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Digestibility of Food Allergens and Nonallergenic Proteins in Simulated Gastric Fluid and Simulated Intestinal Fluid—A Comparative Study

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Information on the comparative digestibility of food allergens and nonallergenic proteins is crucial when stability to digestion is to be used as a criterion to assess the allergenic potential of novel proteins. In this work, we compared the digestive stability of a number of food allergens and proteins of unproven allergenicity and examined whether allergens possess a higher stability than nonallergenic proteins of similar cellular functions, and whether there is a correlation between protein digestibility and allergenicity. The stability of groups of storage proteins, plant lectins, contractile proteins, and enzymes, both allergens and proteins with unproven allergenicity, in a standard simulated gastric fluid and a standard simulated intestinal fluid was measured. Food allergens were not necessarily more resistant to digestion than nonallergenic proteins. There was not a clear relationship between digestibility measured in vitro and protein allergenicity.

KEYWORDS: Digestive stability; food allergens; simulated gastric fluid; simulated intestinal fluid; allergenicity assessment

INTRODUCTION

Food allergens are mostly proteins that invoke IgE-mediated immune reactions (*1*, *2*). Food allergy occurs in 6−8% of children and 1−2% of adults (*3*). Some of the affected individuals can develop life-threatening allergic reactions to the offending food. In the United States, it is estimated that 125−150 people die each year as the result of food anaphylaxis (*4*). Allergic individuals rely primarily on the avoidance of allergenic foods to prevent the occurrence of allergy. The ability to identify foods containing allergens is critical for these individuals.

Advances in biotechnology have brought into the marketplace an increasing number of genetically modified foods containing proteins of nonfood origin. Testing the allergenic potential of these proteins is difficult because there are no validated methods currently available to predict protein allergenicity. The approach taken by the agricultural biotechnology industry to assess the allergenic potential of transgenic proteins is to evaluate whether these proteins possess properties similar to those of known allergens, including comparison of the amino acid sequences and other physicochemical properties (*5*). Among the physicochemical properties usually examined is the stability to digestion in the human gastrointestinal tract (*6*−*11*).

A number of food allergens have been shown to be stable to conditions simulating human gastrointestinal digestion (*12*−*15*). Stability to digestion has thus been considered by many as one of the properties shared by food allergens (*1*, *16*). However, the digestive stability of most allergenic proteins has not been determined. Less is known about the relative stability of food allergens to digestion in comparison with nonallergenic proteins (*13*). Yet such information is crucial to validate the use of digestive stability as a predictive tool for protein allergenicity assessment.

Astwood et al. (*17*) measured and compared the digestive stability of a group of food allergens (most of which were storage proteins) and a group of nonallergenic proteins (all of them enzymes) in a standard simulated gastric fluid (SGF). While some food allergens were stable in SGF for the full 60 min of reaction, others were rapidly degraded within 30 s, although peptide fragments stable for at least 8 min were observed. All the nonallergenic enzymes tested were degraded within 15 s without forming any stable peptide fragments. Astwood et al. concluded that allergens were more stable than the nonallergenic proteins and further proposed that digestive stability can be a parameter to distinguish allergens from nonallergenic proteins.

Certain groups of proteins, such as storage proteins or structural proteins, are inherently more stable to proteolysis in cellular environments than other types of proteins, such as enzymes. It is not clear whether the higher stability observed by Astwood et al. among those food allergens occurred because

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of this inherent differential stability. To determine whether food allergens do possess higher stability to digestion than nonallergenic proteins, it is pertinent to compare the digestibility of proteins, allergens and nonallergens, with similar cellular functions.

The relative allergenicity of a food allergen is customarily measured by the percentage of individuals with allergy to certain foods showing IgE for that specific allergen. Food allergens are generally grouped into major and minor allergens. Major allergens are proteins for which more than 50% of the allergic patients studied have specific IgE. Fuchs and Astwood (*16*) ranked a selected group of egg, milk, and soybean allergens according to their percent allergenicity, compared their digestibility in SGF, and showed that a correlation could be established between the digestive stability of these food allergens and percent allergenicity. It is not clear whether this correlation would hold true if the digestibility of additional food allergens were compared.

In this study, we extended the work of Astwood et al. (*17*) and compared the digestive stability of food allergens and nonallergenic proteins of similar cellular functions. In addition to SGF, a comparative digestion study was also performed in a standard simulated intestinal fluid (SIF). The goal was to determine whether food allergens are more resistant to digestion in SGF and SIF than nonallergenic proteins and whether a correlation exists between a protein's digestibility and its allergenic potential by comparing the digestibility of food allergens with varying percent allergenicity.

A number of food allergens and proteins with unproven allergenicity belonging to each of the four groups (storage proteins, plant lectins, contractile proteins, and enzymes) were subjected to digestion in SGF and SIF. SDS−PAGE analysis was used to measure the rate of protein degradation. The amount of pepsin (or pancreatin) used in the SGF (or SIF) assays was about 10 times that of test proteins (by weight) to ensure sufficient degradation. The effect of changing the relative amount of pepsin and test protein used in a SGF assay on the stability observed was also examined. All the nonallergenic proteins chosen for this study, to the best of our knowledge, have not been reported to be associated with human food allergies.

MATERIALS AND METHODS

Materials. Patatin was purified from potato tubers according to the method of Racusen and Foote (*18*). The protocol details were kindly provided by Dr. David Hannapel at the Iowa State University (Ames, IA). Shrimp tropomyosin was obtained from Drs. Gerald Reese and Samuel Lehrer at the Tulane University Medical Center (New Orleans, LA). Peanut allergens *Ara h* 1 and *Ara h* 2 were obtained from Dr. Gary Bannon at the University of Arkansas for Medical Sciences (Little Rock, AR). Soybean β-conglycinin was kindly provided by Dr. Patricia Murphy at the Iowa State University. Pepsin (catalog no. P6887), pancreatin (catalog no. P1500), and other proteins used in this study were purchased from Sigma Chemical Co. (St. Louis, MO) in the purest form available. Reagents used for SDS−PAGE analysis were obtained from Bio-Rad (Richmond, CA) and Invitrogen (Carlsbad, CA).

SGF Digestion Stability Assay. The protocols described by Astwood et al. (*17*) were followed with some modification. SGF was prepared as described in the United States Pharmacopoeia (*19*) and consists of 3.2 mg/mL pepsin in 0.03 M NaCl at pH 1.2. Aliquots (200 μL) of SGF were placed in 1.5-mL microcentrifuge tubes and incubated in a water bath at 37 °C. Ten microliters of the test protein (5 mg/mL in 0.03 M NaCl) was added to each of the SGF vials to start the digestion reaction. The ratio of pepsin to test protein was about 13:1 (w/w). At intervals of 0, 0.5, 5, 15, 60, and 120 min, 75 μL of 1 N NaOH or 0.2 M $NaCO_3$ was added to each vial to stop the reaction. Next, 70 μL of 5× Laemmli buffer was added to the sample before it was heated for 10 min in a boiling water bath. Samples (15−40 μL) were loaded in a 10−20% tris-tricine ready-made minigel. The gel was run at a constant voltage with tricine buffer according to the manufacturer's instruction. Proteins were visualized by Coomassie Brilliant Blue staining.

SIF Digestion Stability Assay. SIF was prepared as described in the United States Pharmacopoeia (*19*) and consists of 10 mg/mL of pancreatin in 0.05 M KH_2PO_4, pH 7.5. Aliquots (64 μL) of SIF were placed in 1.5-mL microcentrifuge tubes and incubated at 37 °C for 10 min in a water bath. The test protein (10 μL) at a concentration of 5 mg/mL (in 0.05 M KH_2PO_4, pH 7.5) was added to each of the microcentrifuge tubes to start the reaction. The ratio of pancreatin to test protein was about 13:1 (w/w). At intervals of 0, 0.5, 5, 15, 60, and 120 min, 15 μL of 6× Laemmli buffer was added to each tube, and the reaction was immediately stopped by placing the tube in a boiling water bath for 10 min. The samples were then analyzed using the SDS−PAGE procedure described above.

RESULTS

SDS−PAGE Analysis. Figure 1 shows a typical SDS−PAGE analysis of protein degradation in SGF, and **Figure 2** shows a typical SDS−PAGE analysis of protein degradation in SIF. The tricine SDS−PAGE system was able to detect protein fragments with molecular weights as low as 2.5 kDa. Pepsin appeared as a thick band due to its much higher concentration. Pancreatin, a major component of SIF, is a mixture of five enzymes: amylase, trypsin, lipase, ribonuclease, and protease. The stability of the test protein was measured as the last time period for which the protein or degradation fragments could be seen in the gel. It was difficult to determine the stability of those proteins that coeluted with pepsin or any of the five SIF proteins, and they are noted as interferences. **Tables 1** and **2** summarize the digestive stability of all the food allergens and the nonallergenic proteins tested in SGF and SIF.

Comparative SGF Digestibility. As shown in **Table 1**, the stability of food allergens in SGF varied greatly, ranging from 0 to 120 min. Similar ranges of stability were observed for the proteins with unproven allergenicity (**Table 2**). Members of the plant lectin and contractile protein groups showed similar digestibility, irrespective of their allergenicity. For example, the digestibility of shrimp tropomyosin, a major allergen, was similar to those of nonallergenic chicken, bovine, and pork tropomyosins. The digestive stability within the storage protein and enzyme categories, on the other hand, varied greatly. Within the storage protein group, there was no clear indication that food allergens were more resistant to SGF digestion than proteins with unproven allergenicity. A food allergen may be more stable (e.g., bovine β-lactoglobulin vs human α-lactalbumin), equally stable (e.g., soybean trypsin inhibitor vs lima bean trypsin inhibitor), or less stable (e.g., *Ara h* 1 vs zein) to SGF digestion than proteins of unproven allergenicity. Within the enzyme group, most of the allergenic enzymes were as labile to SGF digestion as the nonallergenic enzymes tested.

If the grouping within the storage protein and enzyme groups was narrowed to specific protein families of closely related functions or sequences such as those defined in the Protein Identification Resource (PIR) database (*20*), similarity in SGF digestibility could be observed among members of certain protein families regardless of their allergenicity. For example, bovine α-lactalbumin (an allergen) was as labile to SGF digestion as human α-lactalbumin (a nonallergen). Papain and bromelain, both members of the papain superfamily, were readily digested. The major kiwi allergen actinidin, a member

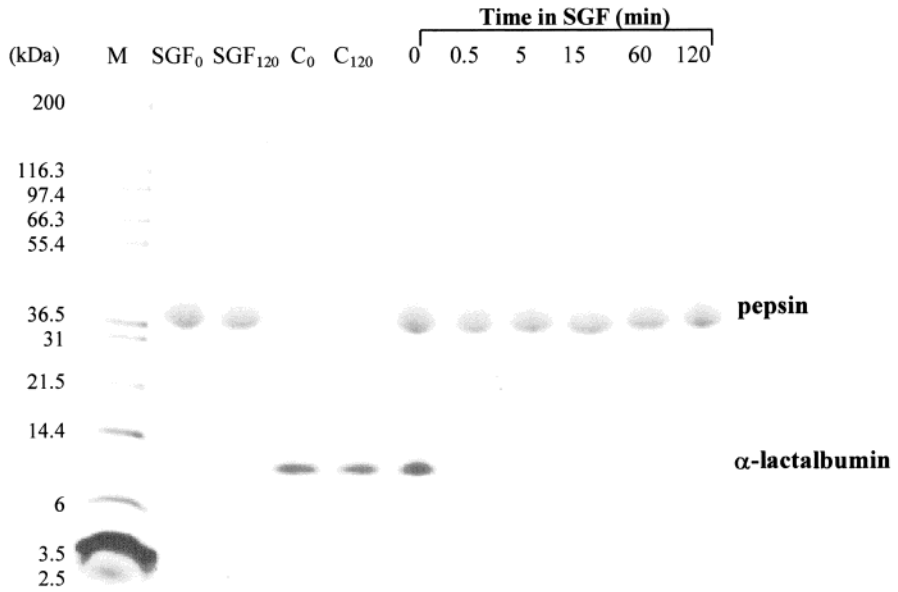

Figure 1. SDS–PAGE analysis of the degradation of bovine α-lactalbumin in SGF. Molecular weight markers (lane M) are indicated on the left-hand side of the gel. SGF and the test protein controls at $t = 0$ and 120 min were run along. The numbers on top denote the incubation times in minutes. The pepsin in SGF and the test protein are labeled at the right-hand side of the gel.

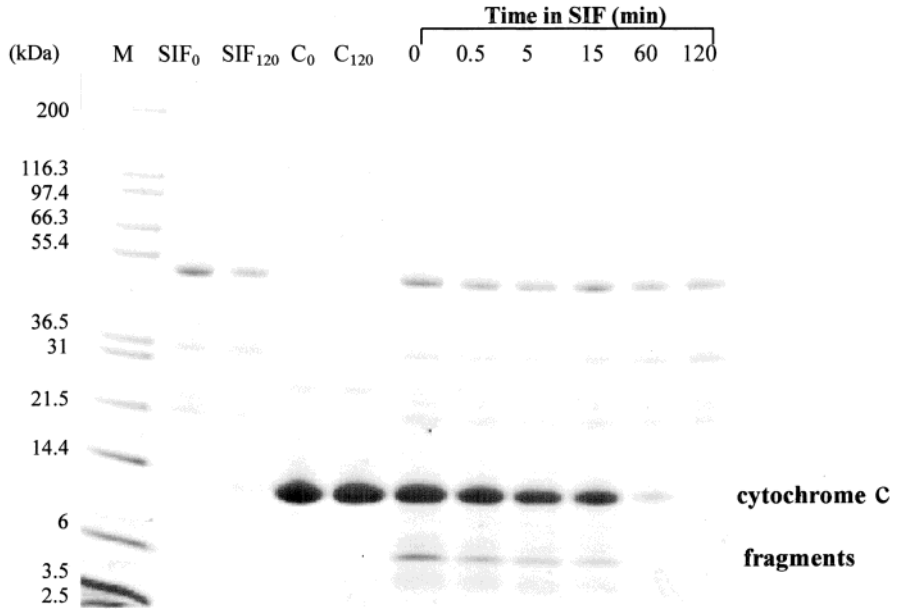

Figure 2. SDS–PAGE analysis of the SIF digestion of cytochrome *c*. Molecular weight markers (lane M) are indicated on the left-hand side of the gel. SIF (in which the pancreatin contained a mixture of five proteins) and test protein controls at $t = 0$ and 120 min were run along. The numbers on top denote the incubation times in minutes. The test protein and the degradation fragments are labeled at the right-hand side of the gel.

of the papain superfamily, was also found to be rapidly degraded in SGF (*21*). Soybean trypsin inhibitor and lima bean trypsin inhibitor showed similar digestibility. Exceptions to this generalization exist, however, as the egg allergen ovomucoid showed less stability than the nonallergenic bovine pancreas trypsin inhibitor. Both proteins share the same Kazal proteinase inhibitor homology. Similarly, egg lysozyme, a member of the lysozyme c superfamily, was very resistant to SGF digestion, whereas bovine and human α-lactalbumins, both members of the lysozyme c family, were rapidly degraded.

Variation in degradation patterns exists among the tropomyosins from different sources, although all degraded rapidly in SGF. The allergenic shrimp tropomyosin formed four fragments that remained clearly visible up to 5 min of digestion (data not shown). On the other hand, the nonallergenic pork, chicken, and bovine tropomyosins each formed a single fragment, which continued to degrade during the SGF reaction. It is not clear whether this difference in degradation pattern is of relevance with respect to the difference in allergenicity exhibited by these proteins.

SGF Digestibility: Major Allergens vs Minor Allergens. To determine whether a correlation exists between the allergenic potential of a protein and its digestive stability, the percent allergenicity for each of the food allergens (based on literature data) is listed in **Table 1**. A higher percent allergenicity suggests a greater allergenic potential. There was no apparent trend to indicate that a protein with a higher percent allergenicity is more resistant to SGF digestion. α-Casein, a major milk allergen to which up to 100% of patients have IgE, was degraded more rapidly in SGF than BSA, a minor milk allergen. The major

Table 1. Stability of Food Allergens in SGF and SIF[a]

protein group	protein source	PIR[b] superfamily	allergenicity[c] (%)	SGF stability (min)	SIF stability (min)
storage proteins					
α-casein	cow's milk	–	100[d] or 56[e]	0	0
β-lactoglobulin B	cow's milk	lipocalin	72[e]	120	5 (5)
β-lactoglobulin A	cow's milk	–	–	0.5	5 (0.5)
BSA	cow's milk	serum albumin	45[e]	0 (120)	120 (120)
α-lactalbumin	cow's milk	lysozyme c	14[e]	0	15
ovalbumin	egg	antithrombin III	100[e]	5	5 (120)
ovomucoid (trypsin inhibitor)	egg	Kazal proteinase inhibitor homology	62–70[f]	0	60
conalbumin	egg	transferrin	51–59[f]	0 (5)	120 (5)
β-conglycinin (α-subunit)	soybean	glycinin	20[e]–25[g]	0	–
β-conglycinin (β-subunit)	soybean	glycinin	75[e]	120	–
Gly m 1	soybean	–	65[e]	2 (8)[h]	–
trypsin inhibitor	soybean	kunitz-type proteinase inhibitor	20[i]	120	120 (120)
Ara h 1	peanut	–	>95[j]	5	15 (60)
Ara h 2	peanut	–	>95[j]	0.5	0.5 (0.5)
patatin	potato tuber	patatin	74[k]	0	0.5
plant lectins					
soybean lectin	soybean	plant lectin	10[e]	5	120 (120)
peanut lectin	peanut	plant lectin	50[i]	5	120 (60)
contractile proteins					
tropomyosin	shrimp	tropomyosin	82[l]	0 (5)	0 (0.5)
enzymes					
lysozyme	egg	lysozyme c	0–44[g]	60	120
lactoperoxidase	cow's milk	myeloperoxidase	67[m]	0	120
papain[n]	papaya	papain	–	0	120
bromelain[o]	pineapple	papain	–	0 (0.5)	120
actinidin	kiwi fruit	papain	100[p]	0[q]	–

[a] Stability was measured as the last time period (in minutes) that the protein could be seen in the SDS–PAGE gel. The number in parentheses is the last time period that one or more degradation fragments were seen in the gel. Percent allergenicity is defined as the percent allergic individuals with IgE specific for that protein (ref 16). –, Not determined. [b] Protein Identification Resource (ref 20). [c] As defined in ref 16. [d] From ref 29. [e] From ref 16. [f] From ref 30. [g] From ref 31. [h] From ref 17. [i] From ref 32. [j] From ref 33. [k] From ref 34. [l] From ref 35. [m] From ref 36. [n] Elicit allergic reactions when ingested as a meat tenderizer (ref 37). [o] Elicit allergic reactions when ingested as a digestive aid (ref 38). [p] From ref 39. [q] From ref 21.

Table 2. Stability of Proteins with Unproven Allergenicity in SGF and SIF[a]

protein group	protein source	PIR[b] superfamily	SGF stability (min)	SIF stability (min)
storage proteins				
α-lactalbumin	human milk	lysozyme c	0	60
zein	corn	zein	120	0.5
trypsin inhibitor	lima bean	Bowman–Birk proteinase inhibitor	120	interference
trypsin inhibitor	bovine pancreas	Kazal proteinase inhibitor homology	120	120
plant lectins				
red kidney bean lectin	red kidney bean	plant lectin	15	120 (120)
pea lectin	pea	plant lectin	5	120 (120)
lentil lectin	lentil	plant lectin	0.5	120 (120)
lima bean lectin	lima bean	plant lectin	5	120
jack bean lectin	jack bean	plant lectin	15 (60)	120 (60)
contractile proteins				
tropomyosin	bovine	–	0 (0.5)	0 (0.5)
tropomyosin	chicken	tropomyosin	0 (5)	0
tropomyosin	pork	tropomyosin	0 (0.5)	0
enzymes				
cytochrome *c*	bovine heart	cytochrome *c*	0	60 (60)
rubisco[c]	spinach leaf	ribulose bisphosphate carboxylase	0	120 (120)
phosphofructokinase	potato tuber	–	0	5 (60)
sucrose synthetase	wheat kernel	–	0	0.5 (60)

[a] Stability was measured as the last time period (in minutes) that the protein could be seen in the SDS–PAGE gel. The number in parentheses is the last time period that one or more degradation fragments were seen in the gel. –, Not determined. [b] Protein Identification Resource (ref 20). [c] D-Ribulose 1,5-diphosphate carboxylase.

egg allergen, ovalbumin, with an allergenicity of 100% showed a lower SGF stability than the minor egg allergen, lysozyme. The major soybean allergen, *Gly m* 1, was found to be less stable to SGF digestion than the soybean trypsin inhibitor, a minor soybean allergen (*17*). Similarly, *Ara h* 1 and *Ara h* 2, both major peanut allergens, showed a lower stability than soybean trypsin inhibitor. Shrimp tropomyosin and patatin, to which 82% of shrimp-allergic individuals and 74% of potato-allergic individuals, respectively, have IgE, also were degraded rapidly. These results suggest that food allergens with high allergenicity are not necessarily more resistant to SGF digestion than proteins with lower allergenicity. It would be difficult to rank the allergenic potential of proteins on the basis of their SGF digestibility.

SGF Digestibility: Effect of Pepsin to Test Protein Ratio. The effect of changing the relative amount of pepsin and test protein used in a SGF assay on the digestibility observed was evaluated. A number of major and minor food allergens and nonallergenic proteins were subjected to digestion with varying weight ratios of pepsin to test protein. **Table 3** summarizes the

Table 3. Effect of Changing the Ratio of Pepsin to Test Protein on the Stability Observed in a SGF Assay[a]

pepsin/test protein ratio (w/w)	stability of test protein in SGF (min)		
	10:1	1:1	1:10
food allergens			
β-lactoglobulin B	120	120	120
ovalbumin	5	60 (120)	120 (120)
papain	0	0 (0.5)	0 (120)
nonallergenic proteins			
zein	60	60 (120)	120
pea lectin	5	120 (0.5)	120 (15)
cytochrome *c*	0	0.5	0.5 (5)
sucrose synthetase	0	0	0 (120)

[a] Stability was measured as the last time period (in minutes) that the protein could be seen in the SDS–PAGE gel. The number in parentheses is the last time period that one or more degradation fragments were seen in the gel.

digestive stabilities observed. Increasing the weight ratio of pepsin to test protein from 0.1 to 10, while it did not affect the degradation of β-lactoglobulin B, affected the degradation rate of other food allergens. At a pepsin/test protein ratio of 0.1, intact ovalbumin along with stable peptide fragments were observed for the full 2 h of reaction. As the pepsin/test protein ratio increased to 10, ovalbumin was degraded within 5 min, and no stable fragments were observed. Similarly, stable fragments were observed after 2 h of reaction for papain but disappeared as the weight ratio of pepsin to test protein increased from 0.1 to 10.

Changes in the pepsin/test protein ratio also affected the rate of degradation of nonallergenic enzymes. For example, sucrose synthetase degraded rapidly in SGF with a pepsin/test protein ratio of 10 without forming any stable fragments; however, peptide fragments stable for more than 2 h were observed when the ratio of pepsin/test protein was reduced to 0.1. These results show that the digestibility of a protein, as determined by the SGF assay, is greatly influenced by the ratio of pepsin and test protein present in the assay. A protein can appear to be resistant or labile to digestion in SGF if different ratios of pepsin to test protein are used.

Comparative SIF Digestibility. The stability to digestion in SIF of both the food allergens and the proteins with unproven allergenicity tested varied greatly, ranging from 0 to 120 min (**Tables 1** and **2**). Food allergens were not necessarily more resistant to SIF digestion than proteins with unproven allergenicity. Some major food allergens showed rather rapid degradation in SIF. For example, *Ara h* 2 was degraded within 0.5 min; shrimp tropomyosin was degraded instantly, although it formed peptide fragments that were stable for 0.5 min. The major milk allergen, β-lactoglobulin B, which was highly resistant to SGF digestion, was relatively labile to SIF digestion.

Some allergens (e.g., ovalbumin, conalbumin, papain, and bromelain) that were labile to SGF digestion seem to be stable in SIF. However, this characteristic was not unique to allergens. A number of nonallergenic enzymes that were readily digested in SGF (e.g., rubisco and cytochrome *c*) also showed high stability in SIF. Some major allergens that were labile to digestion in SGF (e.g., α-casein, shrimp tropomyosin, and patatin) were also labile in SIF.

Similar SIF stability seems to exist among members of certain protein groups, irrespective of their allergenicity. The plant lectins as a group showed a high stability to SIF, whereas the tropomyosins were relatively labile. The SIF stability within the storage protein group varied greatly, although some similarity could be observed among members of certain protein families. All the trypsin inhibitors tested showed similar SIF stability irrespective of their allergenicity. Both members of the papain superfamily, papain and bromelain, also showed similar SIF stability.

A comparison of the SIF stability between major and minor allergens did not indicate a clear correlation between in vitro digestibility and protein allergenicity. Food allergens with high percent allergenicity were not necessarily more resistant to SIF digestion than allergens with low percent allergenicity. A number of major food allergens (e.g., α-casein, β-lactoglobulin B, *Ara h* 2, and shrimp tropomyosin) showed less stability in SIF than some minor allergens (e.g., soybean lectin, peanut lectin, and lysozyme).

DISCUSSION

The stability to digestion in SGF and SIF of the food allergens tested in this study varied greatly, ranging from 0 to 120 min. A similar range of stability was observed among the proteins with unproven allergenicity. In general, we measured a lower SGF stability among the food allergens than that reported by Astwood et al. (*17*). For example, repeated runs on the major peanut allergen, *Ara h* 2, yielded a stability of 0.5 min, whereas a stability of 60 min was reported by Astwood et al. This may be attributed to our use of a greater amount of pepsin, as was evident by the much thicker pepsin band in our SDS−PAGE analysis (**Figure 1**). The ratio of pepsin to test protein used by Astwood et al. was calculated to be about 19 (3.2 μg of pepsin/0.17 μg of test protein). Even though this ratio was similar to that used in the current study, the fact that a thicker pepsin band was observed in this study may be explained by our use of a commercial preparation of pepsin of greater purity than that used in the Astwood study (*22*).

Our data did not indicate that food allergens are more stable to digestion in vitro than proteins with unproven allergenicity. A comparison of the digestibility among proteins within the four functional groups defined in this study (storage proteins, lectins, contractile proteins, and enzymes) showed that food allergens could be more, equally, or less susceptible to SGF and SIF digestion than nonallergenic proteins of similar cellular functions. However, there seems to be some similarity in the SGF and SIF digestibility among members of certain protein families of closely related functions/sequences, regardless of their allergenicity.

Fuchs and Astwood (*16*) indicated that allergens with a low stability in SGF tended to have some stability in SIF. Our study, however, showed that this is not a unique characteristic of food allergens. In addition, food allergens that were rapidly degraded in SGF were not necessarily resistant to SIF digestion, and food allergens that were stable in SGF were not necessarily stable in SIF, either. β-Lactoglobulin B, which was highly resistant to digestion in SGF, was found to be rapidly degraded in SIF. Schmidt et al. (*23*) also found that β-lactoglobulin, while not affected by pepsin hydrolysis, was almost completely digested by pancreatic enzymes.

The observation that resistance to digestion is not a defining characteristic of food allergens has also been made by others. Vieths et al. (*24*) compared the digestibility of peanut and hazelnut allergens in SGF for 2 h followed by a 45-min treatment in duodenal digestion and showed that, while a number of peanut proteins were resistant to digestion, native hazelnut allergens were labile to digestion. Yagami et al. (*21*) showed that vegetable food allergens degraded rapidly in SGF. Kenna and Evans (*25*) compared the digestibility of 17 food allergens with 24 proteins not associated with food allergy in SGF and

found that, although 13 out of the 17 food allergens were partially or completely stable for at least 60 min, 10 out of the 24 nonallergenic proteins showed similar stability in SGF.

Fuchs and Astwood (*16*) suggested that a correlation exists between the allergenicity of a protein and its digestibility in SGF. However, in the current study, a comparison of the SGF and SIF digestibility among food allergens with varying degrees of allergenicity fails to show a clear correlation. Major allergens with high percent allergenicity were not necessarily more resistant to SGF or SIF digestion than allergens with low percent allergenicity.

Overall, the available data do not seem to support the notion that food allergens are more resistant to digestion than nonallergenic proteins, and there does not seem to be a correlation between the digestibility of a protein measured in vitro and its allergenic potential. It would therefore be difficult to use SGF or SIF digestibility as a predictive tool to distinguish potential food allergens from nonallergenic proteins, as suggested by Astwood et al. (*17*), or to rank the allergenic potential of proteins by comparing their digestibility with those of known allergens, as recommended (*26*).

Nonetheless, stability to digestion is still considered by many a relevant parameter for the assessment of the allergenic potential of proteins (*26*, *27*). In vitro digestion assays similar to those used in this study have been applied to assess the allergenic potential of many transgenic proteins introduced into foods (*6*−*11*).

It needs to be cautioned that the digestion stability and thus the perceived allergenic potential of proteins, as determined by the in vitro digestion assays, may be influenced by the assay conditions used. Changes in pH or the relative amounts of enzymes and test proteins used in an assay may affect the relative digestibility measured. We have shown that the major egg allergen, ovalbumin, would appear as stable or unstable, depending on the relative amount of pepsin and test protein used in the SGF assay. Similarly, sucrose synthetase would appear as stable in SGF if the ratio of pepsin/test protein were reduced to 0.1. That changes in pepsin concentration would affect the digestibility measured in a SGF assay was also observed by Astwood et al. (*17*), who showed that the nonallergenic protein rubisco would appear to be a highly stable protein if the pepsin concentration were reduced by 100-fold.

The relative amount of enzyme to food proteins during an actual human digestive process is not known. A great deal of variation seems to exist in the ratio of enzyme to test protein used by different laboratories to study protein digestibility in vitro. Ratios ranging from 1:250 to 5000:1 were reported (*28*). These variations may affect the rate of enzymatic degradation of the proteins in question, and therefore affect the estimated allergenic potential of those proteins. Although the use of digestive stability as a criterion for protein allergenicity assessment has been widely adopted by the agricultural biotechnology industry, there remains a need to establish a globally used standardized assay condition so that direct comparison of assay results can be made.

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