

Elution and adsorptive concentration of Japanese cedar (*Cryptomeria japonica*) pollen allergen in environmental water

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Abstract Elution of Japanese cedar pollen allergens (Cry j I and others) from pollen grains and its adsorptive concentration onto hydrophobic and hydrophilic surfaces were investigated using the surface plasmon resonance technique. Results showed that the allergen elution was obviously enhanced when the ion concentration was higher than that within the human body, indicating that the pollen tend to release its allergen in environmental water having a high ion concentration. However, higher adsorption capacity was observed on hydrophobic surface than hydrophilic surface. These results indicate that water puddles on roadsides beside heavy traffic including large amounts of ion compounds and hydrophobic diesel exhaust particles (DEPs) are a pollen allergen-DEP complex generator. DEPs are easily absorbed into the living body; therefore these mechanisms may be responsible for causing the highest incidence of pollinosis among residents living alongside roads with heavy automobile traffic.

Keywords Japanese cedar pollen allergen; diesel exhaust particles; surface plasmon resonance; water puddle

Introduction

The progress of urbanization generates many water pollution problems such as toxicant discharge to receiving waters through road run-off. Moreover, water puddles on the roadside also contain many harmful compounds and organisms that might impair public health. Many allergic compounds such as plant-derived allergens, especially pollen allergens, are also recruited into such dirty waters as well as suspended particle matter (SPM) such as diesel exhaust particles (DEPs).

The highest incidence of Japanese cedar (*Cryptomeria japonica*) pollinosis was seen among residents living alongside a large road that has heavy automobile traffic all day long (Suzuki *et al.*, 1993). In other words, it is suggested that DEPs have an adjuvant effect for Japanese cedar pollinosis. A schematic diagram of allergic immune response is shown in Figure 1 (Kemedly, 2000). Furthermore, excreted IgE stimulates allergic symptoms, as shown in Figure 2 (Shinohara and Igasaki, 2000). Allergic symptoms are induced by IgE production. There is some evidence that the DEPs induce IgE production in mammals (see, for example, Takafuji *et al.*, 1987). Suzuki *et al.* (1993) revealed that the pyrene in diesel exhaust (commonly adsorbed on the DEP surface) shows adjuvant activity for allergic effect of ovalbumin (OVA) and Japanese cedar pollen allergen to mice. On the other hand, allergens suspend in the atmosphere by attaching to suspended particle matter (SPM) (Ormstad *et al.*, 1998), and it was also revealed that pollen allergens adsorb on fine SPM such as DEPs (Knox *et al.*, 1997). However, up to 30% of inhaled DEPs can be deposited in the alveolar region of the respiratory tract, and a large proportion of it will be phagocytosed by alveolar macrophages (Ormstad *et al.*, 1998).

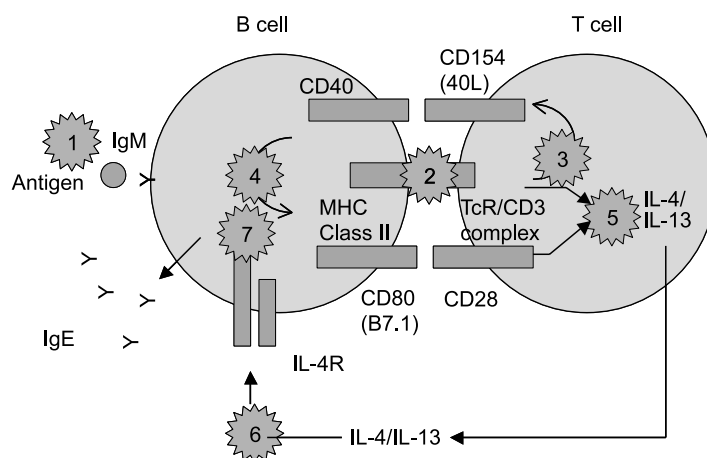


Figure 1 Regulation of immunoglobulin class switching to IgE (Kemeny, 2000). 1: Antigen is taken up by B cells which express IgM, 2: Increased expression of MHC class II peptide in CD4 T cells, 3: Express of CD154, 4: Engagement of CD40 by CD154 induced the express of CD80 which engages CD28, 5: Stimulate the production of IL-4/IL-13, 6: IL-4/IL-13 signal to make IgE, 7: The B cell secretes IgE

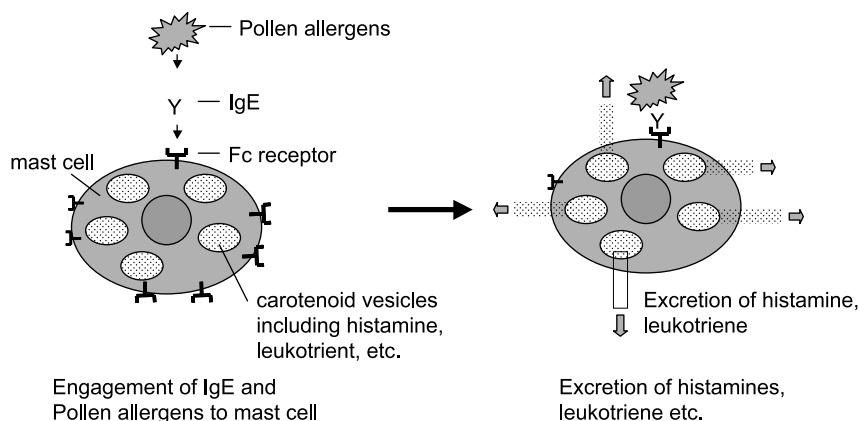


Figure 2 Histamine and leukotriene, which makes the allergic symptoms, release from mast cells (Shinohara and Igasaki, 2000)

In this sense, DEPs can also be considered as a concentrator of the pollen allergen, and it shows a ‘physical adjuvant effect’ on the allergy.

Adsorption will occur where the airborne particles and allergens are in contact and interact under suitable physical conditions (Ormstad *et al.*, 1998). It is suggested that the elution of allergens from pollen grains and adsorption on fine airborne particle would occur in water in atmospheric aerosols. On the other hand, water puddles on the roadside with heavy traffic, where airborne pollen and DEP are enriched, seem to be another allergen-adsorbed DEP generator. Thus, it is important to elucidate the elution properties of allergens from pollens in such low pH and high ion strength waters, and the adsorption properties of the allergen onto hydrophobic surfaces to estimate the physical adjuvant effect of DEPs for Japanese cedar pollinosis. Recently, the surface plasmon resonance (SPR) sensor has been actively investigated as a tool for clarifying the adsorption property onto various surfaces. The mechanism of SPR is depicted in Figure 3. Basically, this technique is applied for measuring the amount of antigen in solution using the

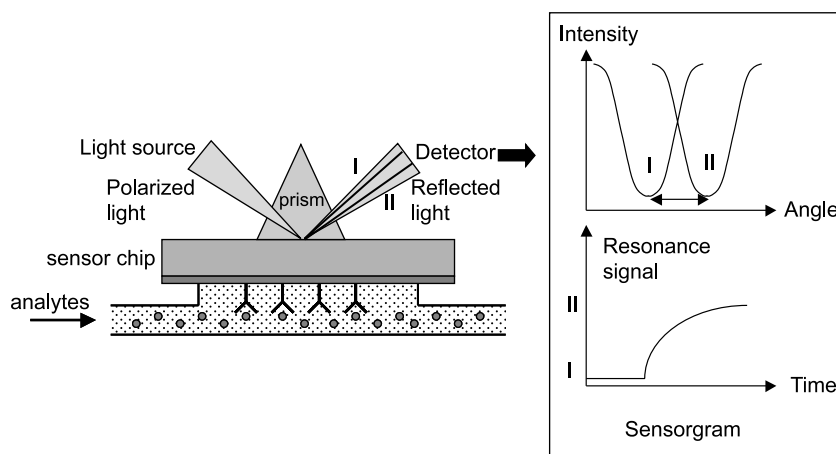


Figure 3 Schematic diagram of a surface plasmon resonance sensor, Biacore X

antigen–antibody interaction. However, combined with the self-assembled monolayer (SAM), it becomes a powerful tool for elucidating various surface phenomena such as adsorption of molecules (Ostuni *et al.*, 2003).

In this research, we evaluated the elution rate of allergens under various pH and ionic concentrations, and adsorptive capacities of them onto hydrophobic and hydrophilic surfaces to confirm this elution-binding mechanism in a condition mimicking the water puddles at heavy traffic roadsides by using an SPR sensor.

Materials and methods

Reagents

Japanese cedar pollen was purchased from the Japan Forest Tree Breeding Association. Japanese cedar pollen allergen (Cry j I) and its polyclonal antibody were purchased from Seikagaku Co. Human IgM, human serum albumin (HSA). OVA and HEPES were purchased from Wako Pure Chemical Co. Octanethiol and 1-hydroxyoctanethiol were purchased from Nacalai Tesque, Inc. and Dojindo Co., respectively.

Surface plasmon resonance sensor

Biacore X (Biacore, Japan) was used as an apparatus to measure the SPR. Its sensor chips were also provided from Biacore. The concentration of allergens was measured from the SPR sensorgrams as well as the adsorption properties of allergens onto various surfaces. To measure the concentration of allergens, IgM and Cry j I antibody were bound on a sensor chip coated with carboxymethyl dextran (CM-5, Biacore). However, to measure the adsorption properties, octanethiol and 1-hydroxyoctanethiol were assembled on a sensor chip having a gold surface (SIA Kit Au, Biacore) using the SAM technique.

Allergen elution experiment

At the first stage of allergic response, allergens bind to IgM on B cell as shown in Figure 1. Thus, the binding property of chemicals to IgM would indicate the allergic potentials of them. Therefore we assumed that the total allergic compounds could be measured with the amount binding to human IgM. In other words, the total allergen assumed to be measured using an IgM-bound sensor chip. However, Cry j I and Cry j II were identified as Japanese cedar pollen allergens. The most abundant allergen is Cry j I, which is a heat-sensitive basic glycoprotein of approximately 40,000 relative molecular

mass (Yasueda *et al.*, 1983). We also measured the Cry j I elution rate using an anti-Cry j I antibody-bound sensor chip.

One gram of cedar pollen was dispersed in 25 mL of PBS buffer. In the pH fluctuated experiment, the pH of the buffer was fluctuated between 6 to 10 without changing the NaCl concentration (1.71 mM). In the ion concentration fluctuation experiment, the NaCl concentration was varied between 1.7 and 176 mM without changing pH (7). The solutions were shaken in a water bath under 20°C, and part of the solution was drawn at scheduled times to measure the concentration of allergens.

Adsorption experiment

Two types of surface, namely a hydrophobic surface coated with $-(CH_2)_7CH_3$ ('C8' below), and a hydrophilic surface coated with $-(CH_2)_7CH_2OH$ ('C8(OH)' below) were prepared using the SAM technique with octanethiol and 1-hydroxyoctanethiol, respectively. Various concentrations of Cry j I in PBS solution were contacted on these two surfaces in a Biacore X flow cell. The amount adsorbed was evaluated from the resultant sensorgrams of surface plasmon resonance (Ostuni *et al.*, 2003).

Results and discussion

Elution of total allergic compounds

Before the experiment, the possibility of measuring the allergic compounds with an IgM-bound sensor chip was elucidated. The human serum albumin, which has no allergic potential for the human body, was injected to Biacore X equipped with an IgM sensor chip. The resultant sensorgram is depicted in Figure 4, which indicates that no obvious plasmon response was observed. However, when ovalbumin, which is a typical allergic compound, was injected, an obvious response of the sensorgram was observed (Figure 5). This result indicates that the IgM fixed chip could detect the total allergic compounds.

The concentration of total allergens in various ionic strength solutions as a function of time is depicted in Figure 6. It is obvious that the elution rate is enhanced in high ionic strength solution, indicating that cedar pollen allergens are easily eluted from pollen grain in high ion concentration water than within human body. Therefore, the pollen tends to release its allergens in environmental water having a high ion concentration such as water puddles on the roadside.

The effect of pH on the elution of total allergens is shown in Figure 7. At high pH, rapid elution of allergens was observed, especially in early stage. However, the rapidly eluted allergens decreased after about 3 hours, possibly because of inactivation, and the

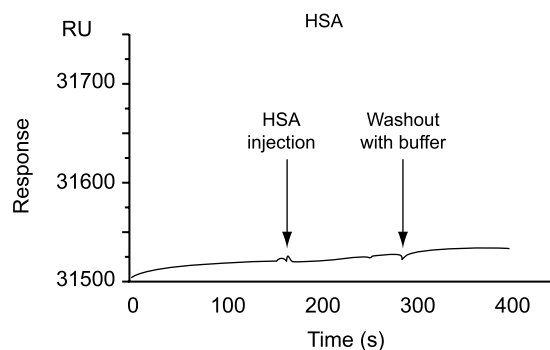


Figure 4 Sensorgram obtained with injection of HSA to IgM bound sensor chip

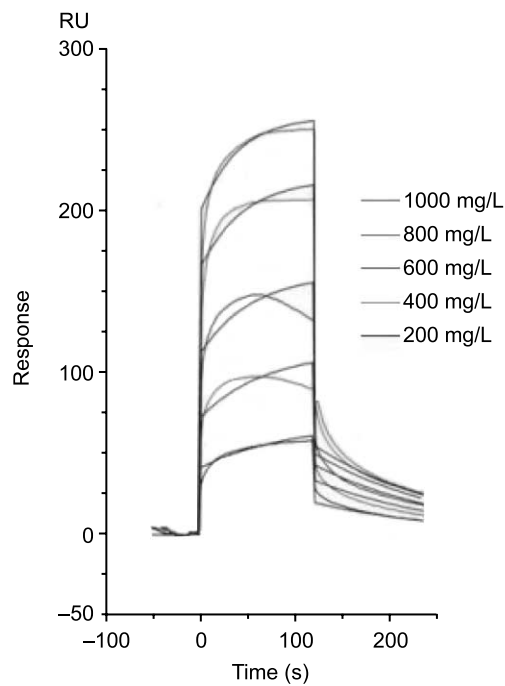


Figure 5 Sensorgram obtained with injection of OVA to IgM bound sensor chip

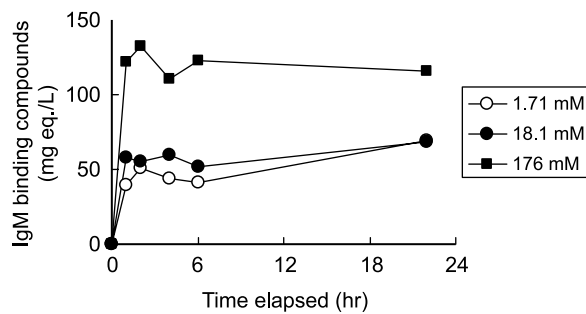


Figure 6 Elution of total allergens from pollen grain in various ionic (NaCl) concentration

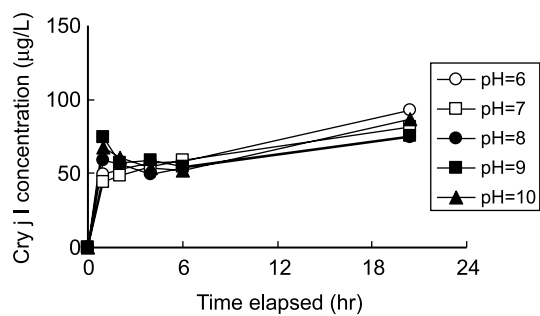


Figure 7 Elution of total allergens from pollen grain in various pH

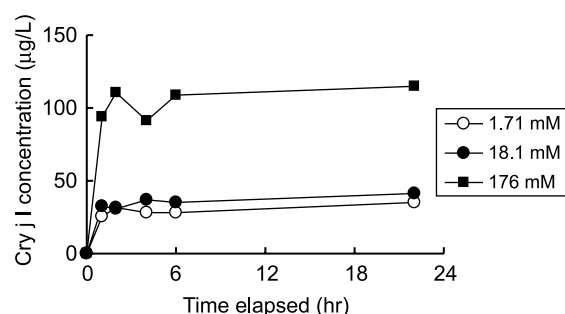


Figure 8 Elution of Cry j I from pollen grain in various ionic (NaCl) concentration

difference in the eluted concentration of allergens between various pH values was diminished.

Elution of Cry j I

Figure 8 shows the elution of Cry j I from pollen grains in various ion strengths. The elution rate was strongly affected by the ion concentration as well as that of total allergens. Compared with Figure 6, it suggests that the eluted allergens mainly composed Cry j I.

The elution in the pH fluctuated experiment is shown in Figure 9. As is distinct from total allergen elution, obvious difference in elution rate between each pH values was not observed, means that the Cry j I elution from pollen grain was not affected by pH in the range 6 to 10. Therefore, it is suggested that the rapid eluted allergen observed in Figure 7 is not Cry j I but another easily inactivated allergen.

Adsorption of Cry j I

Figure 10 shows the adsorption capacity of Cry j I on hydrophobic and hydrophilic surfaces. The hydrophobic (C8) surface has a higher adsorption capacity for Cry j I than the hydrophilic (C8-OH) surface. Cry j I is a glycoprotein having a hydrophobic protein domain and a hydrophilic glycoside domain. The adsorption on the hydrophobic surface could occur by the hydrophobic interaction between the protein domain and the surface. However, the adsorption on hydrophilic surface might result from the hydrogen bond between the glycoside domain and the surface. The difference in adsorption capacity was thus caused by the different adsorption mechanism, and it tends to adsorb on hydrophobic surfaces such as DEPs.

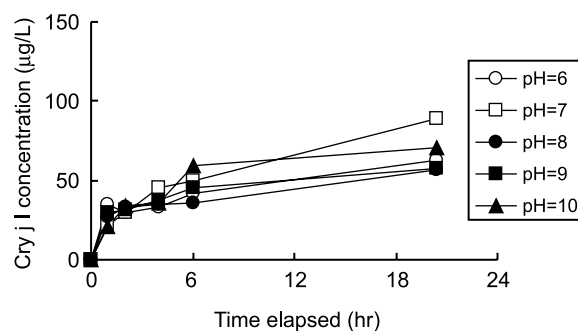


Figure 9 Elution of Cry j I from pollen grain in various pH

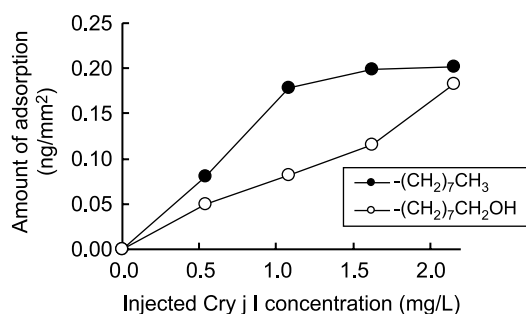


Figure 10 Adsorption of Cry j I on hydrophobic and hydrophilic surfaces

Conclusion

The elution rate of allergens, especially Cry j I, from Japanese cedar pollen grains is strongly affected by the ionic strength of solution, indicating that cedar pollen allergens are easily eluted from pollen grains in environmental water having a high ion concentration, such as water puddles on the roadside. Furthermore, Cry j I can strongly adsorb on hydrophobic surfaces such as DEPs, suggesting that water puddles on roadsides with heavy traffic, which are abundant with pollens and DEPs, should be a pollen allergen-DEP complex generator, and it contributes the highest incidence of pollinosis among residents living alongside roads with heavy traffic.

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