

**ATTACHMENT-36**

**USPTO PATENT FULL-TEXT AND IMAGE DATABASE**

( 8 of 14 )

**United States Patent**  
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**6,190,723**  
**February 20, 2001**

**Neutralization of food allergens by thioredoxin**

**Abstract**

Thioredoxin, a small dithiol protein, is a specific reductant for major allergenic proteins present in widely used foods from animal and plant sources. All targeted allergenic proteins contain disulfide (S--S) bonds that are reduced to the sulfhydryl (SH) level by thioredoxin. The proteins are allergenically active in the oxidized (S--S) state. When reduced (SH state), they lose their allergenicity. Thioredoxin achieved this reduction when activated (reduced) either by NADPH via NADP-thioredoxin reductase (physiological conditions) or by dithiothreitol, a chemical reductant. Skin tests and feeding experiments carried out with sensitized dogs showed that treatment of the food with reduced thioredoxin prior to ingestion eliminated or decreased the allergenicity of the food.

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**Appl. No.:** **046780**

**Filed:** **March 23, 1998**

**Current U.S. Class:** 426/656; 514/2; 530/403

**Intern'l Class:** C07K 001/00

**Field of Search:** 426/656,541,549,574,581 424/94.4 514/2 530/402,345,403  
435/191

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respond to colony disturbance more quickly, in greater numbers and with more stinging (Collins, A. M. et al. (1982) Science 218:72-74). A mass attack by Africanized bees may produce thousands of stings on one individual and cause death. The "killer" bees appeared as a result of the interbreeding between the African bee (*Apis mellifera scutellata*) and the European bee (*Apis mellifera mellifera*). African bees were introduced in 1956 into Brazil with the aim of improving honey production being a more tropically adapted bee. Africanized bees have moved from South America to North America, and they have been reported in Texas and Florida.

In some areas of the world such as Mexico, Brazil, North Africa and the Middle East, scorpions present a life hazard to humans. However, only the scorpions of family Buthidae (genera, *Androctonus*, *Buthus*, *Centruroides*, *Leiurus* and *Tityus*) are toxic for humans. The chemical composition of the scorpion venom is not as complex as snake or bee venom. Scorpion venom contains mucopolysaccharides, small amounts of hyaluronidase and phospholipase, low molecular-weight molecules, protease inhibitors, histamine releasers and neurotoxins, such as serotonin. The neurotoxins affect voltage-sensitive ionic channels in the neuromuscular junction. The neurotoxins are basic polypeptides with three to four disulfide bridges and can be classified in two groups: peptides with from 61 to 70 amino acids, that block sodium channel, and peptides with from 36 to 39 amino acids, that block potassium channel. The reduction of disulfide bridges on the neurotoxins by nonphysiological reductants such as DTT or  $\beta$ -mercaptoethanol (Watt, D. D. et al. (1972) *Toxicol* 10:173-181) lead to loss of their toxicity.

Symptoms of animals stung by poisonous scorpions include hyperexcitability, dyspnea, convulsions, paralysis and death. At present, antivenin is the only antidote for scorpion stings. The availability of the venom is a major problem in the production of antivenin. Unlike snake venom, scorpion venom is very difficult to collect, because the yield of venom per specimen is limited and in some cases the storage of dried venom leads to modification of its toxicity. An additional problem in the production of antivenins is that the neurotoxins are very poor antigens.

The reductive inactivation of snake, bee and scorpion toxins under physiological conditions has never been reported nor has it been suggested that the thiol redox agents, such as reduced lipoic acid, DTT, or reduced thioredoxin could act as an antidote to these venoms in an individual.

Food allergies also represent a long-standing problem important both nationally and internationally. Up to 5% of children under age 12 and 1% of adults are clinically affected in the U.S. population (Adverse Reactions to Foods--AAAI and NIAD Report, 1984, NIH Pub. No. 84-2442, pp.2, 3). In some countries, the figures are higher, and, throughout the world, the problem is considered to be increasing, especially in infants (T. Matsuda and R. Nakamura 1993 Molecular structure and immunological properties of Food Allergens, *Trends in Food Science & Technology* 4, 289-293). The problem extends to a wide range of foods. Food allergies in general have recently achieved an increased profile as a result of the concern about transgenic foods.

Milk represents a significant problem, especially in infants. Wheat and soy allergies are of growing importance as new populations adopt these foods and are of increased concern in pet (especially dog) foods. Beef, rice and egg also cause serious allergies in many individuals and again are of significant concern with respect to pet food.

Many of the major allergenic proteins in the above mentioned foods have intramolecular disulfide (S--S) bonds but so far two treatments have been applied commercially to minimize food allergies: (1) heat, and (2) enzymatic proteolysis. In both cases, success has been only partial. While lowering allergenicity, heat treatment has not eliminated the problem, even in the best of cases, because the responsible proteins are typically heat stable. Moreover, heat lowers product quality by destroying nutritionally important amino acids such as lysine, cysteine and arginine. Enzymatic proteolysis is more successful in reducing

allergenicity, but desirable food properties such as flavor are usually lost and treatment is costly. Therefore a physiologically safe system that would bring about a decrease in or loss of allergenicity when applied to *allergenic foods* without a resulting loss in flavor and nutrition would be extremely valuable.

Certain major pollen allergens are known to be disulfide proteins that are highly resistant to temperature. Two pollen proteins are described as major allergens in ragweed pollen. One is a small protein of 5 kDa, Amb a V, containing four disulfide bridges (Goodfriend, L. et al. (1985), "Ra5G, a homologue of Ra5 in giant ragweed pollen: isolation, HLA-DR-associated activity and amino acid sequence", Mol. Immunol. 22:899-906; Metzler, W. J. et al. (1992), "Determination of the three-dimensional solution structure of ragweed allergen Amb t V by nuclear magnetic resonance spectroscopy" Biochemistry 31:5117-5127; Mole, L. E., et al. (1975), "The amino acid sequence of ragweed pollen allergen Ra5" Biochemistry 14:1216-1220; Metzler, W. J., et al. (1992), "Proton resonance assignments and three-dimensional solution structure of the ragweed allergen Amb a V by nuclear magnetic resonance spectroscopy" Biochemistry 31:8697-8705). This protein is considered to be homologous in both the short and giant ragweed species. The short ragweed protein which is designated Amb a V and the giant ragweed which is now designated Amb t V, both previously called Ra 5, exhibit a 45% sequence similarity.

The other major allergen represents a family of 41 kDa proteins, named Amb a 1.1, Amb a 1.2, Amb a 1.3 and Amb a 1.4. While no disulfide bridges have been described, these proteins contain multiple cysteines (Rafnar, T. et al. (1991), "Cloning of Amb a I (antigen E), the major allergen family of short ragweed pollen" J. Biol. Chem. 266:1229-1236; Griffith, I. J. et al. (1991), "Sequence polymorphism of Amb a I and Amb a II, the major allergens in *Ambrosia artemisiifolia* (short ragweed)" Int. Arch. Allergy Appl. Immunol. 96:296-304). Yet other known allergens are disulfide proteins such as the western ragweed, Amb P 5-A and -B, each 8.5 kDa with three disulfide bridges (Ghosh, B. et al. (1994), "Immunologic and molecular characterization of Amb p V allergens from *Ambrosia psilostachya* (western Ragweed) pollen" J. Immunol. 152:2882-2889) and a short ragweed 11.4 kDa plastocyanin like protein, caUed Ra 3, with one disulfide bridge (Klapper, D. G. et al. (1980), "Amino acid sequence of ragweed allergen Ra3" Biochemistry 19:5729-5734).

The 5 kDa Amb V ragweed pollen proteins have a well-defined structure and the positions of the four intrachain disulfide bonds are precisely known (Metzler, W. J. et al. (1992) Biochemistry 31:5117-5127 and 8697-8705). Previous work has shown that, when reduced under denaturing conditions by chemical agents (urea plus either dithiothreitol or .beta.-mercaptoethanol), the immune response shifts from IgE (allergic) to an IgG (defense) because IgG production is enhanced (Zhu, X. et al. (1995), "T cell epitope mapping of ragweed pollen allergen *Ambrosia artemisiifolia* (Amb a 5) and *Ambrosia trifida* (Amb t 5) and the role of free sulfhydryl groups in T cell recognition" J. Immunol. 155:5064-73).

Pollen allergies are currently being treated by conventional immunotherapy with undenatured pollen extract. However, such treatment, especially in children, carries a certain risk of anaphylactic reactions which are potentially lethal. Consequently, there is a need for an attenuated pollen protein or pollen extract for use in immunotherapy that would reduce or eliminate the possibility of anaphylactic reactions. There is also a need for a physiologically safe system that could determine whether or not an allergen for a particular individual is a disulfide protein. Further, eye drops, nose sprays, aerosols, or dispersants for vaporizers or humidifiers that would alleviate allergy symptoms but also produce less side effects than the currently available products would be extremely valuable.

Two sulfhydryl (SH) groups can often spontaneously react to form an S-S bond in the presence of oxygen. Therefore, the effects of the NADP-thioredoxin system (NTS) and other reductants to decrease allergenicity and increase digestibility of proteins by pepsin may not be permanent. Consequently, there is also a need for the stabilization of proteins, including allergenic proteins reduced by the NTS and