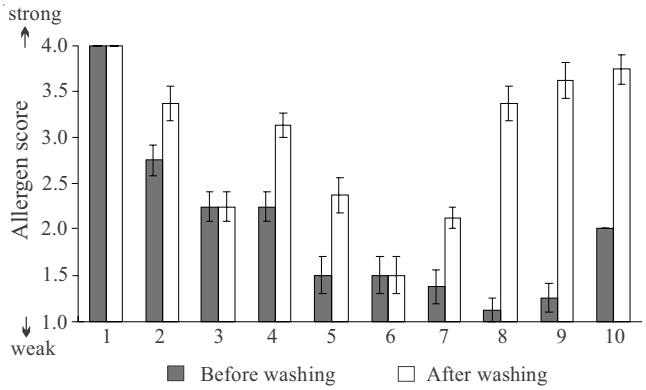

Fig. 3. Deactivation of the mite allergen *Derf2* after water washing. Poly-4-vinyl phenol (p4vp) with molecular weights of 8000 and 20000 was used for the treatment: control (1), LPC/Ac (2), LPC/Me (3), LPC/Et (4), LPG/Et (5), LPG/Me (6), LPG/Et (7), TA/Ac (8), TA/Me (9), TA/Et (10). The abbreviations "Ac," "Me," and "Et" mean acetone, methanol, and ethanol, respectively.

Since lignophenols are more hydrophobic than water-soluble tannic acid, it may be a big advantage if lignophenol-modified fabrics can be used several times. Therefore, the allergen deactivation was tested after washing the fabrics in water. The results are depicted in Fig. 3. Although the efficiency in the case of a tannic acid deactivator without water washing was very effective at deactivating the mite allergen *Derf2*, the deactivation level after washing was much worse. On the contrary, the deactivating effect using lignopyrogallol-modified fabrics with methanol solvents was strongly sustained after water washing. Why the deactivation after washing remained in the case of methanol solvents was not clear. Possibly, the sorption of the lignopyrogallol deactivator onto nonwoven fabric was accelerated by the presence of methanol.

In order to check the reaction of lignophenols with proteins, the interaction between lignopyrogallol and proteins was investigated in water. After centrifugation at 10000 rpm for 1 minute, precipitation could be observed in the case of both the mite allergen *Derf1* and bovine serum albumin, BSA, using lignopyrogallol deactivators. However, although precipitation was seen in the case of bovine serum albumin with tannic acid, it was not in the *Derf1* solution mixed with tannic acid. It is well known that the reaction between protein and polyphenol leads to precipitation. However, when a low concentration of *Derf1* was used, the results obtained with the lignopyrogallol deactivators were different from those of tannic acid. The fact means lignopyrogallol has the ability to interact more strongly with *Derf1* compared to tannic acid. Therefore, it was postulated that lignopyrogallol provides better allergen mitigation. Consequently, it can be confirmed that proteins are strongly adsorbed onto lignopyrogallol from observation of the precipitation of protein/lignopyrocatechol complexes, as shown in the results obtained with tannic acid.

EXPERIMENTAL

Lignophenol and Reagents. Lignophenols, such as ligno-*p*-cresol (LC), lignopyrocatechol (LPC), and lignopyrogallol (LPG), were produced by the reported methods [9]. Tannic acid (TA) and poly-4-vinyl phenol (p4vp, molecular weight 8000 and 20000) were purchased from Sigma-Aldrich Japan Corporation, Tokyo, Japan (high-quality chemical grade). The nonwoven fabrics were obtained from Kureha Corporation, Tokyo, Japan (No. 8056). Recombinant *Derf1* and *Derf2* (*Dermatophagoides farinae*, 25 and 14 kDa) protein mite allergens used in this work were purchased from Seikagaku Corporation, Tokyo, Japan.

Evaluation of Deactivation. The *Derf1* and *Derf2* aqueous solutions (3.1 and 0.83 ng/mL, respectively) were prepared by dissolving the protein in phosphate-buffered saline (PBS). PBS solutions were prepared with ultrapure water, which was purified by an ultrapure water system (Milli-Q Labo, Millipore Corporation) resulting in a resistivity > 18 MΩ cm. The *Derf2* solution contained 0.06% Tween 20 detergent. The deactivation materials containing ligno-*p*-cresol, lignopyrocatechol, lignopyrogallol, tannic acid, and poly-4-vinyl phenol were dissolved in organic solvents with a 2% concentration by weight. Organic solvents such as 80 vol.% methanol (in water), ethanol, and acetone were used for the deactivation treatment.

The nonwoven fabric (20 cm × 15 cm) was immersed in the organic solution with the deactivation materials for 30 min and dried at 30°C for 24 h. Then, the *Derf2* aqueous solution of 0.75 mL was uniformly applied onto the surface of the nonwoven fabric, and the fabric was dried at 30°C for 2 h. Finally, the deactivation was measured by Asahi Dani Scan (Asahi food and healthcare Co. Ltd.). The Asahi Dani Scan checker has a level range of 1 to 4 for mite contamination, and in the checker the level increased up to 4 with worsening mite contamination.

To evaluate *Derf1* deactivation, a sandwich enzyme-linked immunosorbent assay (sandwich ELISA) was used. A 0.1 mL organic solution with 2 wt.% deactivation material concentration was uniformly applied onto the nonwoven fabric (2 cm × 2 cm), and it was dried at 30°C for 2 h. Next, the nonwoven fabric was put into the *Derf1* solution in a cylindrical glass vial (diameter 1 cm, volume 5 mL), and the vial was horizontally shaken for 60 min. Then, the supernatant solution was subjected to ELISA analysis. The absorbance for the assay was measured at 415 nm.

Deactivation after Water Washing. First, the nonwoven fabric (20 cm × 15 cm) was treated according to the above method for the *Derf2* aqueous solution, and then it was washed in pure water for 3 min and dried at 40°C for 12 h. Next, 0.75 mL of *Derf2* aqueous solution was uniformly applied onto the surface of the nonwoven fabric, and it was dried at 30°C for 2 h. Finally, the deactivation was evaluated by Asahi Dani Scan.

Reaction of Deactivation Material with Protein. In order to check the relation of the deactivation material and the protein, BSA (bovine serum albumin) solutions of 1 wt.% concentration and *Derf1* solutions of 3.1 ng/mL concentration were prepared by dissolution in phosphate-buffered saline PBS solution. The *Derf1* concentration is the environmental indoor level that causes the allergy disease. Lignopyrogallol or tannic acid (3 wt% concentration) were dissolved in 80 vol.% methanol in water. Then, 0.3 mL of PBS solution with 1 wt.% BSA or 3.1 ng/mL *Derf1* was mixed with 0.3 mL of 80 vol.% methanol with 3 wt.% deactivation material. After centrifugation at 10000 rpm for 1 min, the precipitation was evaluated for the relation.

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