Development of Allergen Denaturing Agents

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After the development of a mite allergen quick determining system, we carried out the development of allergen denaturing agents. First, tannic acid was evaluated as an allergen denaturing agent using the ELISA method. Then, we investigated various compounds for use as new allergen denaturing agents and identified materials such as rare-earth metal salts and zirconium salts as potential inorganic compounds, and materials such as cationic compounds as potential organic compounds. We also developed applications for these allergen denaturing agents including trigger type liquids and additives for filters and cleaning equipment.

This paper is translated from R&D Report, "SUMITOMO KAGAKU", vol. 2009-II.

Introduction

The origin of the word "allergy" is in the Greek words "allos" (strange) and "ergon" (reaction), and it is a concept that was proposed in 1906. Atopic dermatitis, allergic rhinitis and other diseases that are caused by allergies have recently become major problems. The various allergens that exist in the environment are causes of these allergic diseases. There are many types of allergens, including food allergens such as wheat, eggs and buckwheat, inhalant allergens such as the feces and corpses of mites, pollen, fungi and pet dandruff, and contact allergens such as metals and various chemical substances. Of these, the ones that cause allergy symptoms such as allergic rhinitis are the inhalant allergens, and typical ones are the mite allergens and pollen allergens that are largely contained in the dust in houses (**Table 1**). In houses in particular, allergens from house dust mites are major problems.[1), 2)] In addition, many types of pollen, starting with cedar pollen which flies around in large amounts at the beginning of spring, are allergens that cause the onset of allergic rhinitis, and there are many people who suffer from pollen allergies.[3)] In addition, besides mite allergens and pollen allergens, dandruff and hair from pets such as dogs and cats, and cockroaches and fungi are also known to be allergens.[4)] Many allergens such as these exist in the environment we live in, and in order to reduce the onset of allergic diseases, it is important to know the amount of allergens present in our living environment and also to reduce that amount.

Table 1 Sources of inhalant allergens

Mites (body, feces)	Dust mites, Storage mites
Pollens	Cedar, Ragweed, etc.
Fungi (spore)	*Alternaria, Penicilium,* etc.
Mammals (dandruff, hair)	Pet dandruff, hair (Cats, dogs, etc.)
Insects (body, feces)	Cockroaches, Fleas, Flies, Mosquitoes

Sumika Enviro-Science has developed Mitey Checker® as a simple method for determining the quantity of mite allergens, and it is used as a monitoring kit in schools and other places.[5)] Mitey Checker® is an application of an antigen-antibody reaction using monoclonal antibodies of mites. In general, enzyme-linked immunosorbent assays, which use the same antigen-antibody reactions, are used as methods for the measurement of allergens, and they are used not only for mite allergens but also for cedar pollen and other allergens.

On the other hand, Sumika Enviro-Science has carried out research on chemical agents (allergen denaturing agents) that reduce the allergenic nature of allergens in the environment using these immunological techniques and have found various chemical compounds to have these effects. In this paper, I will report on allergens and methods for measuring them, and further, on the development of allergen denaturing agents.

Allergens in the Environment

1. Mite Allergens

It is said that there are more than 30 types of mites that inhabit indoor spaces, and we can assume that they exist in all homes.

In terms of classifications in the order Acarina, mites are classified as in **Table 2**, and they are roughly classified into dust mites and biting mites. Biting mites are comparatively large in size, and they include the Cheyletidae and other types. Their size is about 0.5 mm, and they do damage by biting humans and animals, but they rarely are the cause of allergies. Dust mites are the ones that cause allergies; their size is about 0.3 to 0.4 mm, and they are the prey of biting mites. Dust mites do not bite humans, but the feces that mites excrete and the corpses of mites cause allergies.

Table 2 Classification of Mites (acarina spp.)

Order	Family	Examples	Notes
Astigmata	Pyroglyphidae Sarcoptidae Acaridae	American house dust mite European house dust mite	Dust mites
Prostigmata Trombidiformes	Trombiculidae Cheyleidae Pyemotidae Tarsonemidae	Chelacaropsis moorei Cheyletus malaccensis Pyemotes tritici Tarsonemus granarius	Biting mites
Mesostigmata	Macronyssidae Dermanyssidae Ascidae Phytoseiidae	Ornithonyssus bacoti Ornithonyssus sylviarum Dermanyssus gallinae Dermanyssus hirundinis	
Cryptostigmata	Phthiracaridae	Phthiracarus japonicus	
Metastigmata	Ixodidae Argasidae	Ixodes ovatus	

Typical species of dust mites are the American house dust mite (*Dermatophagoides farinae*) and the European house dust mite (*Dermatophagoides pteronyssius*). Mite population varies greatly according to the season, and the population increases in June through September when the humidity is high. Since Japan is a temperate region with comparatively high humidity, it is said to be very suitable for mite reproduction.

The mite allergen proteins that cause allergies are fractionated into two types by molecular weight (**Table 3**). The allergen that mainly comes from feces is Der 1(Der f1+Der p1). It is comparatively unstable in heat and decomposes easily. Der 2(Der f2+Der p2) is an allergen that comes from the corpses of mites, and it is comparatively stable (**Table 4**). The role of Der 1 in mites is thought to be that of a digestive enzyme (cysteine protease), but the role of Der 2 is has not been elucidated. There is a correlation between the amounts of Der 1 and Der 2 present in the environment,[6] and it is possible to estimate the mite allergen level present in the environment by measuring either one.

Table 3 Mites causing allergy

	Body length	Allergen
American house dust mite *Dermatophagoides farinae*	0.37~0.44mm	Der f1 Der f2
European house dust mite *Dermatophagoides pteronyssius*	0.29~0.38mm	Der p1 Der p2

Table 4 Mite allergens

Group	Molecular weight	Property	Roles
Group 1 (Der 1)	25kD	Unstable by heat	Cystein protease
Group 2 (Der 2)	14kD	Stable by heat	Unknown

2. Pollen Allergens

Pollen allergens are typical inhalant allergens, and more than 50 types of pollen are known as allergens. Among them, cedar pollen is an important one, and because a large amount flies around at the beginning of spring from February through April, the number of people with allergic rhinitis due to cedar pollen is steadily increasing every year. Cedar pollen is the greatest problem, and other pollens acting as allergens are Japanese cypress, mugwort, ragweed, orchard grass, etc., and we can assume that pollens that cause allergies are flying around throughout the year (**Table 5**). The most progress has been made in research on cedar pollen allergens among the pollen allergens. The main fractions in the allergen proteins in cedar pollen are Cry j1 and Cry j2, and Cry j1 is mainly present in

Table 5 Plants causing allergy

Family	Plants
Taxodiaceae	Japanese cedar (*Cryptomeria japonica*)
Cupressaceae	Japanese cypress
Poaceae	Sweet vernal grass, Orchard grass
Compositae	Mugwort, Ragweed

Table 6 Cedar pollen allergen (*Cryptomeria japonica*)

Group	Molecular weight	Location	Notes
Group 1 (Cry j 1)	40kD	Cellulose membrane of pollen intine layer	Major allergen
Group 2 (Cry j 2)	40kD	Starch grain of cytoplasm	

the outer layer of the pollen wall. Cry j2 is present in starch grains in the cytoplasm (**Table 6**). Cry j1 is an important allergen, and it is generally assayed for monitoring of cedar pollen allergen levels.

3. Other Allergens

There are many types of allergens that cause allergies besides mites and pollen such as fungi, dog, cat and other pet dandruff, cockroaches, midges (Chironomidae) and other insects. Various allergen proteins have been separated from these allergens, and for example, with cockroaches, Bla g1 and Bla g2 are known; Can f1 and Can f2 for dog allergens; Fel d1 and Fel d2 for cat allergens and Alt a1 and Asp f1 for fungi allergens.

Allergen Measurement Methods

Since the amount of allergens present in the environment is minute, it is extremely difficult to measure such amounts by normal analytical methods. Enzyme-linked immunosorbent assays (ELISA) that make use of antigen-antibody reactions are used as the method for measuring minute amounts of allergens. A feature of ELISA is the ability to analyze allergens selectively with a high degree of sensitivity using comparatively simple operations. There are many forms of measurement for ELISA, and there are many derivative methods to make it difficult to classify them at present. The measurement method most often used among these is the sandwich ELISA method. The sandwich ELISA method is a method where the antigen in a sample is reacted with an antibody bound in the solid phase, and next the antibody-enzyme conjugate is reacted. Additionally, the amount of the target antigen is found by coloration using the enzyme for the labeled antibody that has been reacted (**Fig. 1** and **2**). This method uses two different types of antibodies, and because two antigen determining sites are recognized, it has extremely high selectivity. Therefore, it is effective even when many substances other than allergens are also present, as in the measurement of allergens in the environment. The sandwich ELISA was used in evaluations of allergen denaturing agents as will be described in the following.

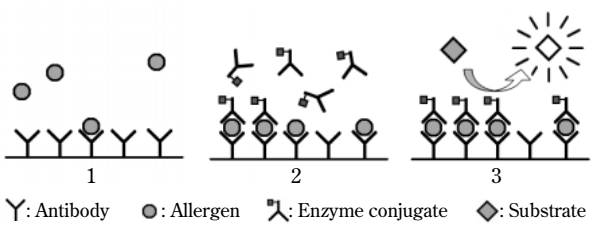

Fig. 1 Image figure of sandwich-ELISA method

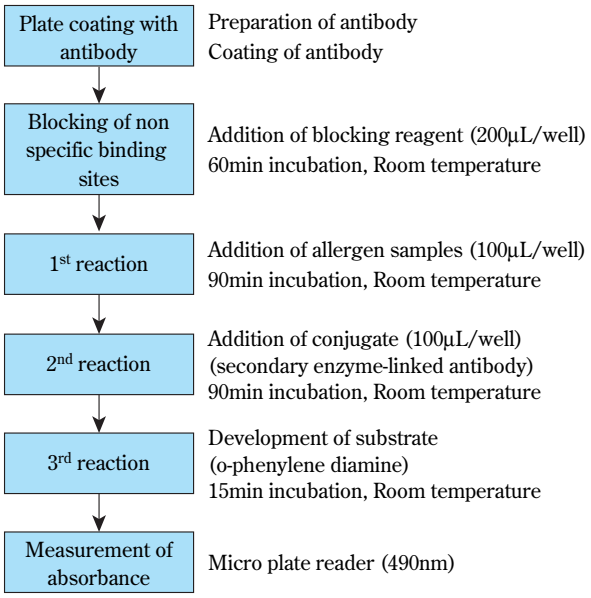

Fig. 2 Protocol of ELISA method

Allergen Denaturing Agents

Sumika Enviro-Science put its Mitey Checker® product on the market in 1998. It makes simple measurements of the mite allergen Der f2 that originates in mite bodies. Mitey Checker® also applies an antigen-antibody reaction using monoclonal antibodies in the same manner as ELISA. It can measure Der f2 selectively with high sensitivity and simplicity. It has become possible to know about the mite allergen levels in public facilities such as hospitals and schools and in typical homes without complicated operations by using Mitey Checker®.[6] When extremely large amounts of mite allergens are detected by measure-

ments of mite allergen levels using Mitey Checker® or ELISA, reducing the mites using mite repellents is an effective measure, but there is a problem in that the remaining corpses and feces of the mites still exist as allergens. In addition, with allergens such as cedar pollen, no means have been achieved other than preventing them from entering rooms or careful cleaning. Cleaning is an effective method for reducing allergens, but since it is difficult to completely remove allergens in the environment with cleaning alone, reducing allergens using chemical agents such as spray or things that reduce their allergenic nature would be desirable. Therefore, we decided to develop chemical agents (allergen denaturing agents) that could reduce the allergenic nature of all of the allergens present in our environment.

Allergens are water-soluble proteins, and they have specific three-dimensional structures that use specific amino acid sequences. Their allergenic nature is expressed through the occurrence of antigen-antibody reactions. Allergens are taken into the body, and in the end express their allergenic nature in the form of a release of histamine from mast cells because of binding with IgE. Preventing allergenic suppression within the body is a task in the medical field. On the other hand, allergen denaturing agents make it so that the allergenic nature of allergens is not expressed by denaturing them while they are present in the environment. In other words, allergen denaturing agents can be thought of as agents that improve the environment.

Some theories have been proposed for the mode of action of allergen denaturing agents, and in one theory, it is thought that the components of the allergen denaturing agent reduce their allergenic nature by binding with allergens and changing the three-dimensional structure of the allergens. Therefore, it is not necessary to decompose the allergens themselves.

Compounds that have an astringent action on proteins are effective as compounds having an allergen denaturing action. Tannic acid is typical of these compounds. Tannic acid is a component contained in various plants, and it is a type of polyphenol. There are polyphenols in many natural allergen denaturing agents, and it can be assumed that many types of plant extracts will exhibit an allergen denaturing effect. Sumika Enviro-Science carried out screening on many types of compounds, not limited to natural products, and has found many compounds that are applicable as allergen denaturing agents (**Table 7**).

Table 7 Allergen denaturing agents

Classification	Allergen denaturing agents	Examples
Inorganic	Alkali-earth metal salts	$CaCl_2$, $SrCl_2$, Calcium Pantothenate, etc.
	Rare-earth metal salts	YCl_3, $LaCl_3$, $CeCl_3$, $DyCl_3$, $HoCl_3$, etc.
	Zirconium salts	$ZrOCl(OH)$, $K_2[Zr(OH)_2(CO_3)_2]$, etc.
	Aluminum salts	$AlK(SO_4)_2 \cdot 12H_2O$, $AlNa(SO_4)_2 \cdot 12H_2O$, etc.
Cationic	Quaternary ammonium salts	Benzalkonium chloride, DDAC, etc.
	Pyridinium salts	Cetylpyridinium chloride, Laurylpyridinium chloride, etc.
Natural extracts (Plants extracts)	*Rhus javanica*	Tannic acid
	Olea europea (Olive leaf)	Olive leaf extracts (Oleuropein)

1. Natural extracts

(1) Tannic acid

Tannic acid, which is a polyphenol component extracted from plants has been known as a type of Chinese medicine since ancient times. It has diverse applications, and is widely used in things such as food products, paper, ink and leather. Tannic acid can be categorized into condensed tannin and hydrolyzable tannin (**Fig. 3** and **4**). In condensed tannin, catechins are joined by carbon-carbon bonds, and these are macromolecules. They are contained in rhubarb in the buckwheat family (Polygonaceae) and cassia and Ceylon cinnamon in the laurel family (Lauraceae). Hydrolyzable tannins are hydrolyzed by acids, alkalis and oxygen, and form polyhydric phenols and polyhydric alcohols. The polyhydric phenols are further categorized into gallotannins and ellagitannins. Tannic acid is a component that is contained in many plants, starting with tea and persimmons, but a quality raw material can be obtained from the nutgalls of the Chi-

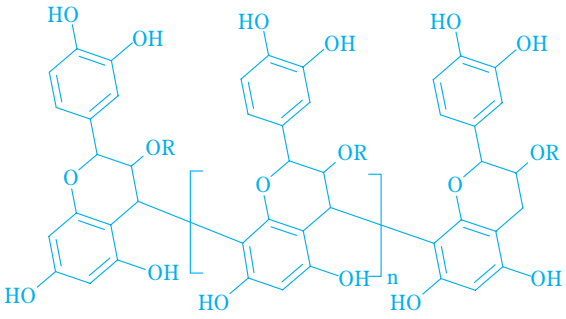

Fig. 3 Structure of tannic acid (Condensed type)

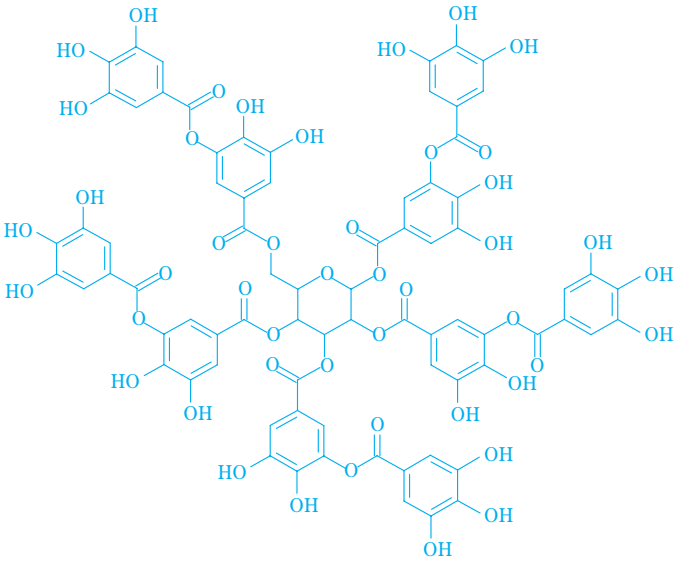

Fig. 4 Structure of Hydrolysable tannic acid (gallotannin) $C_{76}H_{52}O_{46}$

nese sumac, which is in the cashew family (Anacardiaceae), and these contain a high concentration of tannic acid (gallotannin).

It is thought that the protein denaturing action of tannic acid has been known since ancient times, and applications in leather tanning and deproteinization agents for alcoholic beverages make use of this protein denaturing action. This action is effective on allergens, which are one type of protein. It can be used to denature allergens and reduce their allergenic nature. Prof. Green of the University of Sydney in Australia proposed its application as a mite allergen reducing agent in 1986, and spray type and powder type products were marketed as allergen prevention agents in the United States.[7] There was a thought to develop these as products in Japan, but tannic acid dyes things brown, and the coloration progresses over time because of exposure to the sun. A black complex is formed because of iron ions, and there is a serious shortcoming in this coloration, so development was difficult in Japan where applications in the treatment of white futons (Japanese quilted bedding) were expected. However, Sumika Enviro-Science developed applications and marketed products for treating agents in air purification systems and air conditioner filters as applications where coloration was not a problem.

(2) Oleuropein

Polyphenol components are contained in many plants, and it has been reported that polyphenol extracts from various plants may be used as allergen reducing agents. Sumika Enviro-Science has discovered that there is an allergen denaturing effect in oleuropein, which is contained in the components extracted from the leaves of the plants in the privet family and the olive family (**Fig. 5**). Oleuropein is a natural extract, but its coloration is slighter than tannic acids, and it is used in a wide range of applications including such fields as cleaning implements such as handheld wiping tools and wet wipes.

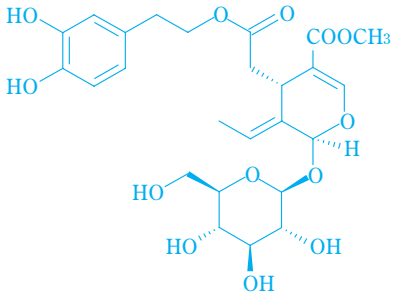

Fig. 5 Structure of Oleuropein

2. Inorganic allergen denaturing agents

Tannic acid is an excellent allergen denaturing agent because of its protein agglutinative action, and it has in the merits of being a natural compound and having extremely high safety. Imparting a brown color and the progression of the coloration because of high temperatures and sunlight would be serious shortcomings. Therefore, there was a need to develop allergen denaturing agents where coloration did not occur, and we searched for an inorganic compound as a candidate for these. In the following we will describe the metal salts that can be used as allergen denaturing agents that we discovered as a result of our search. Various metal salts can be cited as candidates for inorganic allergen denaturing agents, and most of them are thought to denature structures by a chelating action on the peptide bonds of the proteins, etc.[8] Inorganic allergen denaturing agents have the merits of not causing coloration, being stable and not decomposing, as well as having excellent heat resistance. Developments not only for applications in filter materials but also in construction materials (such as building materials and textile products) that can be seen have become possible. In addition, the fact that they do not cause coloration is very suitable for the development of sprays, and products are also being developed overseas for this very large field of application. However, there are shortcomings when they are added to emulsified materials

as in an emulsion; the emulsion may be coagulated, and caution is necessary on this point.

In the following we will describe in detail the metal salts for which allergen denaturing effects have been found.

(1) Second group elements (alkaline earth metals)

The second group elements are beryllium (Be), magnesium (Mg), calcium (Ca), strontium (Sr) and barium (Ba). Excluding toxic beryllium and barium, magnesium salts, calcium salts and strontium salts have a protein solidification action, and for example, calcium chloride and magnesium chloride are used as bitterns in the production of tofu. Of these salts, calcium salts and strontium salts demonstrate particular efficacy as allergen denaturing agents.[9] Calcium salts are commonly used materials, and because they are comparatively safe, they can be developed not only as filter materials, but also as additives in sprays, and various commercial products have been developed. Water-soluble calcium salts are preferable, so salts that are usable include calcium chloride and calcium pantothenate.

(2) Third group elements (rare-earth metals)

The third group elements are known as rare earth salts, and the salts of 16 elements, namely scandium (Sc), yttrium (Y), lanthanum (La), cerium (Ce), praseodymium (Pr), neodymium (Nd), samarium (Sm), europium (Eu), gadolinium (Gd), terbium (Tb), dysprosium (Dy), holmium (Ho), erbium (Er), thulium (Tm), ytterbium (Yb) and lutetium (Lu) can be cited as usable.[10] Rare earth salts are known to have an antibacterial action, and when the author was evaluating the antibacterial activity of lanthanum chloride, a slight turbidity arose in the liquid medium to which the lanthanum salt was added, and it was found unexpectedly that there was precipitation. Therefore, a denaturing action on proteins was presumed and an allergen denaturing function found. Because of their name, one might think that rare earth salts would be "rare," but the amounts present in nature are large, and in particular, there is a wealth of yttrium salts, lanthanum salts and cerium salts. Even lutetium, which is said to be the least present, has a much higher crustal abundance than silver. Among the rare earth salts, the properties of those from lanthanum to lutetium, which are called lanthanoids, closely resemble each other, and this is because the f orbital, which is not the outermost shell, is filled with electrons. Rare earth metal ions are known to have various actions, and the chelating action on organic compounds is an important one. It can be assumed that this effect works effectively in the denaturing of allergens.

The allergen denaturing effect of rare earth salts exhibits a trend that shows effectiveness as water soluble salts, and the effectiveness is lower with water insoluble salts such as phosphates and hydroxides.

Since there is no large difference in the allergen denaturing effect of these rare earth salts, it can be assumed that lanthanum salts, cerium salts and yttrium salts are particularly usable salts in terms of cost advantage. From the aspect of cost, the fact that there is considerably no coloring and the fact that they do not form hydroxides at comparatively high pH, lanthanum salts are advantageous as materials for developing products. Rare earth elements are not essential elements, but they do not have high toxicity and are not highly irritating, and they can be used as treating agents for filter materials, for instance, and as sprays.

(3) Zirconium salts

Zirconyl chloride (ZrOCl (OH)) is a compound that is used as an antiperspirant, and this effect is due to a denaturing action on proteins. In addition, such zirconium compounds are also used as tanning agents for leather, and this makes use of an astringent action on proteins. Zirconyl chloride exhibited an allergen denaturing action. In terms of other zirconium salts, allergen denaturing effects have also been found in compounds such as basic zirconium carbonate, zirconium hydroxide and zirconium acetate.

(4) Aluminum salts

Alum ($(AlK(SO_4)_2 \cdot 12H_2O$) has long been known as an astringent, and it can be used in products such as face lotions. It is also on the list of raw materials for cosmetics. Not only alum, but also aluminum salts such as aluminum sulfate and aluminum chloride exhibit allergen denaturing effects. In particular, alum has high stability, and development as a treating agent for filter materials and sprays can be possible.

3. Cationic allergen denaturing agents

Among bactericides, the bactericidal action is exhibited by various mechanisms such as inhibition of DNA synthesis, destruction of the cell membrane, enzyme blocking, and inhibition of ergosterol synthesis. The protein denaturing action is one of these, and most

cationic compounds are known as bactericides that mainly show a protein denaturing action. Therefore, we can use that protein denaturing action for reduction of allergens. Compounds such as benzalkonium chloride, didecyldimethyl ammonium chloride, polyhexamethylene biguanide, and cetylpyridinium chloride, which are known as cationic bactericides, function as allergen denaturing agents.[11)]

Cationic allergen denaturing agents are mostly bactericides originally, so it is possible for them to express both an allergen denaturing effect and a bactericidal effect at the same time. However, caution must be taken concerning skin irritating properties. In addition, since they are surface activating agents, they have foaming properties, and caution is necessary for foaming during treating onto various materials when such products are used.

Methods for Evaluating Allergen Denaturing Agents

Evaluation of the performance of allergen denaturing agents can be done by reacting a prescribed concentration of an allergen denaturing agent solution with a prescribed amount of an allergen, measuring the remaining amount of the allergen and finding the amount reduced from the initial amount of the allergen. Allergen denaturing agents were evaluated using the spray method (direct method) shown in the following.

Spray Method

(1) A sample of 0.03 g of standard house dust was strewn uniformly on a felt substrate with a diameter of 6 cm and made to adhere.
(2) Approximately 2 g of the allergen denaturing agent solution was sprayed and dried.
(3) Extraction from the felt substrate was carried out with 10 mL of phosphate buffer solution, and the amount of the allergen in the extracted liquid was assayed using ELISA or Mitey Checker®.

Measurements of allergen amounts can be made using ELISA, or for simplicity using Mitey Checker®. It is possible to purchase mite allergens (Der f1, Der f2, Der p1 and Der p2) and cedar pollen allergen (Cry j1) for the standard allergens, but for something close to the allergens present in the current environment, fine dust separated by a mesh from household waste can be used in the case of mite allergens, and cedar pollen can be used as is in the case of cedar pollen allergens. For mite allergens, dust picked up by vacuum cleaners from five typical homes was put through a No. 200 mesh sieve, and the fine dust obtained was prepared for the standard dust.

The amount of mite allergens (Der f2) in the standard dust was measured by ELISA, and a value of 1000 to 1300 μg/g was obtained.

Allergens are denatured by the allergen denaturing agent components and the allergenic nature is reduced, but since the denaturing effect disappears if the denatured allergens are revived by dilution, etc., the denaturing reaction on the allergens using the allergen denaturing agent must be irreversible. Therefore, dialysis was carried out on the liquid mixture of the allergen denaturing agent and the allergen, and whether or not the amount of allergen increased after dialysis was checked. The dialysis method is given below.

Dialysis Method

(1) Approximately 1.5 mL of a mixed liquid of the allergen denaturing agent and the allergen solution was encapsulated in a dialysis tube (Visking tube).
(2) This was immersed in 5 L of physiological saline and left for 18 hours with agitation to carry out dialysis. The physiological saline was changed once during that time.
(3) The amount of allergen in the tube was measured using ELISA or Mitey Checker®.

Inorganic allergen denaturing agents were evaluated using the spray method that used the standard dust, and all of the inorganic salts exhibited a high allergen denaturing effect. In addition, a high level of effect was exhibited after dialysis (**Table 8**). In the spray method,

Table 8 Efficacy of allergen denaturing agents

Agents	Direct method		Dialyzed method	
	Denatured (%) from ELISA	Score* from Mitey Checker®	Denatured (%) from ELISA	Score* from Mitey Checker®
Lanthanum chloride (3%)	94	±	94	±
Cerium chloride (3%)	99	–	90	±
Yttrium chloride (2%)	95	–	96	–
Calcium chloride (3%)	82	–	76	±
Strontium chloride (3%)	84	–	86	±

Amount of initial allergen : 30μg
* – : < 1μg Der 2
± : ~ 5μg Der 2

the standard dust was weighed, and the allergen denaturing agent being tested was sprayed, so the method of operation was somewhat complicated. Therefore, a liquid-liquid method was developed as a simple screening method.

Liquid-Liquid Method

(1) The mite allergen Der f2 or the cedar pollen allergen Cry j1 was diluted with phosphate buffer solution, and an allergen solution with a prescribed concentration was prepared.
(2) Exactly 150 μL of the allergen denaturing agent solution was added to 1 mL of the allergen solution.
(3) After one hour, the amount of allergen in the mixed solution was measured by ELISA or Mitey Checker®.

Since the liquid-liquid method measures the reduction in the amount of allergen using a mixture of a dilute allergen solution and an allergen denaturing agent solution, the dilution can be set freely, so tests may be carried out on allergens with low concentrations. **Table 9** shows examples of evaluations done in this manner. With the liquid-liquid method, it is possible to change the amount of the allergen denaturing agent and the amount of allergens freely. For tannic acid, **Fig. 6** shows the rate of allergen denaturing when the Der f2 concentration was fixed at 900 ng and the tannic acid concentration was varied. At a tannic acid concentration of approximately 0.8%, the denaturing rate for the mite allergen reached approximately 100%.

The liquid-liquid method is simple for evaluating allergen denaturing agents and is extremely effective for screening allergy denaturing agents, but when we consider the conditions where materials treated with allergen denaturing agents are used, we cannot say that it is a state close to reality. In actuality, allergens are often present in the environment as fine powders, and they come into contact with allergen denaturing agents that are in a powdered state. Therefore, a dry method has been proposed as a testing method in a state close to reality.

Table 9 Efficacy of allergen denaturing agents by liquid-liquid method

Allergen denaturing agents	Type	Denatured Der f2 (%)	Denatured Cry j1 (%)
Didecyldimethyl ammonium chloride (10%)	Cationic	97	91
Cetylprydinium chloride (8%)	Cationic	97	91
Laurylprydinium chloride (10%)	Cationic	100	98
Lanthanum chloride (8%)	Inorganic	96	82
Zirconyl chloride (5%)	Inorganic	97	100
Tannic acid (2%)	Organic	100	100

Amount of initial allergen Der f2 : 400ng
Cry j1 : 12.5ng

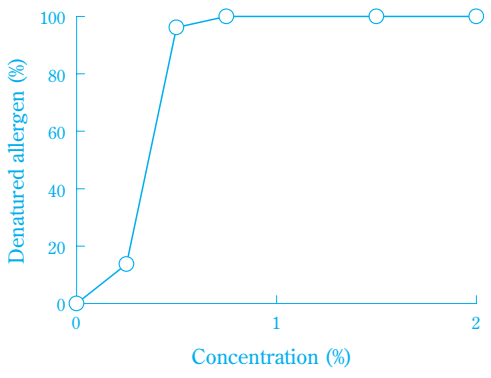

Fig. 6 Efficacy of tannic acid

Dry Method

(1) A 20 cm × 20 cm sample (cloth) treated with an allergen denaturing agent was fixed to a plywood board, and 10 mg of the standard dust (mite allergen) or 3 mg of cedar pollen was strewn on top of that as uniformly as possible.
(2) This was left for 24 hours, then the allergen that had been strewn on the sample was recovered using an electric vacuum cleaner with a dust receiving bag installed.
(3) The allergen was extracted from the dust receiving bag using 10 mL of a PBS buffer solution.
(4) The amount of allergen in the extracted liquid was measured using ELISA or Mitey Checker®.

In addition to the effect of the allergen denaturing agent itself, the opportunity for the allergen and the agent to come into contact affected the results of the evaluation with the dry method. Since the contact was in a dry state, the allergen denaturing rate was generally lower than the effect with the liquid-liquid method. The results of evaluations using nonwoven cloth (filter material) treated with tannic acid and calcium chloride are given in **Table 10**. Since the amount of allergen was measured by mass in the dry method, it was technically difficult to collect minute amounts of the allergen and strew it uniformly on the evaluation sample, so we can assume that work is necessary to make it an evaluation method with a high level of precision.

Table 10 Efficacy of allergen denaturing agents by Dry-method

Agents	Allergen	Detected allergen	Denatured (%)
Filter cloth (Blank)	Der f2	4.4 μg	Standard
Filter cloth treated with tannic acid	Der f2	2.5 μg	43
Filter cloth (Blank)	Cry j1	23 ng	Standard
Filter cloth treated with tannic acid	Cry j1	18 ng	22
Cotton cloth (Blank)	Der f2	1.3 μg	Standard
Cotton cloth treated with Calcium chloride	Der f2	1.1 μg	15

Applications for Allergen Denaturing Agents

It is possible that allergens exist in every place in the environment, and indoors in particular. Mites reproduce easily in carpets, futons and tatami mats, and these are hotbeds for allergens. In addition, a lot of cedar pollen is flying around in spring, and it is highly possible that it will be brought inside.

Therefore, applications for allergen denaturing agents such as the following are conceivable (**Table 11**).

(1) Sprays that are sprayed in the environment indoors for aggressively reducing the allergens
(2) Filter materials for equipment such as air purification systems and air conditioners
(3) Tools and instruments for cleaning
(4) Wallpaper, flooring materials and other construction materials for places where allergens are present
(5) Futons, carpet, pillows, sheets, clothing and other textile products which are present in the indoor environment or which might be present in the indoor environment

Table 11 Usage of allergen denaturing agents

Usage	Examples
Spray	Trigger type, Aerosol type
Filters	Air purifier, Air conditioner, Dust bags
Cleaning equipment	Wet tissues, handy wiper
Interior materials	Floor materials, Wallpaper
Textile	Car seat clothes, Carpets, Masks

The specifications and required standards for products for these applications will be discussed in the following. In addition, Sumika Enviro-Science has developed the various products given in **Table 12** in consideration of these applications.

Table 12 Product list of allergen denaturing agents

Product name	Active ingredient	Usage
Allersave® MAX	Inorganic salts	Spray use
Allersave® T-10	Inorganic salts	Treating use
Allersave® T-50	Natural extract + Inorganic salts	Treating use
Allersave® T-60	Natural extract	Treating use
Allersave® C	Cationic	Treating use
Allersave® L	Natural extract	Treating use

1. Sprays

Allergens can be reduced by applying sprays to carpets, flooring materials, wallpaper, sofas, futons, beds and pillows indoors. Since in this case, the area being sprayed can be seen, the conditions are no occurrences of coloration, no powder after drying and no change of texture. In addition, when textile products such as futons and beds are sprayed, they come into direct contact with humans, so particular consideration must be given to the effects on texture (**Fig. 7**).

Fig. 7 Allersave® MAX

2. Filter Materials

Functions for antibacterial treatment and odor treatment have been added to the function of filtering to air purification systems and air conditioner filters. In addition to these functions, treating allergen denaturing agents has been spreading recently. Actually, filter materials for allergen denaturing agent applications are in the greatest demand at present, and applications not only in indoor environments but also in the air conditioners installed in automobiles have begun. Since these have the properties of concentrating allergenic substances in the air on the filter through air intake, they must be treated with agents that have a high level of effectiveness as allergen denaturing agents. There is not always a condition requiring no coloration, and

since there is no exposure to the sun, components such as tannic acid are used most frequently.

3. Cleaning Tools and Instruments

In terms of cleaning tools, handheld wipers, wet wipers, wet sheets, mops and other everyday products and sundries are used for the general purpose of reducing allergens, and treating allergen denaturing agents can be thought effective. In addition, there is a risk of electric vacuum cleaners releasing allergens once again from their exhaust outlets, and treating the vacuum cleaner bags with allergen denaturing agents is carried out to prevent this.

4. Construction Materials

Wallpaper, flooring materials, ceiling materials and other construction materials can be cited as materials in the indoor environment for being treated with allergen denaturing agents. Wallpaper is being developed with various added functions, such as antibacterial properties, odor elimination, antifouling functions, negative ions, high durability and humidity control, and material makers have started focusing on giving functions for allergen denaturing as one of them. Since in the case of wallpaper, treatment is carried out by a method of application with the binder to the surface layer, it is also possible to treat it with a water based liquid agent, but in the case of floor materials, treatment is done by mixing with surface UV coatings, and a high level of compatibility with the UV coating material is required.

5. Textile Products

There are many textile products, such as carpets, sofas, futons, pillows and stuffed animals, in the indoor environment, and treating these textile products with allergen denaturing agents is effective for reducing the allergens in the environment.

We can also assume that there is a demand for treating clothing with allergen denaturing agents, but there is commonly a demand for washing durability in textile products, so work is necessary for making allergen denaturing agents insoluble in water.

In addition, masks that are used outside are also an important field of application for allergen denaturing agents, and allergen reducing masks are being developed. Masks are directly used on humans, and there are requirements for ones that have a high level of safety and have no odor.

Conclusion

Following the development of the "Mitey Checker®" simple monitoring kit for mite allergens, Sumika Enviro-Science is developing allergen denaturing agents and has found that a variety of compounds are effective. We can assume that allergy diseases will increase in the future, and the reduction of the allergens can be thought of as being extremely important. The point that is being focused on moving forward is the development of compounds with higher activity and a high level of water resistance. It is important to continue looking for new materials through screening, but developing techniques for manufacturing agents such as work on formulation and work on combinations can be thought of as requiring concentrated efforts.

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