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# Phthalocyanine-dyed fibers adsorb allergenic proteins

*Short Communication*

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**Summary.** Phthalocyanine (Pc)-dyed fiber is reported to reduce atopic symptoms in some patients when they use underwear made of the fiber. We investigated the adsorption of allergens on Pc-fiber. Pc-fiber trapped house dust/pollen/food allergens with varied molecular weight and pI. The adsorbed allergens were released in the presence of mild detergent. Pc-fiber did not change the molecular weight or disulfide bonding of the allergens. These observations imply that Pc-fiber is applicable as an "allergen trap" for a wide variety of products.

**Keywords:** Phthalocyanine – Allergen – House dust

## Introduction

Allergen-blocking products are recommended for allergic subjects; for example, special mattresses and pillow cases are used by patients allergic to dust mites, and pollen masks are available for hay fever sufferers. Tightly woven cloth physically blocks pollens and the dead bodies/fecal pellets of mites (Mahakittikun et al., 2003), and decreases allergenic patients' exposure to these allergens. Sometimes, however, allergens are decomposed into small particles, slip through the cloth, and come into direct contact with patients.

The major causes of allergies are allergenic proteins released from pollen and mites. They are highly soluble and may be accessible to patients through sweat. If a fiber that adsorbs allergenic proteins were available, it would have widespread application in allergy-alleviating products such as allergen-proof masks or encasements and underwear for atopic patients.

Clinical observations suggest that the $Fe^{3+}$ complex of phthalocyanine (Pc)-dyed fiber, $FePc(COOH)_4$, was effective in reducing atopic symptoms for some patients when they used underwear, such as stockinette, made of the fiber (Yokozeki and Shirai, 1991; Yokozeki et al., 1996). Pc is reported to interact with some proteins such as cytochrome C (Laia and Costa, 2004) and prion (Caughey et al., 1998), so it is possible that the clinical observations of Pc-dyed fiber alleviating the symptoms of atopic patients are related to the adsorption of allergenic proteins by the fiber. However, there is limited information on the adsorption of protein to Pc. As we describe in this short communication, we investigated the adsorption of several allergens on Pc-dyed fiber.

## Materials and methods

The chemicals and biochemicals were purchased from commercial sources and were of the highest quality available. A convenient tandem-column system, based on a commercially available centrifugal filter device (Microcon, Millipore), was applied to estimate the adsorption of allergenic proteins on a fiber (Fig. 1). It was composed of a sample reservoir (upper column) and a filtrate vial (lower). First, 10 mg of fiber was placed in the sample reservoir. Next, an allergen solution, 20 μg/200 μl, was poured into the sample reservoir, so the fiber was immersed in the solution. After

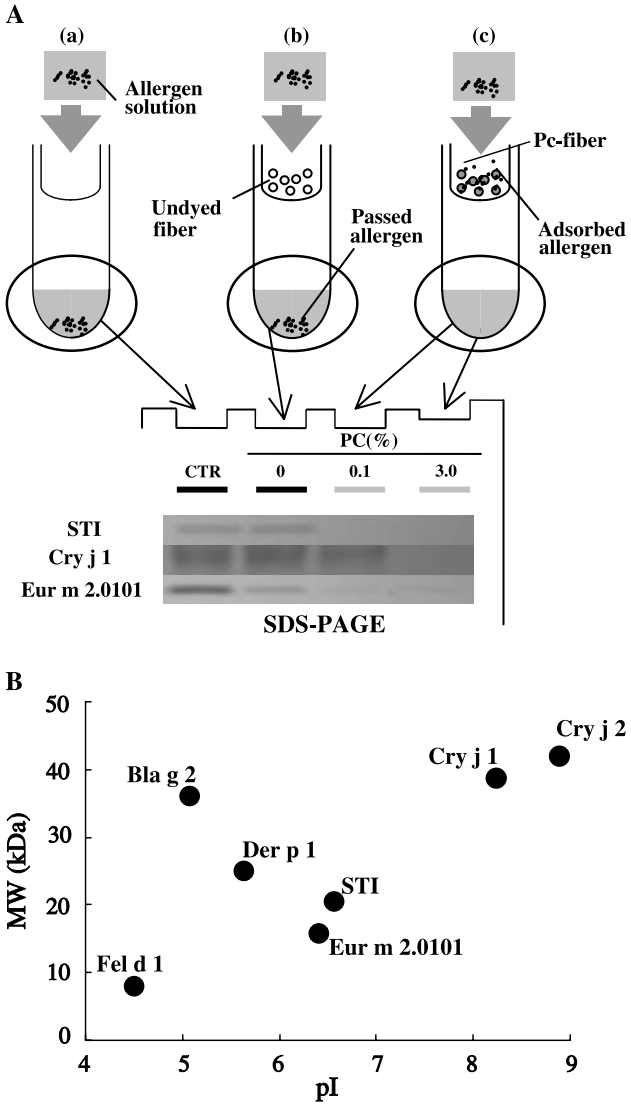


**Fig. 1.** **A** A convenient system for evaluating the adsorption of allergens onto a fiber. Allergen solution was applied in a sample reservoir as follows: (*a*), control; (*b*), the reservoir contained an undyed fiber; (*c*), the reservoir contained phthalocyanine (Pc)-dyed fiber. By comparison of (*b*) and (*c*), it was possible to determine whether the adsorbtion could be attributed to the Pc-dyed fiber. **B** Scatter diagram of allergens adsorbed on the Pc-dyed fiber. *STI* Soybean trypsin inhibitor

gently mixing the fiber in the solution for 10 min at room temperature, the tandem-column system was centrifuged at 5,000 G until the solution in the sample reservoir dropped into the filtrate vial. Then, an aliquot of the solution (30 µl), taken from the filtrate vial, was mixed with ×2 Laemmli sample buffer (30 µl) and applied to SDS-PAGE. After electrophoresis, the gel was stained with Coomassie Blue. The principle was that if the allergenic protein was adsorbed on the fiber, it would remain in the sample vial and would not be recoverable from the filtrate vial. However, if the allergenic protein did not interact with the fiber, the protein could be recovered from the filtrate vial, and the corresponding protein band would then appear on the SDS-PAGE gel. The gel profile was analyzed with a densitometer and the adsorption rate (%) calculated against a control sample in which no fiber was put in the sample reservoir (Fig. 1Aa). In this experiment, to observe the effect of Pc on the fiber, we investigated the adsorption of allergens by Pc-dyed fiber (Fig. 1Ac), Pc-free undyed fiber (b), and a fiber-free column (a).

## Results and discussion

All allergens examined, including Der p 1/Eur m 2.0101 (mites), Fel d 1 (cats), Bla g 2 (cockroach), Cry j 1/Cry j 2 (cedar pollen), and soybean trypsin inhibitor (STI, cereal), were adsorbed effectively on the Pc-dyed rayon fiber (Table 1). The typical adsorption patterns are shown in Fig. 1. The adsorption profile of each allergen was not significantly different whether they were applied individually or in the presence of other allergens (Table 1). Pc-dyed cotton fiber also adsorbed STI (Table 1). As these allergens were not adsorbed on the undyed fiber (except Eur m 2.0101), the adsorption was considered to be attributed to the Pc. This data suggests that Pc-dyed fiber traps a broad spectrum of allergens from house dust, pollen, and food. The adsorbed allergens varied in molecular

**Table 1.** Adsorption of several allergens on Pc-dyed fibers

| Allergens tested | Adsorption (%) on | | |
|---|---|---|---|
| | 0% Pc | 0.1% Pc | 3.0% Pc |
| *Pc-rayon* | | | |
| Allergens applied individually:[a] | | | |
| Bla g 2 (cockroach) | 8.3 ± 5.8 | 97.7 ± 4.0 | 97.5 ± 3.3 |
| Cry j 1 (cedar pollen) | 5.2 ± 3.2 | 20.5 ± 10.4 | 96.4 ± 4.4 |
| Cry j 2 (cedar pollen) | 3.3 ± 1.8 | 14.4 ± 8.7 | 96.1 ± 3.1 |
| Der p 1 (mites) | 12.1 ± 3.2 | 95.4 ± 2.4 | 98.4 ± 2.2 |
| Eur m 2.0101 (mites) | 71.9 ± 16.5 | 93.7 ± 3.7 | 91.5 ± 7.8 |
| Fel d 1 (cats) | 3.2 ± 3.1 | 98.4 ± 3.7 | 91.8 ± 5.0 |
| STI (cereal) | 2.3 ± 2.0 | 97.3 ± 4.2 | 96.7 ± 4.1 |
| Allergen mixtures:[b] | | | |
| Bla g 2/Der p 1/STI | | | |
| Bla g 2 | 7.3 | 82.4 | 99.2 |
| Der p 1 | 13.3 | 94.4 | 97.3 |
| STI | 2.4 | 99.8 | 99.5 |
| Cry j 1/Cry j 2/Eur m 2.0101[b] | | | |
| Cry j 1 | 2.7 | 25.1 | 96.8 |
| Cry j 2 | 3.1 | 18.0 | 96.7 |
| Eur m 2.0101 | 85.0 | 96.4 | 96.9 |
| Adsorption in the presence of collagen or keratin:[b] | | | |
| STI (/collagen) | 2.6 | 97.5 | 98.2 |
| STI (/keratin) | 0 | 97.8 | 97.5 |
| *Pc-cotton*[a] | | | |
| STI | 2.0 ± 2.2 | – | 98.0 ± 1.3 (0.5% Pc) |

Results are expressed in % relative to the recovered amount of allergen in the absence of fiber

[a] Values represent the mean ± SD of five analyses

[b] Experiments were not repeated

*STI* Soybean trypsin inhibitor

weight and pI (Fig. 1B), implying that Pc-dyed fiber is applicable as a trap for a wide variety of allergens. Although the Pc-dyed fiber also adsorbed non-allergenic proteins such as collagen/keratin (both from humans), the adsorption of STI (20 μg) was not mitigated in the presence of collagen or keratin (100 μg each) (Table 1).

Next, the maximum capacity loading of allergens on Pc-dyed fiber was investigated. When the concentration was 0.1% (PC/fiber, w/w), the Pc-fiber was able to contain 20 mg STI per 1 g fiber (data not shown). This large capacity seems to be adequate for an allergen trap; if underwear (200 g, for example) is made of Pc-fiber, it is estimated to have the potential to adsorb 4 g of allergenic proteins. Although Pc-fiber also adsorbed collagen/keratin, the large capacity of the material should allow it to trap allergens even in the presence of other proteins.

We also sought to investigate whether the adsorption of allergens was reversible. First, 40 μg of STI was adsorbed on the Pc-fiber. Next, the fiber was placed in the sample reservoir and several concentrations of SDS solution were poured in the column. After 10 min, the column was centrifuged to see whether STI could be recovered in the filtrate vial. The STI could be recovered completely when the SDS concentration was 0.05% and above (data not shown). This observation suggests that through laundering, Pc-fiber can be used repeatedly as a ''reversible allergen trap'' for such items as underwear or bed encasements.

Accumulating evidence suggests that disulfide bonds play an important role in the allergenicity of proteins (van Milligen et al., 1994; Breiteneder and Mills, 2005). By investigating the changes in disulfide bonding (Yano et al., 2001; Yano and Kuroda, 2003) before and after interactions with Pc-fiber, we confirmed that disulfide bonding was not detectably altered under the conditions tested (data not shown). This observation implies that the clinical effect of Pc-fiber, which consists of alleviating the symptoms of atopic patients, is not related to the reduction of disulfide bonds of allergenic proteins but to the trapping of them. The data presented in this study suggests that Pc-dyed fibers can be used to manufacture products, like underwear, that come into direct contact with the skin.

In conclusion, our preliminary study results suggest that Pc-fiber has the potential to be an effective allergen-trapping material. Further studies are in progress to investigate the industrial applicability of this material.

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