

# Digestibility of allergens extracted from natural rubber latex and vegetable foods

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**Background:** Several cross-reactive allergens are now known to be involved in the defense responses of higher plants. Such proteins are drawing the attention of plant breeders because of their antimicrobial or stress-alleviating activities. Plants genetically modified to express defense-related proteins are being developed. The current concern is focused on the allergenicity of these intentionally expressed proteins.

**Objective:** It is believed that food allergens are proteins resistant to digestion. Digestibility tests have been accepted as an appropriate method for evaluating the allergenicity of newly introduced proteins. In this study we investigated the usefulness of this method for detecting allergens from natural rubber latex and vegetable foods.

**Methods:** Proteins were extracted from rubber latex, potato, and 5 kinds of fruits. Simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) were used for the digestibility test. An aliquot of each digest was periodically withdrawn and analyzed. Allergens were detected with pooled sera from individuals with latex allergy or patients given a diagnosis of oral allergy syndrome.

**Results:** Most latex and vegetable food proteins were digested by the SGF within 4 minutes. Numerous allergens were also decomposed by the SGF within 8 minutes. Although vegetable food allergens were relatively stable in the SIF, kiwi allergens were substantially degraded by the SIF within 16 hours.

**Conclusion:** The pronounced lability of the plant-derived allergens was thought to reflect the discrete sensitization and elicitation processes of patients with latex-fruit syndrome or oral allergy syndrome. These results indicate that the allergenicity of a newly expressed protein should be carefully evaluated according to not only its digestibility but also other important properties. (*J Allergy Clin Immunol* 2000;106:752-62.)

**Key words:** Latex allergy, oral allergy syndrome, cross-reactivity, defense-related protein, genetically modified plant, allergenicity, simulated gastric fluid, simulated intestinal fluid, sensitization, elicitation

Latex allergy is a growing problem among individuals who are in repeated contact with natural rubber products.<sup>1</sup>

## Abbreviations used

OAS: Oral allergy syndrome  
SGF: Simulated gastric fluid  
SIF: Simulated intestinal fluid

This immediate-type allergy is caused by the rubber-tree proteins that remain in latex products.<sup>2,3</sup> One of the most notable features of latex allergy is the patients' cross-reactivity to various fruits and vegetables,<sup>4</sup> a condition often called *latex-fruit syndrome*. The percentage of individuals with latex allergy who have IgE antibodies to a vegetable food is estimated to reach 50% or more.<sup>5</sup> Structurally similar proteins in many kinds of plants must be responsible for such extensive cross-reactivity. We have hypothesized that evolutionarily conserved proteins related to pathogenesis, or rather to defense, in higher plants are the cause of the latex allergy and the accompanying cross-reactivity.<sup>1,6-9</sup> Indeed, several latex allergens reported to date,  $\beta$ -1,3-glucanases (Hev b 2),<sup>9,10</sup> hevein-related proteins (Hev b 6),<sup>11,12</sup> patatin-like proteins (Hev b 7),<sup>13</sup> and hevimines (chitinase/lysozyme),<sup>9,11</sup> are likely to be related to the defense responses of a rubber tree.

Oral allergy syndrome (OAS) is another allergic disorder that is currently occurring with increasing frequency.<sup>14</sup> Many kinds of fruits and vegetables that are not notorious for their allergenicity bother patients with OAS.<sup>14</sup> It is considered that OAS is caused by the cross-reactivity of allergens in pollen and vegetable foods.<sup>15</sup> In previous studies, for example, more than half of the patients with birch pollen allergy were shown to react to fresh apple, celery, or carrot, all of which contain proteins homologous to the major birch pollen allergen (Bet v 1) or birch profilin (Bet v 2).<sup>15-17</sup> Obviously, few patients complain about allergic symptoms solely because of these foods. It is worthwhile to mention that Bet v 1 and its homologues in vegetable foods belong to a group of defense-related proteins (PR-10 family).<sup>18,19</sup>

In addition to the Bet v 1 and its homologues, many cross-reactive plant allergens have a relationship to the defense responses of higher plants.<sup>20</sup> A cross-reactive allergen in avocado, chestnut, and banana has been identified as a class I endochitinase,<sup>21-23</sup> which is a member of the antifungal defense-related protein (PR-3 family).<sup>18</sup> Several fruits in the *Prunoideae* family contain a cross-reactive allergen that is a lipid transfer protein with antifungal activity.<sup>24,25</sup> Some cereal allergens are structural

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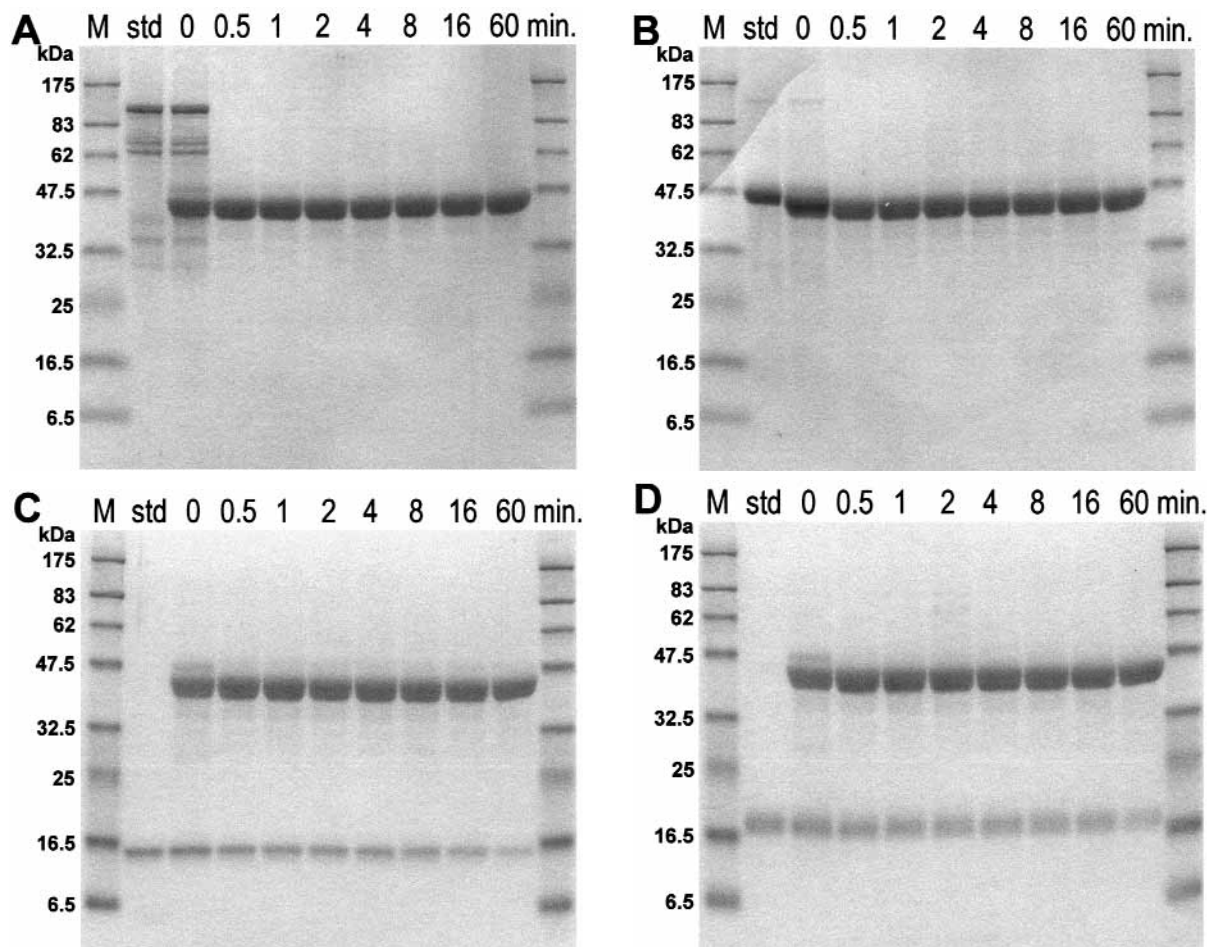


**FIG 1.** Digestibility of representative nonallergic (**A** and **B**) and allergic (**C** and **D**) food proteins in the SGF. Lipoxigenase (**A**, 102  $\mu$ g), acid phosphatase (**B**, 68  $\mu$ g),  $\beta$ -lactoglobulin (**C**, 34  $\mu$ g), or trypsin inhibitor (**D**, 34  $\mu$ g) was dissolved in 200  $\mu$ L of the SGF and incubated at 37°C. A 20- $\mu$ L aliquot of this digest was withdrawn at each of 0.5, 1, 2, 4, 8, 16, and 60 minutes. Each of these aliquots was quickly mixed with a sample buffer (26  $\mu$ L) for SDS-PAGE together with 6.0  $\mu$ L of  $\text{Na}_2\text{CO}_3$  solution (200 mmol/L) and boiled for 5 minutes. After the electrophoresis, separated proteins were visualized by staining with rapid Coomassie stain. The continuous 40-kD bands correspond to pepsin A, which is a constituent of the SGF. In the control experiment (*std*) pepsin A was excluded from the SGF. Molecular weights of the prestained markers (*M*) are shown at the left edges.

ly similar to  $\alpha$ -amylase/protease inhibitors, and some legume allergens are 2S albumins.<sup>20</sup> These proteins are also considered to play defensive roles in addition to their storage function.<sup>18</sup> Because of their evolutionarily conserved structures, defense-related proteins seem to construct plant panallergen families.<sup>26</sup>

Defense-related proteins are drawing much attention among plant breeders because of their antimicrobial and stress-alleviating activities.<sup>27,28</sup> Several of them are going to be intensively expressed in crops. A phytopathogen-resistant tomato variety that constantly expresses antifungal prohevein (Hev b 6.01) is a notable example.<sup>29</sup> In addition, a number of less-toxic agents that induce plants to express defense-related proteins are being explored as another type of agrochemical.<sup>27</sup> These genetically transformed or agriculturally manipulated plants could be

more allergenic to patients with the latex-fruit syndrome or OAS, as recently pointed out by Hänninen et al.<sup>30</sup>

Before being put on the market, genetically modified foods are evaluated for their allergenicity by using several approaches.<sup>31,32</sup> The digestibility of an induced protein in simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) is regarded as a decisive test. It is thought that digestible proteins do not become food allergens because they are thoroughly degraded before being absorbed from the intestine. Astwood et al<sup>33,34</sup> reported that the stability of a protein in the SGF (US Pharmacopoeia) was a significant and valid parameter to distinguish food allergens from non-allergens. None of the food allergens they investigated were completely digested by the SGF within 8 minutes. In contrast, the nonallergenic food proteins were thoroughly decomposed by the identical SGF within 15 seconds.

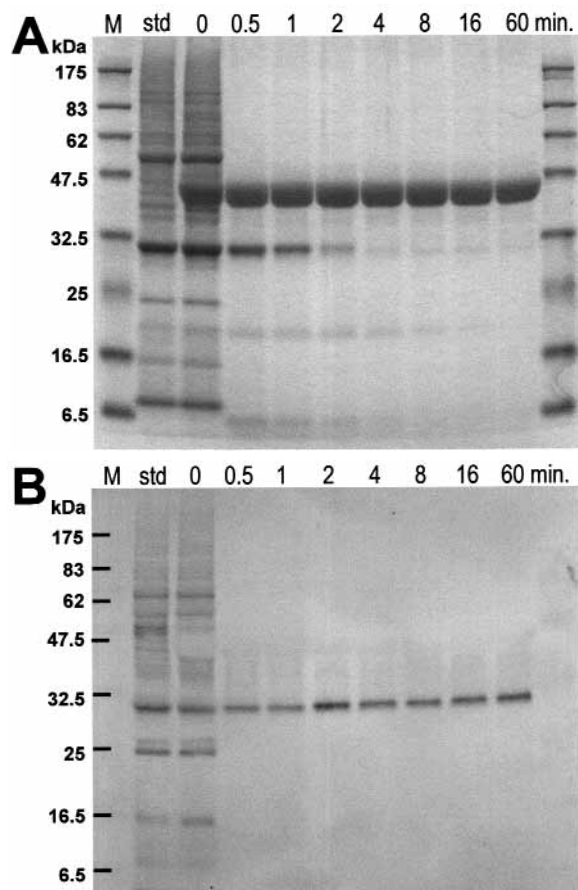


**FIG 2.** Digestibility of latex proteins (**A**) and allergens (**B**) in the SGF. Crude latex protein (680 µg) was dissolved in 200 µL of the SGF and incubated at 37°C. A 20-µL aliquot of this digest was withdrawn at each of 0.5, 1, 2, 4, 8, 16, and 60 minutes. Each of these aliquots was quickly mixed with a sample buffer (26 µL) for SDS-PAGE together with 6.0 µL of  $\text{Na}_2\text{CO}_3$  solution (200 mmol/L) and boiled for 5 minutes. After the electrophoresis, separated proteins were visualized by staining with rapid Coomassie stain (**A**). The continuous 40-kD bands correspond to pepsin A, which is a constituent of the SGF. In the control experiment (*std*) pepsin A was excluded from the SGF. For detection of the allergens (**B**), the separated proteins were transferred onto a membrane and incubated in the pooled serum (diluted 1:10) from patients with latex allergy at 4°C overnight. The IgE antibodies bound to the allergen on the membrane were then reacted with phosphatase-labeled anti-human IgE for 1 hour at 4°C. Finally, the bands of allergenic proteins were visualized by soaking the membrane in a substrate solution for 90 minutes at room temperature. Molecular weights of the prestained markers (*M*) are shown at the left edges.

However, there are notable exceptions. It has been reported that profilin and some Bet v 1-related proteins are not stable when exposed to heat or enzymatic degradation.<sup>35-37</sup> These exceptions have raised a question as to the reliability of allergenicity evaluation by using the SGF. In this study, we investigated the efficacy of the digestibility test for finding cross-reactive food allergens. Proteins extracted from natural rubber latex and vegetable foods were dissolved in the SGF and SIF. Development of the allergen digestion was monitored by immunoblotting with the pooled sera of patients with the latex-fruit syndrome or OAS.

## METHODS

### Sera of allergic patients

Sera were bled from 8 allergic patients who were suspected of having latex allergy on the basis of their clinical history of reaction to natural rubber products, positive RAST results ( $\geq$ class 1, Pharmacia CAP System), and positive results of skin prick tests with crude latex proteins. Four of them had experienced allergic reactions to banana, kiwi, or chestnut. The 8 sera were blended in equal volumes and used for the detection of allergens from nonammoniated latex, avocado, kiwi, banana, and potato. Sera were also sampled from 8 patients diagnosed with OAS. These people had experienced allergic reactions to many fruits, such as melon, peach, kiwi, and apple. Their total IgE levels were very high (2500-21,000 U/mL). All of them had shown positive RAST results ( $\geq$ class 2, Pharmacia CAP System) to orchard grass pollen and Japanese cedar pollen. The 8 sera were mixed in equal volumes and used for the detection of allergens from melon and peach.

### Protein extraction

Crude latex protein was extracted from nonammoniated latex (clone RRIM 600, Malaysia) and precipitated by adding  $(\text{NH}_4)_2\text{SO}_4$  to 90% saturation. Three kinds of latex allergens,  $\beta$ -1,3-glucanases, chitinase/lysozyme, and carboxylesterase, were separated from the crude protein, as previously described.<sup>9</sup> Hevein was purified from the same extract, following the procedure described by Mikkola et al.<sup>21</sup> The identity of the purified hevein was ascertained by liquid secondary-ion mass spectrometry, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, and N-terminal sequence analysis.

Avocado, kiwi, banana, potato, melon, and peach were purchased at local groceries in Tokyo. The edible part of each vegetable food was diced up and homogenized with a blender (Mini Blender, Osaka Chemical Co) for approximately 1 minute. One hundred grams of the homogenate was suspended in 200 mL of precooled (0°C) phosphate buffer (10 mmol/L, pH 7.4) containing NaCl (137 mmol/L), disodium ethylenediaminetetraacetate (2 mmol/L), sodium diethyldithiocarbamate (10 mmol/L),  $\text{NaN}_3$  (3 mmol/L), polyvinylpyrrolidone (2%), and phenylmethylsulfonyl fluoride (1 mmol/L). The suspension was continuously stirred for 2 hours at 0°C and then centrifuged at 17,500g for 1 hour (4°C). The proteins were precipitated by supplementing the supernatant with  $(\text{NH}_4)_2\text{SO}_4$  to 90% saturation. After centrifugation at 17,500g for 30 minutes (4°C), the collected precipitate was dissolved in 20 mL of  $\text{NH}_4\text{HCO}_3$  solution (30 mmol/L), and the residue was filtered off. In the case of the banana and peach suspensions, the supernatants were applied directly to an Amberlite XAD-7 column (2.1 × 20 cm, Organo Co) without using this salting-out procedure. After extensive washing with water, the adsorbed proteins were eluted with 50% methanol and lyophilized. Each protein obtained by salting-out or adsorption chromatography was transferred into a tube of semipermeable membrane (cut-off, 3.5 kD; Spectra Por 3 Spectrum Medical Industries, Inc). The proteins were then dialyzed against tap water for 24 hours, lyophilized, and redissolved in 8.0 mL of PBS (10 mmol/L, pH 7.4). After ultracentrifugation at 120,000g for 5 hours (4°C), the obtained supernatants were stored at -30°C until use. The protein concentration of each sample was determined by using a BCA Protein Assay Reagent kit (Pierce).

### Digestion by SGF

The digestibility of the crude plant proteins and separated latex allergens in the SGF was examined, as described by Astwood et al.<sup>33,34</sup> Briefly, a protein sample (680 µg of crude proteins or 34-680 µg of separated latex allergens) was dissolved in 200 µL of prewarmed SGF (US Pharmacopoeia) containing 0.32 wt/vol per-

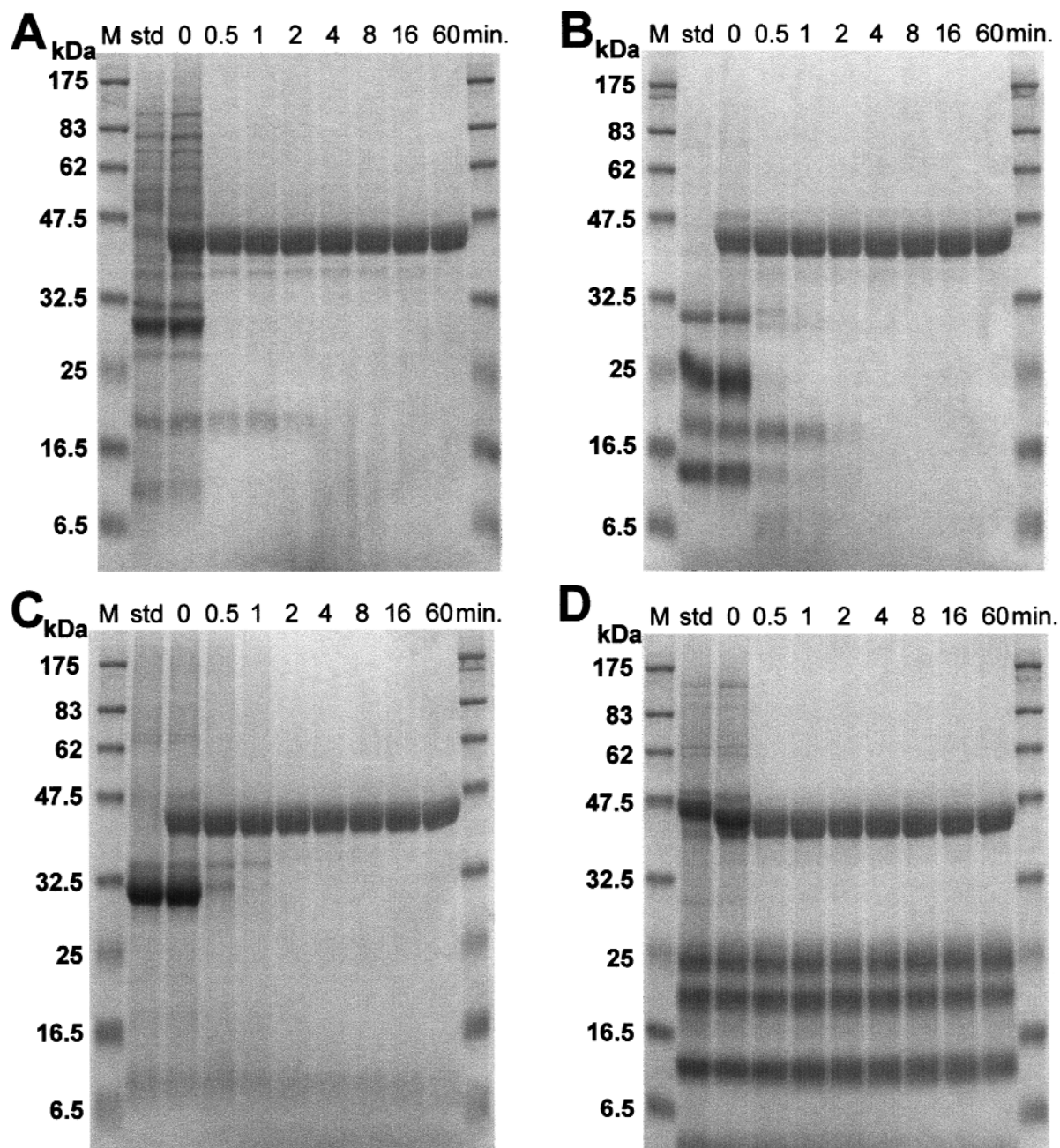


**FIG 3.** Digestibility of avocado (**A**), kiwi (**B**), banana (**C**), potato (**D**), melon (**E**), and peach (**F**) proteins in the SGF. Refer to the legend of Fig 2 for the experimental methods and conditions. All proteins were detected by staining with rapid Coomassie stain.

centage of pepsin A (Sigma Chemical Co). Digestion proceeded at 37°C with continuous rocking, and an aliquot (20  $\mu$ L) of this digest was periodically withdrawn (at 0.5, 1, 2, 4, 8, 16, and 60 minutes). These aliquots were quickly mixed with 26  $\mu$ L of a sample buffer (containing 2% 2-mercaptoethanol and 4% SDS) for SDS-PAGE together with 6.0  $\mu$ L of  $\text{Na}_2\text{CO}_3$  solution (200 mmol/L). The mixture was then boiled for 5 minutes and stored at  $-30^\circ\text{C}$  until further analyses. The reducing agent was removed from the sample buffer when hevein was the analyte. For the 0-minute digestion, each protein sample (68  $\mu$ g of crude proteins) was first dissolved in 26  $\mu$ L of the sample buffer for SDS-PAGE.

It was then mixed with 26  $\mu$ L of the SGF that had been inactivated by boiling for 5 minutes after neutralization with  $\text{Na}_2\text{CO}_3$  solution (200 mmol/L). In the control experiments each protein sample was dissolved in the SGF that did not contain pepsin A and then treated as described above.

The digestibility of two representative food allergens, milk lactoglobulin and soybean trypsin inhibitor, was examined in parallel to assess the activity of the SGF. Soybean lipoxygenase and potato acid phosphatase were selected as the representative nonallergenic food proteins. These proteins, which were used for comparison, were purchased from Sigma Chemical Co.

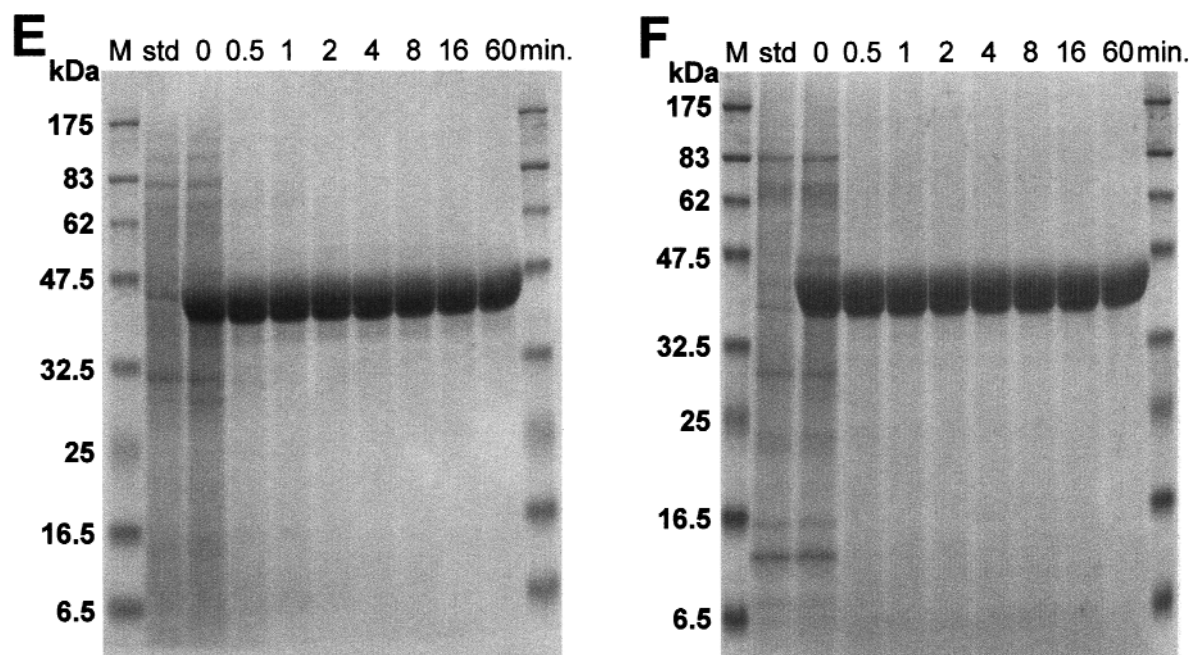


FIG 3. Continued.

### Digestion by SIF

Crude vegetable food proteins (340  $\mu$ g) were dissolved in 130  $\mu$ L of SIF (US Pharmacopoeia) containing 1.0 wt/vol percentage of pancreatin (Pancreatin USP, Sigma Chemical Co) and then incubated at 37°C with continuous rocking. An aliquot of this solution (26  $\mu$ L) was periodically withdrawn (at 0.25, 1, 4, and 16 hours) and quickly boiled for 5 minutes after mixing with 26  $\mu$ L of a sample buffer (containing 2% 2-mercaptoethanol and 4% SDS) for SDS-PAGE. These pretreated digests were stored at -30°C until further analysis. For the 0-hour digestion, crude vegetable food proteins (68  $\mu$ g) were dissolved in 52  $\mu$ L of the SIF that had been inactivated by mixing and boiling with the same amount of the sample buffer for SDS-PAGE.

### SDS-PAGE and immunoblotting

Pretreated digests (7.65  $\mu$ L) were loaded on a slab gel (12.5% acrylamide), and a constant current of 10 mA was applied. The migration buffer contained TRIS (25 mmol/L), glycine (192 mmol/L), and SDS (0.1%). The separated proteins were fixed by incubating the gel in 12% trichloroacetic acid for 90 minutes and visualized with rapid Coomassie stain (Diversified Biotech). Prestained Protein Marker (Broad Range, New England Biolabs Inc) was used for estimating the molecular weights of the electrophoresed proteins.

The digestibility of allergens was analyzed by immunoblotting using the pooled sera from either patients with latex allergy or individuals diagnosed with OAS. Pretreated digests (0.765  $\mu$ L) were loaded on a slab gel for SDS-PAGE, as described above. After the electrophoresis, proteins were transferred onto a polyvinylidene difluoride membrane (Immobilon-P, Millipore Co) by applying a constant current of 65 mA for 90 minutes with a semidry-type blotting apparatus. To block the unoccupied sites, the membrane was incubated for 2 hours at 40°C with 0.5% casein dissolved in TRIS-buffered saline (20 mmol/L, pH 7.6). Thereafter, the transferred proteins were reacted overnight at 4°C with one of the pooled sera diluted (1:10) in the blocking buffer. After washing with TRIS-buffered saline containing 0.05% Tween-20 (2 times by 2 minutes and then 3 times by 5 minutes), the membrane was incubated for 1 hour at 4°C with alkaline phosphatase-labeled anti-human IgE anti-

bodies (Kirkegaard & Perry Laboratories, Inc) diluted 1:1500 in the blocking buffer. After repeated washings, the membrane was soaked in a substrate solution (Substrate Kit III, Vector Laboratories, Inc) for 90 minutes at ambient temperature. Reproducibility of the results was ascertained by repeating the entire experiments, from extraction to detection of the allergens, at least two separate times.

## RESULTS

### Digestibility of representative nonallergic and allergic food proteins in the SGF

Both representative nonallergenic food proteins, lipoxygenase and acid phosphatase, were thoroughly digested by the SGF within 30 seconds (Fig 1, A and B). In contrast, the representative food allergens, lactoglobulin and trypsin inhibitor, were not completely digested by the identical SGF within 1 hour (Fig 1, C and D). These results coincided with the findings of Astwood et al.<sup>33,34</sup>

### Digestibility of latex proteins and allergens in the SGF

Most latex proteins were digested by the SGF within 4 minutes. Only faint protein bands were detected after a 1-hour digestion (Fig 2, A). Immunoblotting with the pooled serum of patients with latex allergy showed that most latex allergens were also digested by the SGF within 30 seconds (Fig 2, B). As an exception, one latex allergen with a molecular weight of 28 kd was resistant to the SGF. Three separated latex allergens,  $\beta$ -1,3-glucanases (Hev b 2, 35 to 38 kd), hevine (30 kd), and carboxylesterase (46 kd), were digested by the SGF within 2 minutes (data not shown). However, one latex allergen (28 kd) concomitantly separated with the carboxylesterase was very stable in the SGF. Its migrated position agreed with the allergen band clearly visualized even after a 1-hour digestion of the

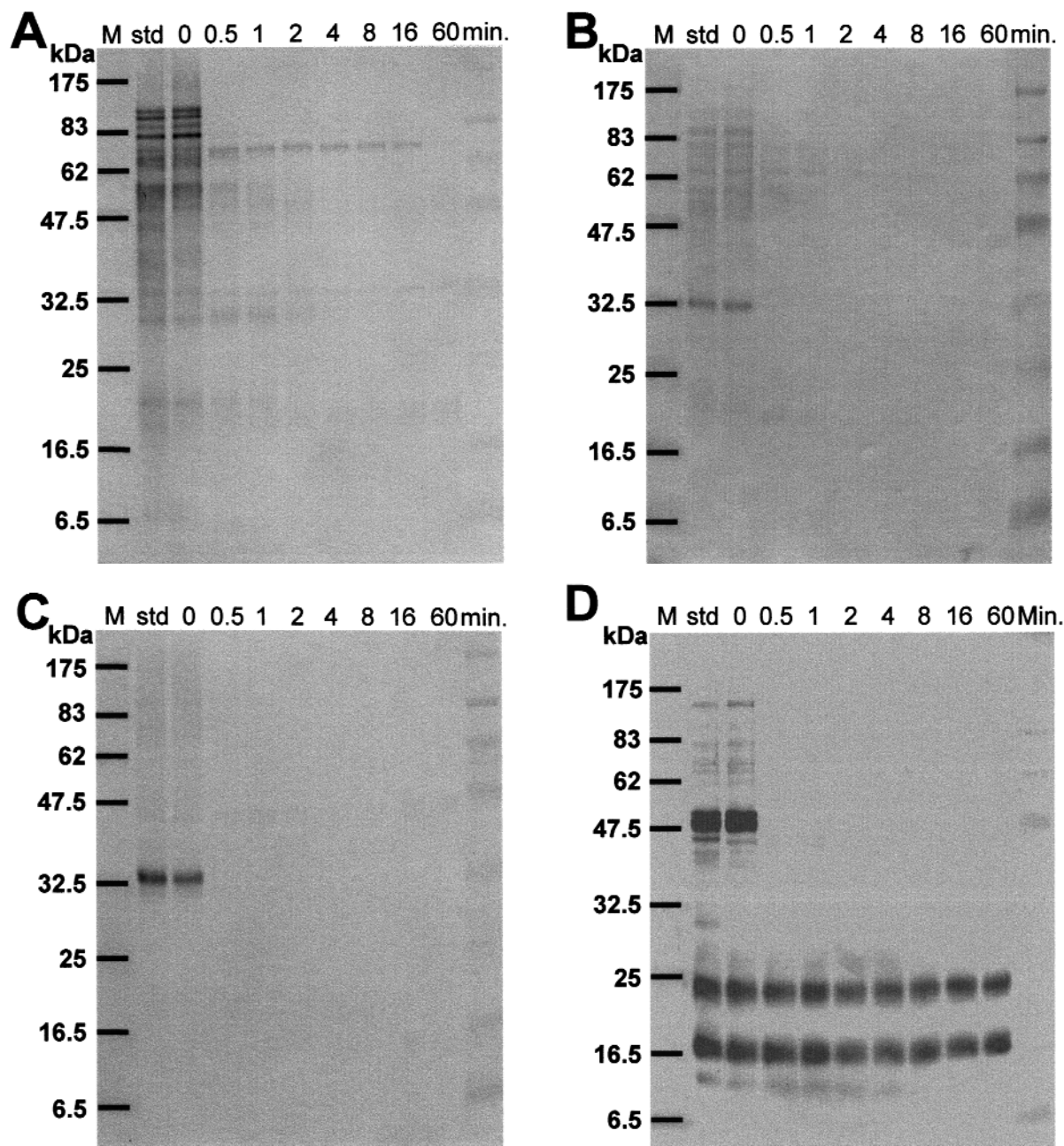


**FIG 4.** Digestibility of cross-reactive vegetable food allergens in the SGF. Refer to the legend of Fig 2 for the experimental methods and conditions. Avocado (**A**), kiwi (**B**), banana (**C**), and potato (**D**) allergens were detected by applying the pooled serum from patients with latex allergy. Pooled serum from patients with OAS was used for the visualization of melon (**E**) and peach (**F**) allergens.

crude latex protein. Hevein (Hev b 6.02, 4.7 kd) was also stable in the SGF and not completely digested within 1 hour (data not shown). However, hevein was hardly detected irrespective of its purity when the SDS-PAGE was carried out under reducing conditions, as in Fig 2.

#### Digestibility of vegetable food proteins and allergens in the SGF

Almost all the proteins extracted from avocado and kiwi were digested by the SGF within 4 minutes (Fig 3,

A and B). In correspondence with this protein staining, rapid degradation of the avocado and kiwi allergens was demonstrated by immunoblotting with the pooled serum of patients with latex allergy (Fig 4, A and B). Most cross-reactive allergens from these fruits were digested by the SGF within 4 minutes. The proteins extracted from banana also disappeared quickly (Fig 3, C). Cross-reactive banana allergens at 33 kd survived in the SGF for no more than 30 seconds (Fig 4, C). In contrast, potato proteins were relatively stable in the SGF (Fig 3, D).

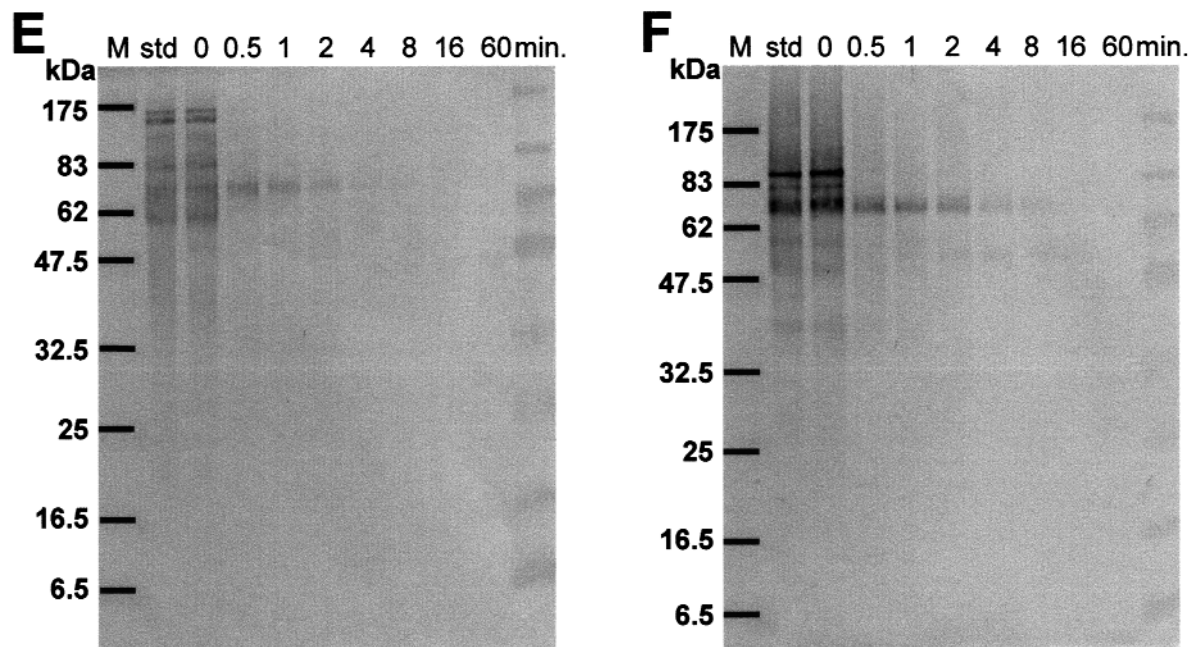


FIG 4. Continued.

Several protein bands (10, 20, and 24 kD) were clearly detected, even after a 1-hour digestion by the SGF. Among these SGF-resistant potato proteins, two antigens (17 and 24 kD) were specifically recognized by the IgE antibodies of the patients with latex allergy (Fig 4, D). However, the major allergen bands around 47 kD were broken down by the SGF within 30 seconds.

Many proteins extracted from melon and peach were digested by the SGF within 30 seconds (Fig 3, E and F). The allergenic proteins recognized by the IgE antibodies of patients diagnosed with OAS were also digested by the SGF within 8 minutes (Fig 4, E and F). None of the allergens persisted in the SGF for 16 minutes. These results were consistent with the above-mentioned lability of many latex and vegetable food allergens recognized by the IgE antibodies of patients with latex allergy.

#### Digestibility of cross-reactive vegetable food allergens in the SIF

The stability of the vegetable food allergens in the SIF was also examined (Fig 5). Many vegetable food allergens were gradually decomposed by the SIF. In particular, kiwi allergens were substantially digested within 16 hours (Fig 5, B). The other vegetable food allergens were not thoroughly degraded by this time.

#### DISCUSSION

The digestibility of IgE-reactive proteins extracted from natural rubber latex and vegetable foods was investigated in this study. Vegetable food allergens were relatively resistant to the SIF defined by US Pharmacopoeia. We cannot properly evaluate this result at this stage because detailed information about the stability of food allergens to the SIF has not yet been published. Remark-

ably, almost all of the IgE-reactive vegetable food proteins, except for two potato allergens, were digested by the SGF (US Pharmacopoeia) within 8 minutes. Contrary to the findings of Astwood et al,<sup>33,34</sup> these results imply that the SGF is not effective for finding potential food allergens. As a possible explanation for this discrepancy, we note that patients with latex-fruit syndrome or OAS have distinctive sensitization and elicitation processes, which might render the SGF ineffective as a determinant of food allergens.

Immediate-type allergic reactions elicited by food proteins to which the patient has been sensitized perorally are generally called food allergies. Typical food allergens are peroral sensitizers and, at the same time, peroral inducers of allergic symptoms.<sup>38</sup> Food proteins having both the ability to sensitize and the ability to induce symptoms are often called *complete food allergens*.<sup>38</sup> Complete food allergens are stable to digestion<sup>38,39</sup> because they must be absorbed from the intestine and recognized by the immune system of the person to be sensitized. Therefore digestibility testing with the SGF is regarded to be an effective and appropriate way to find such stable food allergens.

In our experiments, however, most of the IgE-reactive proteins from vegetable foods were readily digested by the SGF (Fig 4). Because these allergens cannot sensitize perorally, they cannot be considered complete food allergens. Nevertheless, these IgE-binding proteins are expected to provoke allergic reactions in patients with latex-fruit syndrome or OAS. To interpret this seeming discrepancy, the cross-reactivity between inhalant or contact allergens and food allergens must be taken into account.<sup>40,41</sup> Food proteins that can perorally induce allergic symptoms, even though they are digestible and cannot perorally sensitize people, are often called *incomplete food allergens*.<sup>38</sup>

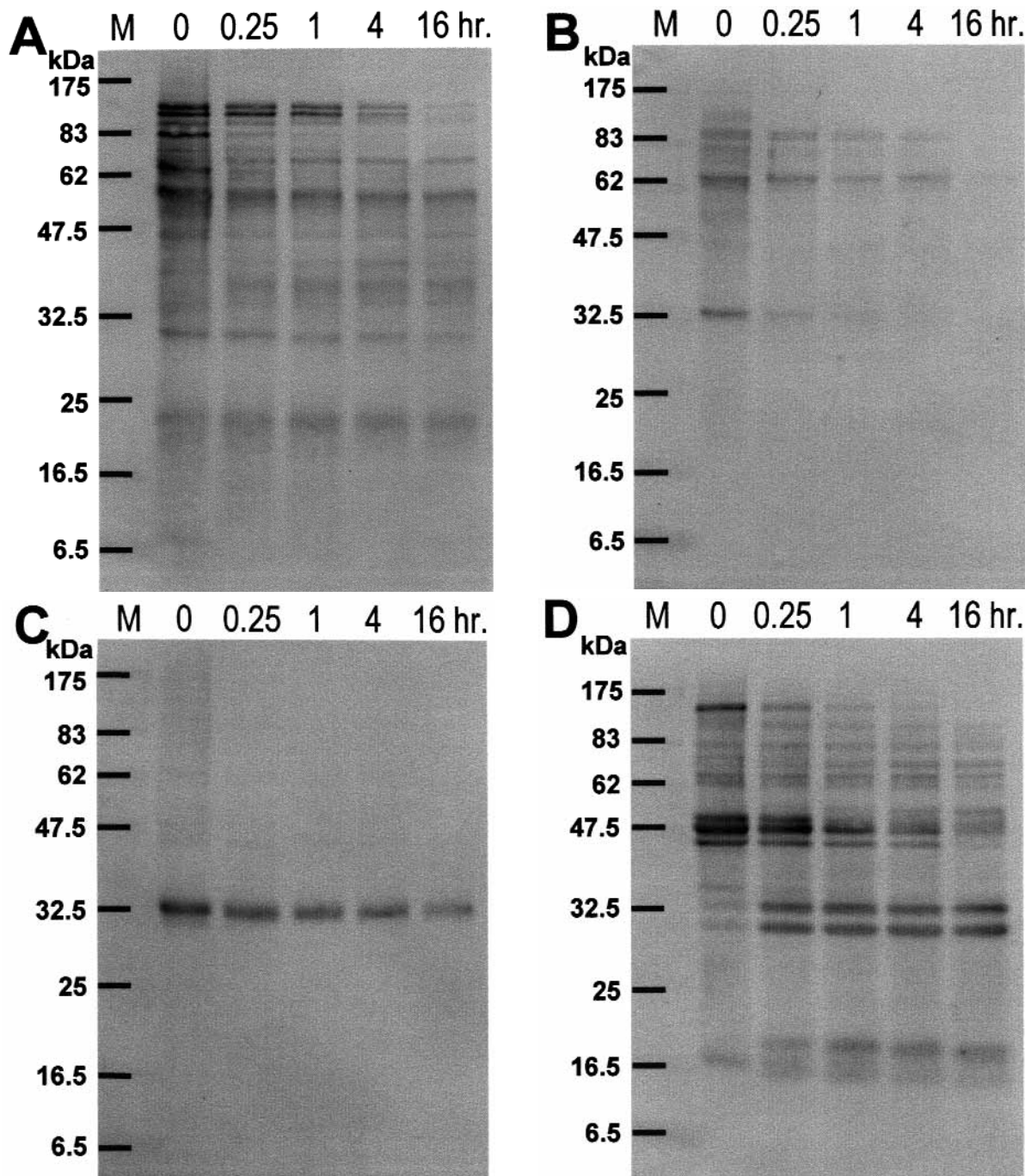


**FIG 5.** Digestibility of cross-reactive vegetable food allergens in the SIF. Crude vegetable food proteins (340  $\mu$ g) were dissolved in 130  $\mu$ L of the SIF and incubated at 37°C. A 26- $\mu$ L aliquot of this digest was withdrawn at each of 0.25, 1, 4, and 16 hours and quickly boiled for 5 minutes together with a sample buffer for SDS-PAGE. For the 0-hour digestion (lane 0), previously inactivated SIF was added to each sample. After the electrophoresis, the separated proteins were transferred onto a membrane and reacted with a pooled serum (diluted 1:10) at 4°C overnight. Allergens from avocado (**A**), kiwi (**B**), banana (**C**), and potato (**D**) were detected by applying the pooled serum from patients with latex allergy. Allergens from melon (**E**) and peach (**F**) were detected by using the pooled serum from patients diagnosed with OAS. Molecular weights of the prestained markers (*M*) are shown at the left edges.

Indigestibility is not a prerequisite for a food protein to induce allergic symptoms. Digestible food allergens, as well as indigestible food allergens, have the ability to elicit allergic reactions perorally.<sup>35-37,42</sup> Incomplete food

allergens are supposed to provoke allergic reactions on the basis of their cross-reactivity to the homologous inhalant, contact, or peroral sensitizers.<sup>36,41,43</sup> For example, patients presensitized by inhaling birch pollen aller-

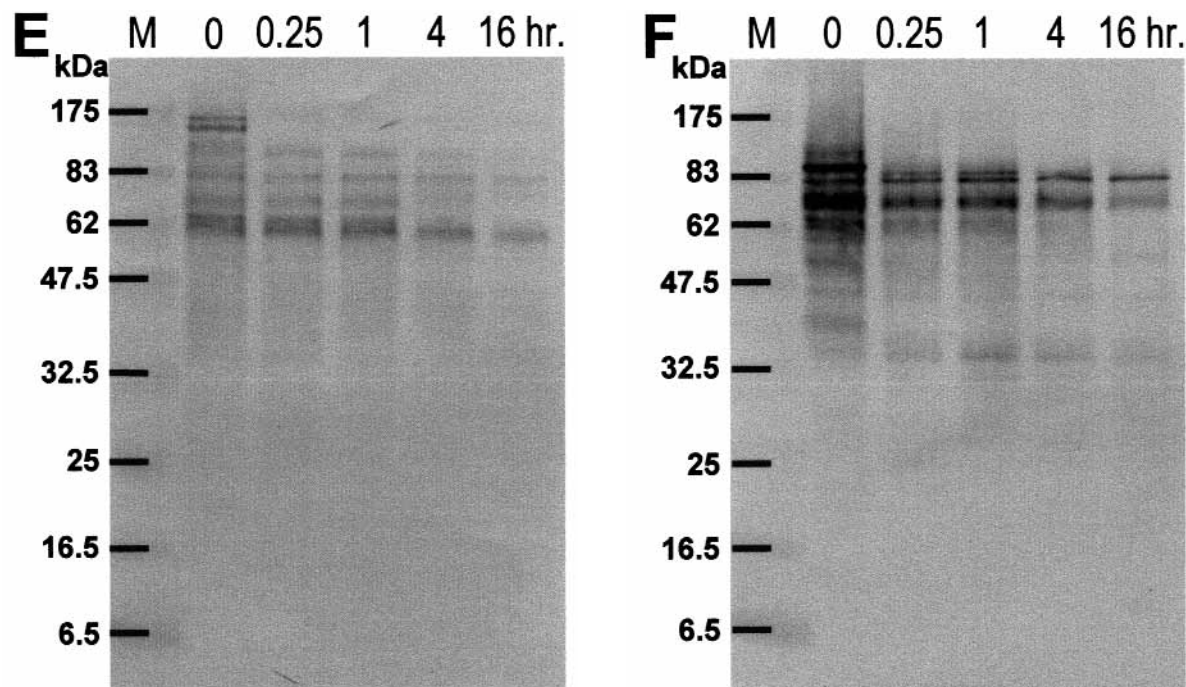


FIG 5. Continued.

gens, such as Bet v 1 and birch profilin, often experience OAS to various vegetable foods containing Bet v 1-related proteins or profilin,<sup>16,17</sup> which are not stable under condition of heat or enzymatic degradation.<sup>35-37</sup> Because of their susceptibility to digestion, incomplete food allergens are hardly recognized by the intestinal immune system and cause little problems in nonsensitized individuals.<sup>36</sup> Such digestible food allergens can provoke allergic symptoms only in patients who have already been sensitized by the cross-reactive allergens.

The major sensitization routes of latex allergy are presumed to be inhalation of an allergen-adsorbing powder and direct contact with latex products. Judging from these sensitization courses, indigestibility is not a requirement for a latex protein to be recognized by the immune system. In the present study, many of the latex allergens were indeed digestible proteins. Moreover, the cross-reactive allergens from avocado, kiwi, and banana were also digested easily. These readily degradable food proteins would have little ability to sensitize perorally. In actuality, only a very small number of patients complain of allergic reactions to exclusively avocado or banana.<sup>23</sup> Even so, the structurally related avocado and banana proteins are predicted to cause allergic reactions in patients presensitized by the homologous latex allergens; that is, they are incomplete food allergens for patients with latex allergy. This sensitization and subsequent elicitation process of the latex-fruit syndrome is comparable with that of OAS accompanying pollinosis.<sup>16,17,21</sup> For the patients who have already been sensitized by latex or pollen allergens, food allergens are not confined only to indigestible proteins (complete food allergens). Any food proteins that cross-react to the inhalant or contact sensitizers should also have

the potential to provoke allergic symptoms. The lability of such incomplete food allergens may explain why the allergic reactions tend to be restricted to the oral cavity (OAS) and why many patients with pollen allergy can eat cooked vegetables without any troubles. As an exception, hevein (Hev b 6.02) was stable in the SGF, although it was susceptible to reducing reagents and hardly detected under the usual SDS-PAGE and immunoblotting conditions. Individuals with latex allergy and IgE antibodies to this small antifungal protein (4.7 kd) might experience generalized symptoms when they ingest plant proteins that include a hevein domain (eg, one of the class I endochitinases).<sup>21-23</sup>

The striking difference between our experimental results and those reported by Astwood et al<sup>33,34</sup> is most likely attributable to the type of food allergens examined. Astwood et al examined the digestibility of only those food allergens that are suspected to sensitize perorally (complete food allergens). They did not investigate the digestibility of food allergens that cross-react to pollen or latex allergens and cause allergic symptoms in pollen- or latex-sensitized patients. We investigated the digestibility of not only suspected peroral sensitizers (complete food allergens) but also cross-reactive elicitors (incomplete food allergens). The complete food allergens (eg, lactoglobulin and soybean trypsin inhibitor) were stable in the SGF (Fig 1, C and D), as reported by Astwood et al. By contrast, the cross-reactive food allergens were not always resistant to the SGF (Fig 4). Considering the difference between the sensitization and elicitation processes of a complete food allergen and those of an incomplete food allergen, we can rationalize their differential susceptibility to the digestion. Vieths et al<sup>44</sup> have recently reported that the allergenicity of peanut proteins was

very persistent during digestion, whereas native birch pollen-related hazelnut allergens appeared to be relatively labile under the identical conditions. Wigotzki et al<sup>45</sup> have also shown that IgE-binding proteins in hazelnut were not very stable to *in vitro* digestion with proteolytic enzymes. The latest experimental results published from these two groups are compatible with our findings.

Many cross-reactive vegetable allergens have been isolated and sequenced. Some of them are now known to play roles in the defense responses of higher plants.<sup>20,26</sup> On the other hand, defense-related proteins, including pathogenesis-related proteins, will soon be deliberately expressed in crops designed to resist various stresses.<sup>27,28</sup> Novel transgenic tomato plants coexpressing antifungal endochitinases and  $\beta$ -1,3-glucanases are notable examples.<sup>46</sup> Lipid transfer proteins and 2S storage albumins are also being expressed in genetically modified plants resistant to fungal pathogens.<sup>27</sup> It is natural that the public should be anxious about the safety of foods containing such intentionally induced proteins. As demonstrated here, a digestibility test with the SGF alone cannot fully predict the allergenicity of a protein. Even readily degradable food proteins are able to provoke allergic symptoms in presensitized patients on the basis of their cross-reactivity.<sup>35-37</sup> Together with the other characteristics of known allergens, sequence homology,<sup>47</sup> structural conservatism,<sup>48</sup> and reactivity to the IgE antibodies of appropriate patients, the allergenicity of an intentionally expressed protein must be assessed individually and carefully.<sup>49,50</sup>

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