

# Identification and immunologic characterization of an allergen, alliin lyase, from garlic (*Allium sativum*)

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**Background:** Garlic (*Allium sativum*) is one of the most common relishes used in cooking worldwide. Very few garlic allergens have been reported, and garlic allergy has been rarely studied.

**Objective:** The aim of the study was to identify allergenic proteins in garlic and to investigate their importance in allergies to other *Allium* species (leek, shallot, and onion).

**Methods:** A crude extract of garlic proteins was separated by SDS-PAGE and 2-dimensional electrophoresis; immunoblotting was then performed with the use of individual and pooled sera from patients with garlic allergy, and the major IgE-binding proteins were analyzed by amino acid sequencing and mass spectrometry. The putative allergens were further purified by chromatography; the antigenicity, allergenicity, and IgE-binding cross-reactivity of the purified protein were then studied by immunoblotting, periodate oxidation, skin tests, and IgE-binding inhibition assays.

**Results:** A major allergen, alliin lyase, was identified by mass spectrometry and Edman sequencing and purified to homogeneity through the use of a simple 2-step chromatographic method. Skin tests showed that the purified protein elicited IgE-mediated hypersensitive responses in patients with garlic allergy. Periodate oxidation showed that carbohydrate groups were involved in the antigenicity, allergenicity, and cross-reactivity. Garlic alliin lyase showed strong cross-reactivity with alliin lyases from other *Allium* species, namely leek, shallot, and onion.

**Conclusions:** Alliin lyase was found to be a major garlic allergen in a garlic-allergic group of patients in Taiwan. The wide distribution of alliin lyase in *Allium* suggests it may be a new cross-reactive allergen. (*J Allergy Clin Immunol* 2004;113:161-8.)

**Key words:** Proteomics, allergen, lectin-binding reactivity, cross-reactivity, alliin lyase, garlic, *Allium sativum*

## Abbreviations used

MALDI-TOF-MS: Matrix-assisted laser desorption/ionization-time of flight mass spectrometry  
PSD: Postsource decay  
PVDF: Polyvinylidene difluoride  
TBST: Tris-buffered saline containing 1% Tween-20

In recent years, it has been recognized that ingestion of, contact with, or exposure to certain members of the liliaceae family, namely garlic, onion, yucca, Easter lily, leek, tulip, and asparagus, can trigger allergic symptoms, including urticaria, dermatitis, rhinitis, and anaphylaxis in susceptible individuals.<sup>1-10</sup> Garlic is one of the most common relishes used in cooking worldwide. It has also been used since ancient times by many cultures as a folk remedy for a variety of illnesses.<sup>11,12</sup> Although garlic allergy is not rare, it has been little studied. We recently discovered that a small population of patients in Chayi County, in the mid-southern region of Taiwan, was allergic to garlic. These patients presented several allergic symptoms, such as rhinitis, urticaria, dermatitis, and asthma, and all had lived near garlic fields, worked or played near a garlic store, and eaten garlic over a long period of time. We therefore used this group of patients to identify allergens in garlic to help in the diagnosis and treatment of garlic allergy and to study cross-reactivity of these antigens with potential allergens from other *Allium* species.

There have been few studies aimed at identifying garlic allergens. With the advent of proteomics, a combination of the process of characterizing proteins separated by 2-dimensional electrophoresis, the development of protein database searches and identification algorithms for mass spectrometry data,<sup>13,14</sup> has greatly simplified protein identification. In the current study, we describe the use of an efficient and rapid approach, combining proteomics and immunologic methods, for the identification and characterization of the major allergens of garlic.

## METHODS

### Patient sera and preparation of *Allium* crude extracts

Serum samples were obtained from 15 patients with garlic allergy in the God's Help Hospital, Chiayi, Taiwan, and stored in aliquots at -70°C. Allergy was confirmed by clinical history and diagnosis and

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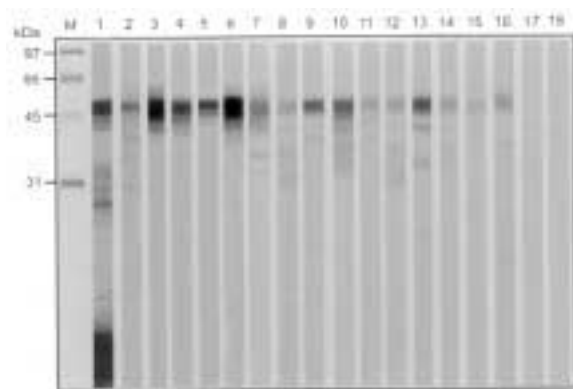


**FIG 1.** Binding of patient IgE to crude extract of *Allium sativum* on immunoblots. Lane 1, Coomassie blue-stained blot. Lanes 2 through 16, Immunoblots with serum samples from patients allergic to garlic, Nos. 1 through 15, respectively. Lanes 17 and 18, Immunoblots with normal serum samples. Lane M, Molecular weight markers.

characterized by use of the Pharmacia CAP system (Amersham Biosciences, Uppsala, Sweden) for measuring specific IgE antibodies.<sup>15</sup> Serum samples from nonatopic individuals were used as control. Garlic (*Allium sativum*), shallot (*Allium ascalonicum*), leek (*Allium porrum*), and onion (*Allium cepa*) were purchased from a local supermarket and homogenized for  $6 \times 30$  seconds, with an interval of 30 seconds in a Polytron (CH-6010, Kinematica, Kriens/Lucern, Switzerland) in ice-cold 50 mmol/L phosphate buffer, pH 7.5, containing 1 mmol/L phenylmethylsulfonyl fluoride, 1 mmol/L calcium chloride, and 0.1 mmol/L dithiothreitol (10 mL of buffer per gram of material). The homogenate was filtered through two layers of gauze and the filtrate centrifuged at 27,000g for 15 minutes at 4°C. The supernatant solution (crude extract) was used in the IgE binding assay and for the purification of IgE-binding proteins.

### SDS/PAGE and immunoblotting

Samples of crude extract (10 µg of protein) were separated by 12.5% SDS-PAGE and electroblotted onto polyvinylidene difluoride (PVDF) membranes (Millipore, Bedford, Mass); IgE-binding proteins were detected as described previously.<sup>16</sup> The patients' sera used for immunoblotting were diluted 5-fold.

### Two-dimensional analysis

Crude garlic extract (0.4 mg of protein) was precipitated by adding trichloroacetic acid to a final concentration of 20% and leaving the sample for 15 minutes at 4°C before centrifugation at 12,000g for 15 minutes at 4°C and vacuum-drying of the pellet on a SpeedVac (SC110, Savant, Farmingdale, NY). The pelleted material was analyzed by 2-dimensional (2-D) electrophoresis following by immunoblotting, as described previously,<sup>16</sup> using pooled serum from patients 1 through 4.

### N-terminal microsequencing

After 2-D electrophoresis, the blotted proteins were visualized with the use of Coomassie brilliant blue R (Sigma, Steinheim, Germany); the protein spots corresponding to those recognized by the above serum pool from garlic allergy patients were excised and subjected to N-terminal sequence analysis in a Procise 494 protein sequencer (Applied Biosystems, Foster City, Calif).

### In-gel digestion for mass spectrometry

The protein spots recognized by the above serum pool from patients with garlic allergy were excised and subjected to in-gel

tryptic digestion, as described previously,<sup>17</sup> then the peptides produced were analyzed on a matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF-MS) Voyager DE-STR workstation (PerSeptive Biosystems, Framingham, Mass). Derivatized peptides of interest were isolated by using timed ion selection and analyzed by postsource decay (PSD) MS/MS.

### Protein identification

The peptide mass fingerprint and PSD MS/MS database searches were performed on the NCBI<sub>nr</sub> nonredundant protein database with the use of the MS-Fit and MS-Tag program (Protein Prospector; UCSF, San Francisco, Calif), using the masses obtained by MALDI-TOF-MS analysis.

### Purification of alliin lyase

Alliin lyase from garlic, leek, shallot, and onion was purified as described previously,<sup>18,19</sup> with some modifications. Briefly, the crude extract was brought to 35% saturation with solid ammonium sulfate, stirred slowly for 1 hour at 4°C, then centrifuged at 27,000g for 15 minutes at 4°C. The pellet was resuspended in 20 mmol/L phosphate buffer, pH 7.5; the suspension was dialyzed extensively against the same buffer at 4°C and centrifuged at 27,000g for 15 minutes at 4°C. The supernatant solution was applied to a HiTrap Q Sepharose Fast Flow column (0.4 × 2 cm, Amersham Biosciences), which was then eluted with a 15-mL linear gradient of 0 to 1.0 mol/L NaCl in 20 mmol/L phosphate buffer, pH 7.5, and 1-mL fractions were collected and tested for IgE binding on immunoblots. Fractions positive for IgE binding were pooled, concentrated to 1 mL, and applied to a Sephacryl S-100 HR column (1.6 × 60 cm, Amersham Biosciences), preequilibrated in 20 mmol/L phosphate buffer containing 0.15 mol/L NaCl, pH 7.5, and elution performed with the use of the same buffer at a flow rate of 0.5 mL/min. Fractions with IgE binding activity were pooled, concentrated, and stored at -70°C.

### Antiserum preparation

A female New Zealand White rabbit was injected intradermally at 20 sites with a total of 100 µg of garlic alliin lyase (purified as described above) in 1 mL of 50 mmol/L phosphate-buffered saline, pH 7.2 (PBS) emulsified with 1 mL of Freund complete adjuvant (Sigma, Steinheim, Germany); boosters of 100 µg in 1 mL of PBS emulsified with 1 mL of Freund incomplete adjuvant (Sigma, Steinheim, Germany) were given intradermally at weeks 3 and 6. The rabbit was bled 10 days after the last boost, and the serum was used for immunoblotting experiments.

### Lectin binding

Lectin binding was assessed as described previously.<sup>20</sup> After electrophoresis and electrotransfer of purified garlic alliin lyase, PVDF strips bearing 5 µg of protein were blocked and washed, then incubated for 1 hour at room temperature, with constant shaking with biotinylated concanavalin A (Con A), *Maclura pomifera* agglutinin, or *Phytolacca americana* agglutinin (Lectin-Link kit; Genzyme, Boston, Mass). Binding of lectins was detected with the use of avidin-conjugated horseradish peroxidase (HRP), 2-amino-9-ethyl-carbazole (AEC), and hydrogen peroxide.

### Periodate treatment

Periodate treatment of the allergen was performed as described,<sup>20</sup> with certain modifications. Briefly, PVDF membrane strips with bound electroblotted purified garlic alliin lyase were oxidized for 18 hours at 4°C, using sodium periodate (20 mmol/L, pH 4.5). After washing with distilled water, 50 mmol/L sodium borohydride was added and the sample was incubated for 30 minutes at 22°C. Control samples were treated identically, except that periodate was omitted.

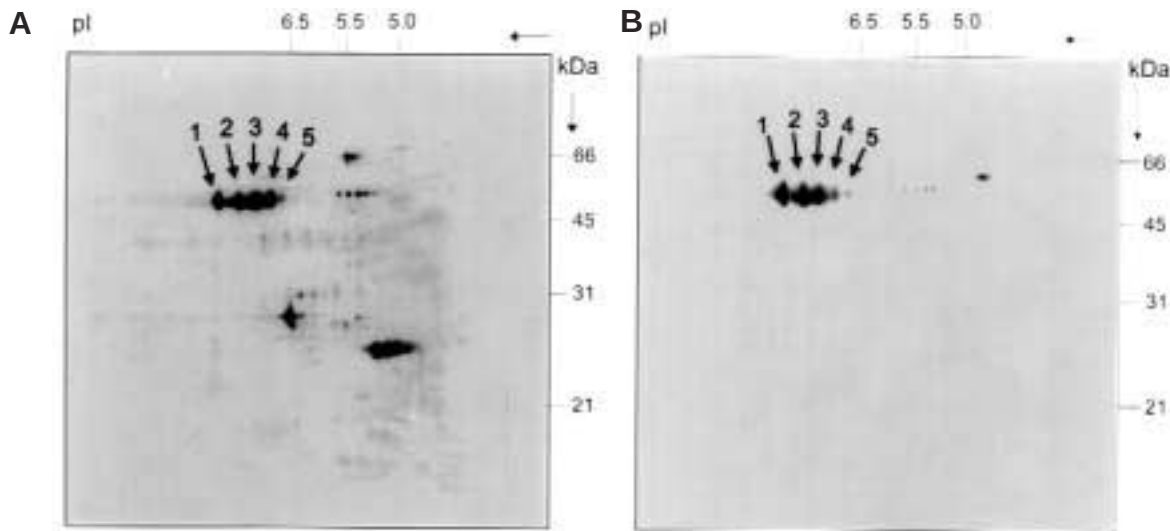


**FIG 2.** Two-dimensional electrophoresis and immunoblot analysis of *A sativum* extract. **A**, Coomassie blue-stained blot. **B**, Immunoblot probed with pooled serum from patients 1 through 4. Arrows (**A**) indicate protein spots corresponding to the IgE-binding spots (**B**).

### Intradermal skin test

The intradermal skin test was performed as described previously,<sup>21</sup> using 100  $\mu$ L of  $2 \times 10^{-8}$ ,  $1 \times 10^{-8}$ , and  $2 \times 10^{-9}$  mol/L purified garlic alliin lyase in PBS, with PBS as the negative control. Skin responses (wheals and erythema) were measured 15 minutes after injection. Wheals with a diameter of at least 3 mm greater than that produced by the negative control were regarded as positive responses.<sup>22</sup>

### Cross-reactivity of alliin lyases from different *Allium* species

Cross-reactivity of alliin lyases was tested as described previously.<sup>23</sup> Crude extracts from leek, shallot, and onion were prepared as described above, then electrophoresed (10  $\mu$ g/lane) on SDS gels and electrotransferred to a PVDF membrane. Antigenic cross-reactivity was studied by using the rabbit anti-garlic alliin lyase antiserum (preparation described above, diluting 30,000-fold), with bound antibodies being detected with HRP-conjugated anti-rabbit IgG antibodies (Jackson ImmunoResearch, West Grove, Pa) and AEC in acetate buffer (50 mmol/L, pH 5.0). Binding of individual serum samples from patients with garlic allergy was also tested.

### IgE-binding inhibition assay

An IgE-binding inhibition assay was performed as described by Yu et al.<sup>24</sup> Briefly, for ELISA cross-inhibition studies, a serum pool from 6 patients (Nos. 2 through 5, 8, and 9) displaying high IgE reactivity to garlic alliin lyase was used. Microtiter plates (Costar, Cambridge, Mass) were coated for 16 hours at 4°C with 100  $\mu$ L of purified garlic alliin lyase (0.01  $\mu$ g/ $\mu$ L) in PBS, pH 7.9, then washed. Aliquots of the serum pool, previously incubated overnight at 4°C with different concentrations of purified alliin lyase from garlic, leek, shallot, or onion, were added to the wells for 16 hours at 4°C. Bovine serum albumin (BSA) was used as the negative control. After washing, the plates were incubated with alkaline phosphatase-labeled goat anti-human IgE antibodies (BD PharMingen, San Diego, Calif); color development was then performed for 30 minutes, with para-nitrophenylphosphate (Sigma Chemical, St Louis, Mo) used as substrate, and absorbance at 405 nm was measured. All assays were performed in triplicate.

## RESULTS

### Detection of IgE-binding proteins in crude garlic extract

As shown in Fig 1, when 15 serum samples from patients allergic to garlic were tested for IgE binding to the crude garlic extract by immunoblotting, all had positive results (samples from patient Nos. 1 through 15 were used in lanes 2 through 16, respectively), whereas samples from nonallergic control subjects did not (lanes 17 and 18). The apparent molecular weights of the IgE-binding components ranged from 31 to 60 kDa. A component with a molecular weight of approximate 56 kDa was detected by all 15 sera. Other IgE-binding components of various molecular weights were detected at frequencies of less than 30%, for example, serum samples 3, 6, 10, and 13 gave a positive reactions to a 42-kDa component.

### Two-dimensional IgE immunoblotting

Two-dimensional electrophoresis after by immunoblotting was used to characterize the proteins in the crude garlic extract. Coomassie blue staining of the separated components showed a relatively simple protein profile, with only a few major proteins being detected (Fig 2, A), whereas immunoblotting showed at least 6 different IgE-reactive spots, with molecular weights of 56 to 60 kDa and pIs ranging from 5.0 to 8.5, the most reactive spots having a molecular weight of 56 kDa and pIs ranging from 6.5 to 8.5 (Fig 2, B).

### N-terminal sequencing and mass spectrometry

After 2-D electrophoresis, electroblotting, and Coomassie blue staining, one of the major IgE-reactive protein spots, spot 1, with a molecular weight of 56 kDa

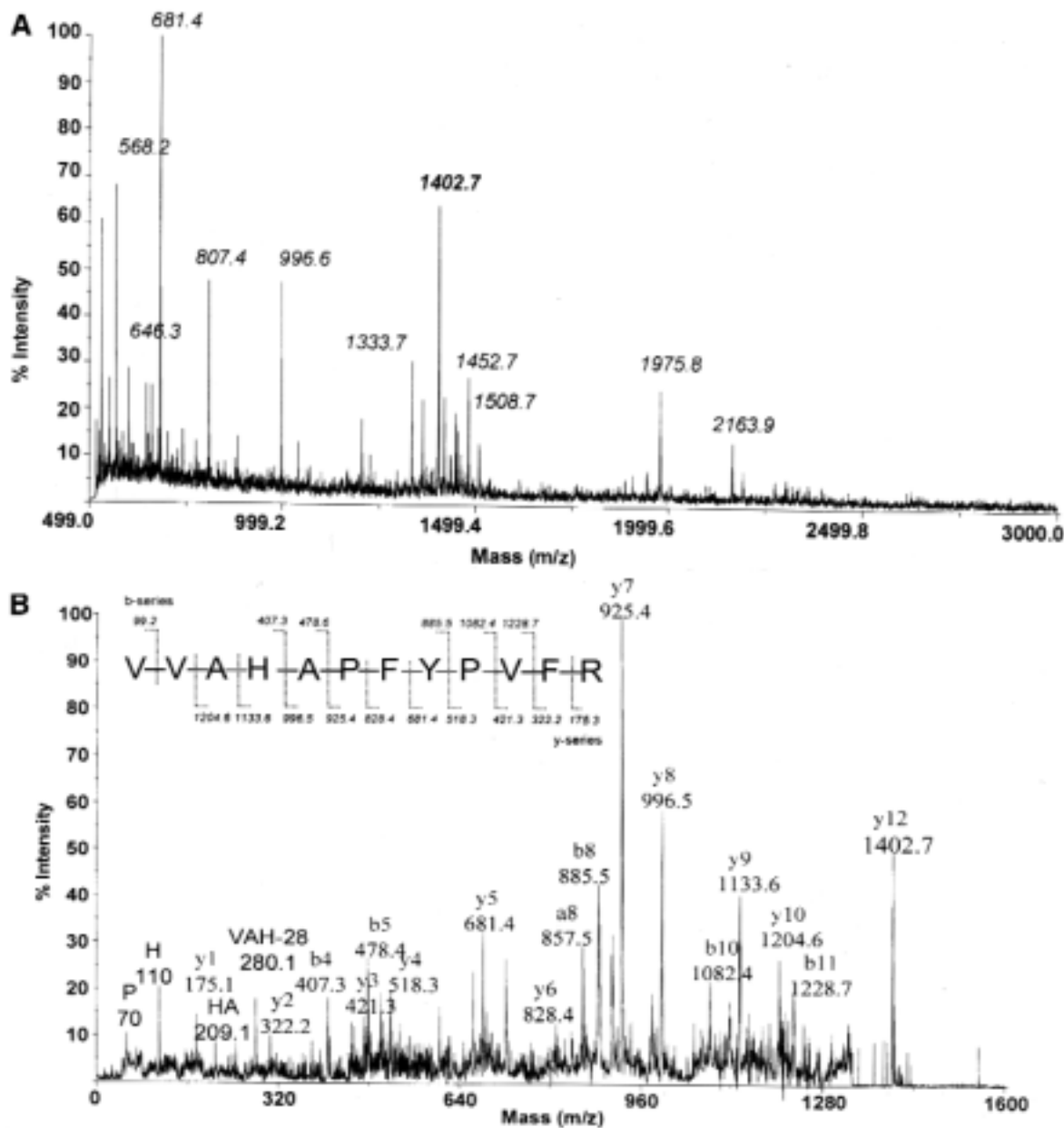


**FIG 3.** MALDI-TOF mass spectrometry analysis. **A**, MS profile of the spot 1 digest. **B**, PSD MS/MS spectrum of derivatized tryptic peptide with mass of 1402.7 Da (see **A**); peptide sequence ions from the amino-terminus (*b* series) and carboxy-terminus (*y* series) are indicated.

and a pI of 8.5, was excised. N-terminal amino acid sequencing gave the sequence KMTWTMKAAEEAEAVAN, which is identical to the N-terminal sequence of the mature form of garlic alliin lyase.<sup>25</sup> Spots 1 through 5 were then subjected to in situ digestion with trypsin, and the peptide fragments were eluted and analyzed by MALDI-TOF-MS. The MS profile of spot 1 is shown in Fig 3, A; spots 2 through 5 had similar MS profiles (data not shown). By comparison with established databases, the protein showing the highest correlation with spots 1 through 5 was found to be alliin lyase from *Allium sativum*,<sup>26</sup> with the coverage of the spots ranging from 17% to 41% (data not

shown). Subsequent PSD MS/MS analysis identified the sequence of the 1,402.7 Da fragment in spot 1 as VVAHAPFYPVFR (Fig 3, B), identical to residues 196 through 207 of alliin lyase from *Allium sativum*.<sup>26</sup>

### Isolation and immunogenicity of native alliin lyase

Alliin lyase was purified from garlic, leek, shallot, and onion by two chromatographic steps on HiTrap Q-Sepharose and Sephacryl S-100. The SDS-PAGE protein profiles of samples taken during the purification of the garlic crude extract are shown in Fig 4, A. The purified

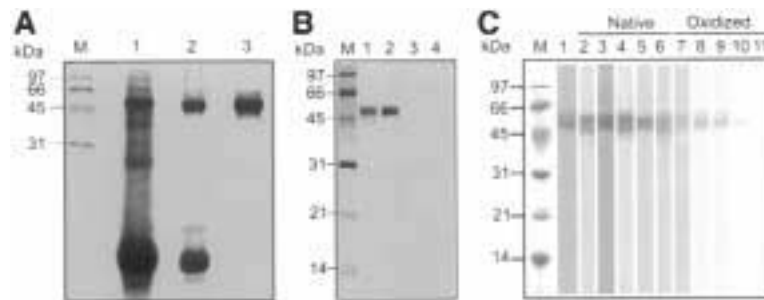


**FIG 4.** Purification and characterization of garlic alliin lyase. **A**, Coomassie blue-stained SDS gel of crude garlic extract (lane 1) and IgE-reactive pools from the HiTrap Q-Sepharose column (lane 2) and the Sephacryl S-100 HR column (lane 3). **B**, Lectin binding to purified garlic alliin lyase. Alliin lyase (5  $\mu$ g per lane) was electrophoretically stained with Coomassie blue (lane 1) or reacted with labeled Con A (lane 2), *Maclura pomifera* agglutinin (lane 3), or *Phytolacca americana* agglutinin (lane 4). **C**, Effect of periodate on IgG and IgE binding to purified garlic alliin lyase on immunoblots; Coomassie blue-stained blot (lane 1) and immunoblots of untreated (lanes 2 through 6) and periodate-treated (lanes 7 through 11) alliin lyase reacted with rabbit anti-garlic alliin lyase antiserum (lane 2 and 7) or with individual serum samples from patients 1 (lanes 3 and 8), 2 (lanes 4 and 9), 3 (lanes 5 and 10), and 5 (lanes 6 and 11). *M*, Molecular weight markers.



**FIG 5.** Skin test with purified garlic alliin lyase. Purified alliin lyase (100  $\mu$ L) at concentrations of  $2 \times 10^{-8}$  mol/L (spot 1),  $1 \times 10^{-8}$  mol/L (spot 2), or  $2 \times 10^{-9}$  mol/L (spot 3) was injected intradermally into the forearm of a patient with garlic allergy; wheal-and-flare responses were assessed after 15 minutes.

protein was identified as alliin lyase by peptide mass fingerprinting and N-terminal sequencing (data not shown). Since garlic alliin lyase is reported to be a glycoprotein,<sup>26</sup> binding of three lectins, Con A, *M pomifera* agglutinin, and *P americana* agglutinin, was examined, and only Con A was found to bind (Fig 4, B, lanes 2 through 4). The binding specificity of these lectins suggests that garlic alliin lyase contains carbohydrate with free terminal  $\alpha$ -D-mannopyranoside or  $\alpha$ -D-glucopyranoside residues.

The involvement of carbohydrate groups in the antigenic and allergenic determinants of garlic alliin lyase was investigated by periodate oxidation of PVDF membranes bearing the purified garlic protein, followed by immunoblotting. The results with untreated and periodate-treated alliin lyase and rabbit anti-garlic alliin lyase antiserum and individual serum samples from 4 patients allergic to garlic (Nos. 1, 2, 3, and 5) are shown in Fig 4, C. The markedly reduced binding of rabbit IgG and human IgE to oxidized alliin lyase indicated that the car-

bohydrate group(s) on the protein was involved in its antigenicity and allergenicity. Staining of the treated and untreated strips before antibody reaction showed that periodate treatment did not result in loss of protein from the PVDF membrane (data not shown).

### Skin test

To further characterize the allergenicity of purified garlic alliin lyase in vivo, a quantitative intradermal skin test was performed on 12 subjects allergic to garlic and 4 nonatopic subjects as normal control subjects. Table I summarizes the characteristics of the 12 patients studied. All 12 patients and none of the control subjects gave a positive response with  $1 \times 10^{-8}$  mol/L of purified garlic alliin lyase (Table I), whereas 58% (7/12) also gave a positive response with the lower concentration of  $2 \times 10^{-9}$  mol/L (data not shown). An example of a positive wheal-and-flare response in one of the allergic patients (No. 2) is shown in Fig 5. These results showed that purified gar-

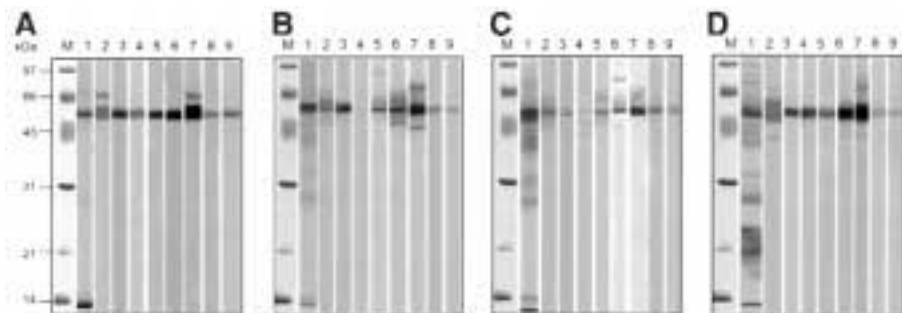


**FIG 6.** Cross-reactivity of antibody binding to alliin lyases from different members of the *Allium* family. Crude extract proteins from garlic (**A**), leek (**B**), shallot (**C**), or onion (**D**) were electroblotted onto membranes, then stained with Coomassie blue (lane 1) or immunoblotted with rabbit antiserum against garlic alliin lyase (lane 2) or with individual serum samples from 7 garlic-sensitized patients (lanes 3 through 9; patients 2, 3, 4, 5, 8, 9, and 12, respectively). M, Molecular weight markers

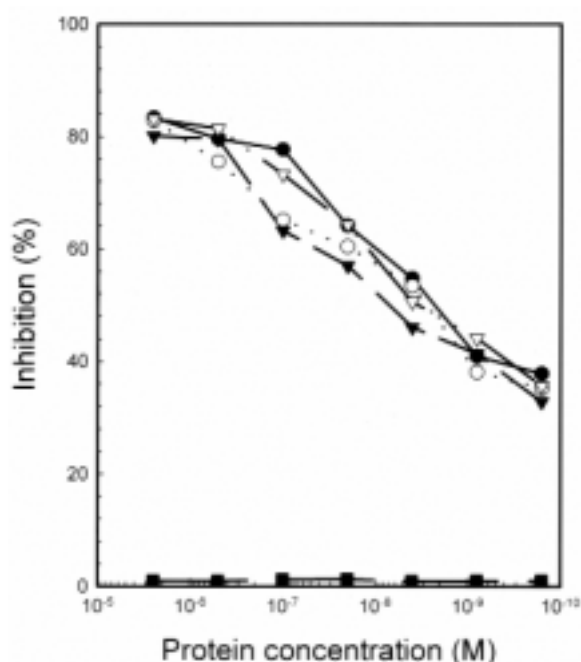


**FIG 7.** Inhibition of IgE binding to purified garlic alliin lyase by purified alliin lyases from garlic, leek, shallot, and onion. Samples of a pool of serum samples from 6 garlic-alliin lyase-reactive patients (Nos. 2 through 5, 8, and 9) were incubated overnight at 4°C with different concentrations of purified alliin lyase from garlic (filled circle), leek (filled triangle), shallot (open circle), onion (open triangle), or BSA (filled square); mixtures were then tested for binding to ELISA plates coated with purified garlic alliin lyase.

lic alliin lyase could elicit specific IgE-mediated hypersensitivity responses in patients allergic to garlic and indicate that alliin lyase is an important allergen of garlic.

#### Cross-reactivity and IgE-binding inhibition assay of alliin lyases from different *Allium* species

Crude extracts from shallot, leek, and onion were tested for antigenic and allergenic cross-reactivity by using rabbit anti-garlic alliin lyase antiserum and individual serum sam-

ples from 7 garlic-sensitive patients. Immunoblotting showed that with all test extracts, the rabbit anti-alliin lyase serum reacted strongly with proteins with molecular weights of 56 to 60 kDa (Fig 6, B through D, lane 2). In addition, similar-sized proteins in the crude extracts from other *Allium* species were recognized by the sera from all of the patients allergic to garlic (Fig 6, B through D, lanes 3 through 9), except sample 3, which did not bind to leek and shallot extracts (Fig 6, B and C, lane 4), implying that these *Allium* species show allergenic cross-reactivity. We also found that the carbohydrate on alliin lyases contributed to the IgE-binding cross-reactivity (data not shown).

To confirm that the same population of IgE antibodies recognized both antigens, competitive ELISA experiments were performed by preincubating sera containing garlic-alliin lyase-reactive IgE with the different purified alliin lyases before adding the samples to ELISA plates coated with purified garlic alliin lyase. As shown in Fig 7, IgE binding to garlic alliin lyase was inhibited in a dose-dependent manner by leek, shallot, and onion alliin lyases but not by BSA, demonstrating cross-reactivity of the anti-garlic alliin lyase IgE antibodies with the alliin lyase from these other *Allium* species.

#### DISCUSSION

Hypersensitivity to garlic can cause contact dermatitis,<sup>27,28</sup> rhinoconjunctivitis and asthma,<sup>29-31</sup> urticaria,<sup>32</sup> and anaphylaxis after ingestion of young garlic.<sup>33</sup> However, few studies have been performed to examine the allergenicity of garlic. To date, only 2 garlic proteins with molecular weights of approximately 12 kDa<sup>33</sup> and 54 kDa<sup>10</sup> have been shown to bind serum IgE from patients allergic to garlic on immunoblots. In addition, these allergens have not been isolated or characterized. In the current study, immunoblot analysis using sera from patients allergic to garlic indicated that the 56-kDa alliin lyase was a major IgE-binding protein. On 2-D immunoblots, the major 56-kDa IgE-reactive group of proteins had pIs ranging from 6.5 to 8.0, suggesting that these 5 IgE-reactive proteins might share identical or similar IgE epitopes. However, further detailed epitope mapping is required.

TABLE I. Clinical characteristics of patients with garlic allergy

| Patient No.        | Sex    | Age (y) | CAP class for garlic | Symptoms to garlic | Serum total IgE (IU/mL) | Skin response (purified alliin lyase):<br>1 × 10 <sup>-8</sup> mol/L |
|--------------------|--------|---------|----------------------|--------------------|-------------------------|----------------------------------------------------------------------|
| 1                  | Male   | 15      | 2                    | AD, AR, AS, UR     | 219                     | 11*/35†                                                              |
| 2                  | Male   | 16      | 2                    | AD, AR, UR         | 89                      | 17/62                                                                |
| 3                  | Male   | 10      | 1                    | AD, AR             | 747                     | 13/32                                                                |
| 4                  | Male   | 7       | 2                    | AD, AR             | 1388                    | 7/28                                                                 |
| 5                  | Male   | 35      | 2                    | AD, AR, AS, UR     | 128                     | 12/38                                                                |
| 6                  | Male   | 27      | 2                    | AD, AS, UR         | 1914                    | 18/65                                                                |
| 7                  | Male   | 8       | 2                    | AD, AR, AS         | 409                     | 6/20                                                                 |
| 8                  | Male   | 12      | 2                    | AR                 | 157                     | 7/28                                                                 |
| 9                  | Female | 34      | 2                    | AD, AS, UR         | 1149                    | 11/34                                                                |
| 10                 | Male   | 48      | 2                    | AD, AR, AS         | 76                      | 10/32                                                                |
| 11                 | Male   | 11      | 1                    | AD, AR             | 3198                    | 8/28                                                                 |
| 12                 | Male   | 35      | 2                    | AD, AR             | 128                     | 12/34                                                                |
| Control<br>(n = 4) |        |         | 0                    | —                  | ND                      | —                                                                    |

\*Wheal diameter (mm); †Erythema diameter (mm); —, negative response.

AD, Atopic dermatitis; AR, allergic rhinitis; AS, asthma; UR, urticaria; Control, nonatopic subjects; ND, not determined.

The N-terminal sequence of alliin lyase from garlic<sup>25</sup> is highly homologous to those of alliin lyase from onion<sup>34</sup> and shallot,<sup>25</sup> deduced from the cDNA sequences, and from leek,<sup>35</sup> determined by N-terminal sequencing. Moreover, the sequence of the 1,402.7 Da fragment, VVAHAPFYPVFR, identified by PSD MS/MS analysis, represents a highly conserved region in alliin lyase from garlic,<sup>26</sup> onion,<sup>25</sup> shallot,<sup>25</sup> and Chinese chives (*Allium tuberosum*).<sup>18</sup>

Alliin lyase has four putative N-glycosylation sites<sup>26,34</sup> and has been shown to be glycosylated at Asn146 and to form a complex with a garlic mannose-specific lectin.<sup>36</sup> In this study, purified garlic alliin lyase was recognized by Con A but not by *M pomifera* agglutinin or *P americana* agglutinin, indicating that it contains carbohydrate with free terminal  $\alpha$ -D-glucopyranoside or  $\alpha$ -mannopyranoside residues but no or few  $\alpha$ -galactopyranoside or N-acetylglucosaminopyranoside residues.

Carbohydrate epitopes can bind human IgE from allergic subjects and play a role in cross-reactivity between allergens from unrelated sources.<sup>37,38</sup> In this study, we found that carbohydrate groups contributed to the binding of IgG and IgE to alliin lyase from garlic and other *Allium* species. The effect of the carbohydrate moieties on cross-reactivity will be evaluated in further studies.

IgE cross-reactivity and IgE-binding inhibition analyses demonstrated that garlic alliin lyase showed a high cross-reactivity with alliin lyase from leek, shallot, and onion, suggesting that this molecule is a common plant allergen. Further investigations on this class of allergens are underway.

The molecular basis of the interaction between food allergens and the immune system is not clear, and there are many unanswered questions about the pathogenic and physiologic events that follow ingestion of food capable of initiating an IgE-mediated response. Standardization of allergens will be required to determine the molecular basis of the IgE response and for the development of new diagnostic and therapeutic strategies.<sup>39-41</sup> A combined

proteomic and immunologic approach provides a powerful tool for the identification of allergenic proteins from different food sources and should facilitate further studies of the mechanism involved in food allergen sensitization.

## REFERENCES

1. Rafaat M, Leung AK. Garlic burns. *Pediatr Dermatol* 2000;17:475-6.
2. Asero R, Mistrello G, Roncarolo D, Amato S. A case of onion allergy. *J Allergy Clin Immunol* 2001;108:309-10.
3. Munno G, Giannoccaro F, Riva G, Antonicelli L. Allergy to yucca. *Allergy* 2001;56:921-2.
4. Piirila P, Kanerva L, Alanko K, Estlander T, Keskinen H, Pajari-Backas M, et al. Occupational IgE-mediated asthma, rhinoconjunctivitis, and contact urticaria caused by Easter lily (*Lilium longiflorum*) and tulip. *Allergy* 1999;54:273-7.
5. Cadot P, Tits G, Bussels L, Ceuppens JL. Asthma and hand dermatitis to leek. *Allergy* 2001;56:192-3.
6. López-Rubio A, Rodríguez J, Crespo JF, Vives R, Daroca P, Reano M. Occupational asthma caused by exposure to asparagus: detection of allergens by immunoblotting. *Allergy* 1998;53:1216-20.
7. Escibano MM, Muñoz-Bellido FJ, Serrano P, de la Calle A, Conde J. Acute urticaria after ingestion of asparagus. *Allergy* 1998;53:622-3.
8. Navarro JA, del Pozo MD, Gastaminza G, Moneo I, Audicana MT, Fernandez de Corres L. *Allium cepa* seeds: a new occupational allergen. *J Allergy Clin Immunol* 1995;96:690-3.
9. Kawane H. Bronchial asthma caused by onion. *J Allergy Clin Immunol* 1995;96:568.
10. Anibarro B, Fontela JL, De La Hoz F. Occupational asthma induced by garlic dust. *J Allergy Clin Immunol* 1997;100:734-8.
11. Mirelman D, Monheit D, Varon S. Inhibition of growth of *Entamoeba histolytica* by allicin, the active principle of garlic extract (*Allium sativum*). *J Infect Dis* 1987;156:243-4.
12. Adetumbi MA, Lau BH. *Allium sativum* (garlic): a natural antibiotic. *Med Hypotheses* 1983;12:227-37.
13. Wilkins MR, Gasteiger E, Tonella L, Ou K, Tyler M, Sanchez JC, et al. Protein identification with N- and C-terminal sequence tags in proteome projects. *J Mol Biol* 1998;278:599-608.
14. Yates JR. Database searching using mass spectrometry data. *Electrophoresis* 1998;19:893-900.
15. Ewan PW, Coote D. Evaluation of a capsulated hydrophilic carrier polymer (the ImmunoCAP) for measurement of specific IgE antibodies. *Allergy* 1990;45:22-9.
16. Chow LP, Su NY, Yu CJ, Chiang BL, Shen HD. Identification and expression of Pen c 2, a novel allergen from *Penicillium citrinum*. *Biochem J* 1999;341:51-9.
17. Moritz RL, Eddes JS, Reid GE, Simpson RJ. S-pyridylethylation of intact

- polyacrylamide gels and in situ digestion of electrophoretically separated proteins: a rapid mass spectrometric method for identifying cysteine-containing peptides. *Electrophoresis* 1996;17:907-17.
18. Manabe T, Hasumi A, Sugiyama M, Yamazaki M, Saito K. Alliinase S-alk(en)yl-L-cysteine sulfoxide lyase from *Allium terberosum* (Chinese chive): purification, localization, cDNA cloning and heterologous functional expression. *Eur J Biochem* 1998;257:21-30.
  19. Krest I, Keusgen M. Quality of herbal remedies from *Allium sativum*: differences between alliinase from garlic powder and fresh garlic. *Planta Medica* 1999;64:139-43.
  20. Su SN, Shu P, Lau GX, Yang SY, Huang SW, Lee YC. Immunologic and physicochemical studies of Bermuda grass pollen antigen BG60. *J Allergy Clin Immunol* 1996;98:486-94.
  21. Nelson HS, Oppenheimer J, Buchmeier A, Kordash TR, Freshwater LL. An assessment of the role of intradermal skin testing in the diagnosis of clinically relevant allergy to timothy grass. *J Allergy Clin Immunol* 1996;97:193-201.
  22. Burks W. Current understanding of food allergy. *Ann N Y Acad Sci* 2002;964:1-12.
  23. Leung PS, Chow WK, Duffey S, Kwan HS, Gershwin ME, Chu KH. IgE reactivity against a cross-reactive allergen in crustacea and mollusca: evidence for tropomyosin as the common allergen. *J Allergy Clin Immunol* 1996;98:954-61.
  24. Yu CJ, Lin YF, Chiang BL, Chow LP. Proteomics and immunological analysis of a novel shrimp allergen, Pen m 2. *J Immunol* 2003;170:445-53.
  25. Van Damme EJ, Smeets K, Torrekens S, Van Leuven F, Peumans WJ. Isolation and characterization of alliinase cDNA clones from garlic (*Allium sativum* L) and related species. *Eur J Biochem* 1992;209:751-7.
  26. Rabinkov A, Zhu XZ, Grafi G, Galili G, Mirelman D. Alliin lyase (Alliinase) from garlic (*Allium sativum*): biochemical characterization and cDNA cloning. *Appl Biochem Biotechnol* 1994;48:149-71.
  27. Cronin E. Dermatitis of the hands in caterers. *Contact Dermatitis* 1987;17:265-9.
  28. Kanerva L, Estlander T, Jolanki R. Occupational allergic contact dermatitis from spices. *Contact Dermatitis* 1996;35:157-62.
  29. Falleroni AE. Occupational asthma secondary to inhalation of garlic dust. *J Allergy Clin Immunol* 1981;68:159-60.
  30. Seuri M, Taivanen A, Ruoppi P, Tukiainen H. Three cases of occupational asthma and rhinitis caused by garlic. *Clin Exp Allergy* 1993;23:1011-4.
  31. Lybarger JA, Gallagher JS, Pulver AW, Litwin A, Brooks S, Bernstein L. Occupational asthma induced by inhalation and ingestion of garlic. *J Allergy Clin Immunol* 1982;69:448-54.
  32. Asero R, Mistrello G, Roncarolo D, Antonietti PL, Falagiani P. A case of garlic allergy. *J Allergy Clin Immunol* 1998;101:427-8.
  33. Pérez-Pimiento AJ, Moneo I, Santaolalla M, de Paz S, Fernández-Parra B, Domínguez-Lázaro AR. Anaphylactic reaction to young garlic. *Allergy* 1999;54:626-9.
  34. Lancaster JE, Shaw ML, Joyce MD, McCallum JA, McManus MT. A novel alliinase from onion roots. Biochemical characterization and cDNA cloning. *Plant Physiol* 2000;122:1269-79.
  35. Peumans WJ, Smeets K, Van Nerum K, Van Leuven F, Van Damme EJ. Lectin and alliinase are the predominant proteins in nectar from leek (*Allium porrum* L) flowers. *Planta* 1997;201:298-302.
  36. Rabinkov A, Wilchek M, Mirelman D. Alliinase (alliin lyase) from garlic (*Allium sativum*) is glycosylated at ASN146 and forms a complex with a garlic mannose-specific lectin. *Glycoconj J* 1995;12:690-8.
  37. Hiemori M, Bando N, Ogawa T, Shimada H, Tsuji H, Yamanishi R, et al. Occurrence of IgE antibody-recognizing N-linked glycan moiety of a soybean allergen, Gly m Bd 28K. *Int Arch Allergy Immunol* 2000;122:238-45.
  38. Iacovacci P, Pini C, Afferni C, Barletta B, Tinghino R, Schinina E, et al. A monoclonal antibody specific for a carbohydrate epitope recognizes an IgE-binding determinant shared by taxonomically unrelated allergenic pollens. *Clin Exp Allergy* 2001;31:458-65.
  39. Platts-Mills TA, Chapman MD. Allergen standardization. *J Allergy Clin Immunol* 1991;87:621-5.
  40. Van Ree R. Analytic aspects of the standardization of allergenic extracts. *Allergy* 1997;52:795-805.
  41. Vieths S, Hoffmann A, Holzhauser T, Muller U, Reindl J, Haustein D. Factors influencing the quality of food extracts for in vitro and in vivo diagnosis. *Allergy* 1998;53:65-71.