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Kiwellin, a Modular Protein from Green and Gold Kiwi Fruits: Evidence of in Vivo and in Vitro Processing and IgE Binding

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Kiwellin, an allergenic protein formerly isolated from green kiwi fruit, has been identified as the most abundant component of the gold kiwi species. A protein named KiTH, showing a 20 kDa band on reducing SDS-PAGE and 100% identity with the C-terminal region of kiwellin, has been identified in the extract of the ripe green species. In vitro treatment of purified kiwellin with the protease actinidin from green kiwi fruit originated KiTH and kissper, a recently described pore-forming peptide. Primary structure analysis and experimental evidence suggest that kiwellin is a modular protein with two domains. It may undergo in vivo proteolytic processing by actinidin, thus producing KiTH and kissper. When probed with sera recognizing kiwellin from green kiwi fruit, KiTH showed IgE binding, with reactivity levels sometimes different from those of kiwellin. The IgE-binding capacity of kiwellin from gold kiwi fruit appears to be similar to that of the green species.

KEYWORDS: Kiwellin; KiTH; kiwi fruit; protein processing; allergen

INTRODUCTION

The increasing spread on the international market and the availability of new species of kiwi fruit are associated with a growing interest in its influence on human health. Literature reports describe several investigations on possible health-promoting effects of this fruit, such as cardiovascular protective properties (*1*), cytotoxicity for tumor cell lines and antimicrobial activities against human pathogens (*2*), protection against oxidative DNA damage (*3*), and laxation in elderly people (*4*). However, none of the molecules responsible for these biological effects has been identified so far, and only a low number of kiwi fruit proteins, such as actinidin (*5*), pectin methylesterase inhibitor (*6*), and pectin methylesterase (*7, 8*), have been isolated from the natural source and characterized. In contrast, a greater number of papers describe negative effects on human beings, due to allergic reactions with a wide range of symptoms including generalized urticaria and anaphylaxis (*9, 10 11*).

Despite the remarkable number of published studies, the available information on kiwi fruit allergens is still scanty, and sometimes discrepancies concerning their number and relevance have been reported (*9*). Beyond the differences associated with the study population under investigation, it is conceivable that, depending on the experimental procedures used and the fruit characteristics (e.g., the ripening stage), the protein extracts may be variable in both the number and amount of the components. In fact, different patterns of IgE-binding proteins showing up to 12 reactive bands, with molecular masses ranging approximately from 6 to 67 kDa, have been observed upon Western blotting analysis (*9, 12*). Several IgE-binding components of kiwi fruit extracts have been identified as IgE-binding bands (*11*). Nevertheless, few of them have been characterized. Actinidin (*13*) and a thaumatin-like protein (*14*), showing 30 and 24 kDa bands in SDS-PAGE and designated Act d 1 and Act d 2, respectively, were purified and their properties investigated.

More recently, the identification in green kiwi (GrK) fruit extracts of kiwellin, a member of a new class of proteins, showing a 28 kDa IgE-binding band upon Western blotting analysis, has been described (*15*). According to the mass spectrometry data, elucidation of the primary structure by direct protein sequencing provided a sequence-deduced molecular mass of approximately 20 kDa for this protein. The identification in the ripe kiwi fruit of a 39-residue peptide, named kissper (*16*), showing 100% identity with the N-terminal region of kiwellin, suggested that it could originate from the in vivo processing of kiwellin. Kissper is a proteolysis-resistant peptide with pH-dependent and voltage-gated pore-forming and ion-channeling activities in model synthetic planar lipid membranes.

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Here we describe (i) the identification of kiwellin in gold kiwi fruit (GoK) extract, (ii) the identification in ripe GrK extract and characterization of KiTH, a protein component showing a 20 kDa band upon reducing SDS-PAGE and 100% identity with the C-terminal region of kiwellin, (iii) an investigation of the in vivo and in vitro proteolytic processing of kiwellin, and (iv) the IgE binding of these proteins by Western blot.

MATERIALS AND METHODS

Kiwi fruits, *Actinidia deliciosa* cv. Hayword (green kiwi fruit, GrK) and *Actinidia chinensis* cv. Hort 16A (gold kiwi fruit, GoK), were from a local farm. Trypsin and pepsin were from Boheringer (Boehringer Mannheim GmbH, Germany); bovine serum albumine, dithiothreitol, Tris, and 4-vinylpyridine were from Sigma (Milan, Italy); and DEAE-cellulose (type DE52) was from Whatman (Brentford, U.K.). HPLC-grade acetonitrile was from Baker (Phillipsburg, NJ). Bromochloroindolylphosphate (BCIP) and nitroblue tetrazolium (NBT) were from Bio-Rad (Segrate, Italy). Sequencer grade reagents were from Applied Biosystems (Foster City, CA). Chromatographic columns PD-10, Mono-Q HR 10/10, and phenyl-Superose HR 5/5 were from Amersham-Pharmacia (Uppsala, Sweden). All other reagents were of the highest commercially available quality.

Kiwi Fruit Protein Extracts. Ripe kiwi fruit were collected at the end of October and, after peeling, homogenized in water. After centrifugation at 10400*g* for 20 min, the supernatant, representing the soluble fraction (*15*), was collected for further analysis. The pellet, containing the cell wall fraction, was resuspended in 0.5 M NaCl and homogenized again. After centrifugation, the supernatant (cell wall salt extract) (*15*) was collected. A total protein extract was obtained by homogenizing kiwi fruit in 0.5 M NaCl and collecting the supernatant obtained after centrifugation.

SDS-PAGE and Electroblotting. Extracts and purified proteins were subjected to reducing 15% SDS-PAGE. Following electrophoresis, proteins were either stained with Coomassie Brilliant Blue or transferred onto PVDF membranes. For N-terminal amino acid sequence analysis, transferred proteins were stained with Coomassie Brilliant Blue, and the protein bands, after excision, were subjected to automated Edman degradation. For immunoblotting analysis, the transferred proteins were stained with ponceau red.

IgE Immunoblot. Serum samples were obtained after informed written consent from kiwi-allergic subjects on the basis of the Institutional ethical committee approval of the study protocol. Kiwi allergy was established on the basis of suspected clinical history or positive skin prick test (wheal diameter of >3 mm) with a commercial extract or by the prick−prick test technique using the fresh fruit and with positive IgE detection on CAP system (>0.35 kUa/l, following the manufacturer's instructions, Phadia, Uppsala, Sweden). IgE immunoblotting was carried out by using the blocking solution and goat anti-human IgE contained in the Enzallergy Specific E kit (Bioallergy, Fiumicino, Italy).

After a washing with the blocking solution, membranes were incubated with primary antibodies (1:4 dilutions of sera of kiwi allergic subjects) for 3 h. IgE−allergen complex was detected by incubation with secondary antibody, that is, a 1:20 dilution of goat anti-human IgE conjugated to alkaline phosphatase, for 18 h, followed by incubation with BCIP/NBT solution prepared according to the manufacturer. Nonspecific binding of the anti-IgE antibody conjugate was estimated in a similar blotting procedure, omitting the incubation step with patient serum.

Kiwellin and Kissper Purification. Kiwellin was purified as described by Tamburrini et al. (*15*) from GrK and GoK cell wall protein extracts.

The peptide kissper, the primary structure of which corresponds to the N-terminal region of kiwellin (residues 1–39), was purified from ripe GrK as described by Ciardiello et al. (*16*). Attempts to detect kissper in GoK were unsuccessful.

KiTH Purification. The GrK soluble fraction, dialyzed against 10 mM Tris-HCl, pH 8.0, was loaded on a DE52 column (2.5 × 18 cm), equilibrated in the same buffer. The column was eluted with 0.5 M NaCl in the equilibrating buffer, and aliquots of the collected fractions were analyzed by reverse-phase HPLC. The fractions containing KiTH were dialyzed against 10 mM Tris-HCl, pH 8.0, and then loaded on a Mono-Q HR 10/10 column equilibrated in the same buffer. The column was eluted by a linear gradient from 0 to 0.3 M NaCl. The fractions from Mono-Q were analyzed by reverse-phase HPLC, and those containing KiTH were pooled and loaded on a phenyl-Superose HR 5/5 column, equilibrated in buffer A (50 mM sodium phosphate buffer, pH 7.0, containing 1.7 M ammonium sulfate). The elution was carried out by a linear gradient from 0 to 100% buffer B (50 mM sodium phosphate, pH 7.0).

Protein concentrations were determined by the Bio-Rad Protein Assay, using calibration curves made with bovine serum albumin. When needed, protein samples were concentrated by ultrafiltration using Centriplus YM-3 filters (Amicon, Millipore, Bedford, MA).

Amino Acid Sequencing of KiTH. Denaturation and alkylation were carried out by dissolving the peptide at a concentration of 2 mg/mL in 0.5 M Tris-HCl (pH 7.8) containing 2 mM EDTA and 6 M guanidine hydrochloride. Dithiothreitol (10-fold molar excess over the thiol groups of the protein) was added under nitrogen atmosphere, and the solution was kept at 37 °C for 1 h. 4-Vinylpyridine (5-fold molar excess over the total thiols) was then added under nitrogen atmosphere, and the mixture was kept in the dark at room temperature for 30 min. At the end of the reaction, excess reagents were removed by gel filtration on a PD-10 column equilibrated with 0.1% trifluoroacetic acid (TFA).

Tryptic digestions on pyridylethylated samples (0.5 mg/mL) were carried out at an enzyme/protein ratio of 1:20 (w/w) in 1% ammonium bicarbonate at 37 °C for 2 h.

Reverse-phase HPLC of kiwi extracts and of peptides derived from chemical or enzymatic cleavage was performed on a Vydac C_8 column (0.21 × 25 cm), using a Beckman (Fullerton, CA) System Gold apparatus. Elution was accomplished by a multistep linear gradient of eluant B (0.08% TFA in acetonitrile) in eluant A (0.1% TFA) at a flow rate of 1 mL/min. The eluate was monitored at 220 and 280 nm. The chromatographic peaks were manually collected, vacuum-dried, and redissolved in 0.1% trifluoroacetic acid containing 20% acetonitrile, and, after mass spectrometry analysis, they were sequenced.

Amino acid sequencing was performed with an Applied Biosystems Procise 492 automatic sequencer (Applied Biosystems, Foster City, CA), equipped with online detection of phenylthiohydantoin amino acids. Protein sequence analyses were performed using software available on the ExPASy Proteomics Server (www.expasy.org).

MALDI-TOF mass spectrometry was carried out on a PerSeptive Biosystems (Framingham, MA) Voyager-DE Biospectrometry Workstation. Analyses were performed on premixed solutions prepared by diluting samples (final concentration = 5 pmol/μL) in 4 volumes of matrix, namely, 10 mg/mL α-cyano-4-hydroxycinnamic acid in 60% acetonitrile containing 0.3% TFA.

Purification of Actinidin. Actinidin was purified from the soluble fraction of GrK fruit by ion exchange chromatography on DE-52 and Mono-Q columns, following the experimental conditions described for the purification of KiTH. The actinidin-containing fractions were identified by SDS-PAGE and N-terminal amino acid sequencing.

Proteolysis of Kiwellin by Actinidin. Purified GrK and GoK kiwellin was incubated with GrK actinidin at an enzyme/protein ratio of 1:4 (w/w), at 37 °C, in 0.1 M sodium phosphate buffer (pH 6.0) containing 25 mM β-mercaptoethanol. The kiwellin concentration in the incubation mixture was 1 mg/mL. At the beginning ($t = 0$) and at the end ($t = 6$ h) of the incubation, aliquots were removed and either diluted with loading buffer for SDS-PAGE analysis or analyzed by reverse phase HPLC.

Glycosylation Analysis. Glycosylation analysis was carried out by the DIG Glycan Detection Kit (Roche Diagnostics, Indianapolis, IN), following the manufacturer's instructions.

RESULTS

Kiwi Extract Analysis. SDS-PAGE analysis of GrK and GoK total extracts revealed significant differences in the protein pattern of the two species (**Figure 1**). Electroblotting onto PVDF membrane and N-terminal amino acid sequencing allowed the identification of the already known GrK proteins actinidin

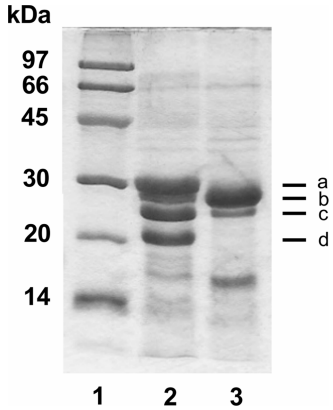

Figure 1. SDS-PAGE of green (lane 2) and gold (lane 3) kiwi fruit total extracts. Lane 1 represents molecular weight markers. Actinidin (**a**), kiwellin (**b**), TLP (**c**) and KiTH (**d**) were identified by N-terminal amino acid sequencing.

(LPSYVDWRSA), kiwellin, and thaumatin-like protein (TLP) (ATFNIINN), showing 30, 28, and 24 kDa bands, respectively.

A further major component, giving a band at 20 kDa upon SDS-PAGE, was also observed in the GrK extract. N-Terminal sequencing of this electroblotted protein produced 15 residues of a double sequence. Analysis of both sequences revealed 100% identity with the region starting either from the residue T^{40} or from the residue S^{38} of kiwellin (**Figure 2**). The yields of the former and latter sequences were 76 and 24%, respectively. The 20 kDa component was named KiTH, where "Ki" are the first two initials of kiwi, and "TH" are the two first amino acid residues of the most abundant form of this protein found in GrK.

N-Terminal sequencing of the GoK 28 kDa band allowed the identification of kiwellin (ISSCNGPCRD) as the major protein component of this species. Furthermore, sequence analysis of this large band revealed the presence of actinidin (LPSYVDWRSA) as a comigrating minor component producing a 30-fold less abundant sequence and showing a molecular mass very similar, if not identical, to that of GrK actinidin (30 kDa). TLP was also detected at a lower extent with respect to GrK. In GoK, a further protein band of about 17 kDa was observed. N-Terminal amino acid sequencing of the electroblotted band did not produce any sequence, suggesting a blocked N terminus and thus preventing its identification.

Protein Purification. Following the experimental procedures described under Materials and Methods, GrK kiwellin, GoK kiwellin, KiTH, and kissper appeared to be pure upon SDS-PAGE and amino acid sequencing analysis.

The estimated amounts of KiTH and kissper were approximately 4 and 1 mg (both corresponding to approximately 0.25 μmol) per 100 g of fruit, respectively. Therefore, it appears that the two polypeptides are present in ripe GrK in similar molar amounts.

Amino Acid Sequencing. Twenty residues of the N-terminal region of purified GoK kiwellin were elucidated by automated Edman degradation. Comparative analysis of the sequence showed 100% identity with the corresponding region of GrK kiwellin.

N-Terminal amino acid sequencing of purified KiTH proceeded for 25 residues and showed a double sequence, one of which lacked the first two residues. The tryptic hydrolysate of KiTH was analyzed by reverse-phase HPLC (data not shown), and the purified peptides were sequenced. The complete sequence was established by alignment of the tryptic fragments with the amino acid sequence of kiwellin. KiTH contained 150 amino acid residues (**Figure 2**), and the sequence-derived molecular mass was 15.835 kDa, which is lower than that shown under reducing SDS-PAGE (20 kDa) but in agreement with the value (16 kDa) obtained upon SDS-PAGE carried out under nonreducing conditions (data not shown). Moreover, glycosylation analysis did not show the presence of glycans linked to KiTH.

Sequence Analysis. Analysis of the primary structure of KiTH showed 100% identity with the C-terminal region of kiwellin corresponding to either the residues 40–189 or the residues 42–189 (**Figure 2**). Therefore, KiTH may derive from the hydrolysis of the peptide bond either between T^{39} and T^{40} (cleavage site 1) or between H^{41} and S^{42} (cleavage site 2) of kiwellin (**Table 1**).

A homology search in Data Bank carried out by FASTA3 showed that both KiTH and kissper display high identity with Grip 22 (*17*) from grape and the putative kiwellin from potato. **Figure 2** shows that kissper and KiTH can be aligned with the N- and C-terminal regions, respectively, of both the homologous putative proteins. The pattern of the 14 cysteine residues of kiwellin is highly conserved in the homologous sequences aligned in **Figure 2**. The high identity of GrK kiwellin shared with the proteins from potato and grape (>70%) decreases in the region bearing the sites of proteolytic processing by actinidin.

However, unlike kissper, KiTH displays high identity (>50%) with a group of genome-derived sequences of hypothetical proteins of unknown function from *Oryza sativa* and *Medicago truncatula* (**Figure 2**). Alignment highlights that, despite the high conservation of the region corresponding to KiTH, the upstream region, corresponding to kissper, is not conserved and, at least partially, lacking.

In Vitro Proteolytic Processing of Kiwellin by Actinidin. Purified GrK and GoK kiwellins were incubated with the protease actinidin from GrK. Upon SDS-PAGE analysis (**Figure 3**), kiwellin samples showed a double band, suggesting the presence of isoforms. Among the digestion products obtained after 6 h of incubation, a band at 20 kDa, lacking in the controls, appeared. Electroblotting onto PVDF membrane and N-terminal amino acid sequencing of the first 10 residues of the 20 kDa band produced three sequences, allowing the identification of the cleavage sites and the estimation of the relative abundance of the digestion products (see **Table 1**). Both KiTH forms identified in GrK extracts, deriving from cleavages at sites 1 and 2, were detected. Furthermore, a third form of KiTH, showing two additional residues at the N terminus and deriving from the hydrolysis of the peptide bond between R^{37} and G^{38} (cleavage site 3), was detected.

A second aliquot of the digestion products was analyzed by reverse-phase HPLC. The chromatographic peaks were manually collected and analyzed by N-terminal amino acid sequencing. On the basis of the elution times from HPLC and the obtained sequences, three forms of kissper were detected (data not shown), identified as the expected products of the proteolytic processing of GrK and GoK kiwellins by actinidin.

IgE-Binding Assay. Sera of patients allergic to kiwi fruit were tested to select those showing reactivity to kiwellin. Several nonreacting sera were detected and used as negative controls (not shown). Six individual sera containing IgE specific to GrK kiwellin were identified and used to probe KiTH and GoK kiwellin in IgE immunoblot experiments. All of the selected sera recognized kiwellin from GoK (**Figure 4B**) and KiTH from GrK (**Figure 4A**). Comparative analysis of immunoblot results showed very similar reactivities of the kiwellin samples from the two species, whereas serum 2 (**Figure 4A**) showed higher

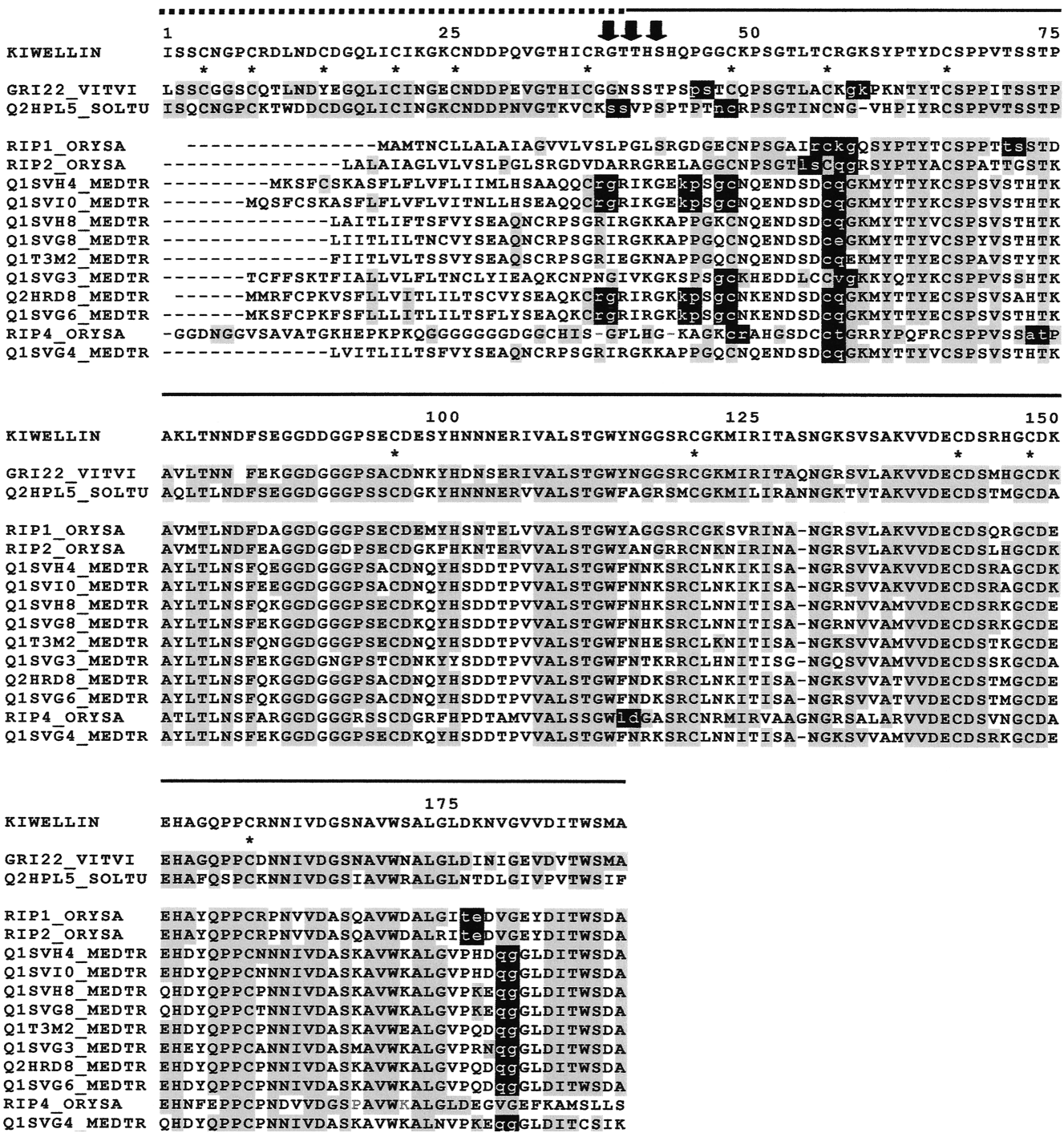

Figure 2. Alignment of the amino acid sequence of kiwellin from green kiwi fruit (*A. deliciosa*) with those of homologous putative proteins from grape (GRI22), potato (Q2HPL5), *O. sativa* (RIP1, RIP2, RIP4), and *M. truncatula* (Q1SVH4, Q1SVI0, Q1SVH8, Q1SVG8, Q1T3M2, Q1SVG3, Q2HRD8, Q1SVG6, Q1SVG4). Residues conserved in the kiwellin sequence are gray-shaded. Dark shaded lower case letters indicate the boundary amino acid residues of gapped regions introduced by the Fasta3 software (http://www.expasy.org/) into the query sequence and excised from both query and hit sequences to ensure a contiguous query string. Dotted and continuous lines indicate the regions corresponding to kissper and KiTH, respectively. Asterisks and arrows indicate the kiwellin cysteine residues and the sites cleaved by actinidin, respectively.

reactivity with kiwellin and lower reactivity with KiTH and serum 5 (**Figure 4A**) showed an opposite behavior.

DISCUSSION

Kiwellin was first identified in GrK and described as an allergen and one of the major protein components of this fruit (*15*). Kiwellin has now been identified in the GoK species, where it appears as the most abundant protein component. Actinidin, the presence of which in GoK was previously suggested on the basis of immunochemical data (*11*), has been identified by N-terminal amino acid sequencing. In line with the results reported by Nieuwenhuizen et al. (*18*), actinidin was detected in much lower amount in GoK than in GrK.

Another abundant protein component of ripe GrK fruit extract, not observed in the unripe fruit (data not shown) or in the GoK species, was detected on SDS-PAGE as a 20 kDa band. Elucidation and analysis of the primary structure of the purified component, named KiTH, revealed 100% amino acid sequence

Table 1. Forms of KiTH Found in Kiwi Extracts and in Vitro after Digestion of Kiwellin by Actinidin

source		N-terminal amino acid sequence	cleavage site[a]	abundance (%)
GrK	in vivo	THSHQPGGCK	1	76
		SHQPGGCKPS	2	24
	in vitro	THSHQPGGCK	1	12
		SHQPGGCKPS	2	12
		GTTHSHQPGG	3	76
GoK	in vivo	none	none	
	in vitro	THSHQPGGCK	1	27
		SHQPGGCKPS	2	15
		GTTHSHQPGG	3	58

[a] Site 1, $T^{39}T^{40}$; site 2, $H^{41}S^{42}$; site 3, $R^{37}G^{38}$.

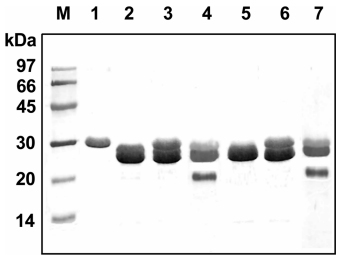

Figure 3. SDS-PAGE analysis of kiwellin from GrK (lanes 3 and 4) and GoK (lanes 6 and 7) after 0 and 6 h, respectively, of incubation with actinidin. Controls were actinidin without kiwellin (lane 1) and kiwellin from GrK (lane 2) and GoK (lane 5) without actinidin. The amounts of actinidin and kiwellin were 2.5 and 10 μg, respectively.

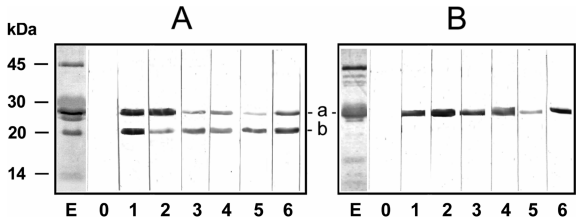

Figure 4. IgE immunoblot of kiwellin (a) and KiTH (b) from GrK (**A**) and of kiwellin (a) from GoK (**B**), probed with six individual sera (lanes 1–6) and with buffer as a negative control for the antihuman IgE antibody (lane 0). The proteins were purified from the natural source. IgE binding to total extracts of GrK (**A**) and GoK (**B**) was probed with a pool of the six individual sera (lane E).

identity with the C-terminal region (residues 40–189) of kiwellin. Recently, a pore-forming peptide, named kissper, showing 100% identity with the N-terminal region (residues 1–39) of kiwellin, was identified in GrK (*16*). The isolation in GrK extracts of KiTH and kissper in approximately stoichiometric amounts suggested that they both were produced following a proteolytic cleavage of kiwellin. KiTH and kissper were not detected in GoK extracts, although we cannot exclude the presence of very low, undetectable amounts. The observation that their presence in GrK was correlated with a high amount of actinidin suggested a possible involvement of this protease (*19, 20*) in their generation. Experimental data here described demonstrate that KiTH and kissper are actually produced following the in vitro proteolytic processing of purified GrK and GoK kiwellin by GrK actinidin. Both KiTH forms found in the GrK extracts, deriving from in vivo cleavage between T^{39} and T^{40} (site 1) and between H^{41} and S^{42} (site 2) of kiwellin, were also identified as products of in vitro digestion by actinidin. A third form of KiTH, showing two additional residues at the N terminus, was obtained after in vitro enzymatic cleavage of kiwellin, suggesting a significant effect of the enviromental conditions on the specificity of the proteolytic action. Furthermore, the results obtained suggest that the environmental conditions may also affect the ratio of the digestion products.

Purified GoK kiwellin was cleaved in vitro by GrK actinidin originating in a similar ratio as the same products obtained from GrK kiwellin. Therefore, the apparent absence of KiTH and kissper in GoK extracts can be correlated to the low level of the protease actinidin, rather than to different structural features of GoK kiwellin. Alignment of kiwellin with homologous sequences from other sources shows that the region containing the three peptide bonds cleaved by actinidin is the less conserved. It is conceivable that low structural constraints and high molecular flexibility may characterize this region, making it sensitive to proteolytic cleavages by actinidin. Treatment of kiwellin and KiTH with the proteases pepsin and trypsin produced very complex hydrolysates with high levels of fragmentation detected upon SDS-PAGE (data not shown), whereas kissper was resistant to proteolysis (*16*). Moreover, HPLC analysis of the reaction products showed that treatment of kiwellin did not produce KiTH or kissper (data not shown). Unlike pepsin and trypsin, actinidin does not produce degradation of kiwellin, but specifically cuts peptide bonds leading to the separation of the two protein domains kissper and KiTH.

Although actinidin was identified more than 40 years ago as a thiol proteinase (*21*), kiwellin is the first natural substrate, from the same biological source, to be identified for this enzyme. In fact, like most of the known proteases (*22*) (http://merops.sanger.ac.uk), the only information available so far on the actinidin activity and specificity derives from studies performed on synthetic substrates. The characterization of protease specificity on natural substrates appears as an important goal for the understanding of biochemical processes occurring in biological systems as well as for application purposes, such as the preparation, under controlled conditions for the protease activity, of standardized allergen extracts or extract mixtures with known and reproducible composition, potency, and stability (*23*).

Analysis of the KiTH primary structure, carried out by homology search, showed significant identity with hypothetical proteins of unknown function from grape (*17*), potato, *O. sativa*, and *M. truncatula*. Kissper also showed high sequence identity with the grape and potato homologous proteins, but not with the group of *O. sativa* and *M. truncatula* hypothetical sequences, which lack the region corresponding to this peptide. On the other hand, unlike KiTH, kissper has some structural and functional features in common with the pore-forming peptides (*16*), including thionins and defensins, displaying the so-called EGF-like cysteine-rich motif, which are involved in defense mechanisms against pathogens. It could be suggested that kissper and KiTH are two independent protein modules, fused together into kiwellin, which can be separated following in vivo or in vitro processing by actinidin.

Similar to kiwellin from GrK (*15*), the homologous protein from GoK displays IgE-binding capacity. Both proteins were detected by the same sera, and even the levels of the signal on Western blotting were comparable, thus suggesting conservation of the IgE-binding epitopes. All of the sera also detected KiTH. However, one of them recognized KiTH much better than kiwellin, whereas another one did the opposite. These results suggest that (i) kiwellin may have a hidden IgE-binding epitope that becomes available in KiTH, following the removal of kissper, and (ii) kissper might be an IgE-binding epitope by itself. Further analyses are needed to explain the immunological

relationships between the newly identified components and clinical features of kiwi allergy and to define whether processing might enhance the sensitizing and triggering capabilities of kiwellin in such clinical conditions.

Different patterns of proteins and IgE-binding bands upon SDS-PAGE and immunoblotting analysis of protein extracts have been reported for kiwi fruit (*9, 10, 14, 24, 25*). This could be at least partially ascribed to proteolytic events that, beyond gene expression variability, may provide a significant contribution to proteome formation and, therefore, to the composition of the extracts. In this framework, the results reported in this study contribute to the solution of the protein and allergen puzzle of kiwi fruit.

ABBREVIATIONS USED

BCIP, bromochloroindolylphosphate; GrK, green kiwi fruit; GoK, gold kiwi fruit; NBT, nitroblue tetrazolium; TFA, trifluoroacetic acid; TLP, thaumatin-like protein.

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