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Physico-chemical features of the environment affect the protein conformation and the immunoglobulin E reactivity of kiwellin (Act d 5)

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Summary

Background Allergy diagnostic systems sometimes give false positive or negative results. In this respect, the influence of protein conformational changes on the allergen-IgE interaction sites is worthy to be investigated.

Objective To investigate the influence of different experimental conditions on the structural properties and IgE reactivity of kiwellin (Act d 5) as a model system.

Methods Act d 5 was purified from the natural source. To study its conformational features, experiments of circular dichroism (CD) in different media were performed. The IgE reactivity was investigated by skin testing, immunoblotting and ISAC microarray system, in a population of kiwifruit allergic subjects.

Results CD experiments indicated that Act d 5 has a mainly helical structure and the conformation is strongly affected by the experimental conditions. The protein is more structured in low polarity media and at acidic pH values, similar to those of the natural source. Eleven subjects of 29 (38%) allergic to kiwifruit were positive to purified natural Act d 5 by skin test. Among them, three patients (10%) showed a reaction only to Act d 5 at pH 4.5, and three (10%) showed a reaction only to the allergen in standard neutral conditions. No one of the 11 subjects with positive skin test recognized Act d 5 immobilized on the ISAC system. Eight of nine subjects detected Act d 5 by IgE immunoblotting. One subject did not recognize the sequence epitopes of Act d 5 in IgE immunoblotting experiments and reacted to the skin test only when the allergen was in acidic conditions.

Conclusions and Clinical Relevance The conformation and IgE reactivity of Act d 5 are affected by the physico-chemical characteristics of the solvent. These findings suggest that the assay conditions influence the results of the diagnostic systems by modulating the pattern of exposed antigenic epitopes.

Keywords Act d 5, circular dichroism, conformation, kiwellin, kiwifruit, pH, skin prick test

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Introduction

Several *in vitro* and *in vivo* test systems are available and currently used to investigate the cause of individual

allergic reactions. Usually, data from more than one test system are comparatively evaluated in the attempt to reach a reliable diagnosis [1]. False positive and negative responses are frequently observed and can be due to many causes that need to be investigated to improve the predictive value of diagnostic tests [1–3]. Discrepancies observed among tests based on allergen extracts are very often attributed to the variability of extract composition [4–8]. Although the difficulties to obtain standardized extract composition may affect the diagnostic test responses, false positive and negative results can be observed even with purified allergenic proteins.

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Data from extensive IgE testing using the ISAC 103 system have been obtained following manuscript submission. Among 19,699 tests, 105 (0.53%) were IgE positive for Act d 5, ranging between 0.1 and 24.97 kU/l. Unless very low in prevalence, the data suggest the presence of epitopes that are recognized also in the current conformation on the chip.

Kiwellin is a cystein-rich protein identified few years ago as a major protein component of green kiwi fruit (*Actinidia deliciosa*) [9]. It has been described as an IgE-binding protein [9, 10] and named Act d 5 by the Allergen Nomenclature Sub-committee (<http://www.allergen.org>) of the International Union of Immunological Societies (IUIS). It has a molecular weight of 20 kDa, calculated on the basis of the amino acid sequence and confirmed by mass spectrometry analysis, although in SDS-PAGE it shows a band at approximately 28 kDa [9].

Structural properties and experimental evidence suggest that Act d 5 is a modular protein with two domains [11]. It may undergo *in vivo* and *in vitro* proteolytic processing by kiwi fruit actinidin (Act d 1), thus producing KiTH and kissper. The former is a 16 kDa protein, showing a 20 kDa band upon reducing SDS-PAGE and corresponding to the C-terminal region of Act d 5; the latter is a 4 kDa peptide, corresponding to the N-terminal region of Act d 5, showing pH-dependent and voltage-gated pore-forming activity in model synthetic planar lipid membranes [12].

Serological tests showed that Act d 5 is specifically recognized by IgE of some patients allergic to kiwi fruit. The first study performed to investigate the allergenic properties of Act d 5 involved two patients reporting significant symptoms upon ingestion of kiwi [9]. One of them needed hospital care after kiwi fruit ingestion; however, he showed a negative skin prick test (SPT) performed with commercial kiwi extract. In contrast, serological tests demonstrated that the serum of that subject contained specific IgE for Act d 5, as evaluated by performing the CARLA analysis (Radim SpA, Pomezia, Roma) with the purified and biotinylated kiwi protein, and IgE immunoblotting analysis confirmed this result [9]. Interestingly, the analysis of the protein composition of the commercial extract producing the negative SPT response revealed the presence of significant amounts of Act d 5 (unpublished data). Therefore, although the subject had IgE specific for Act d 5 and this protein was present in the test system, nevertheless Act d 5 was not able to produce a positive SPT. This observation suggested the hypothesis that the interaction between Act d 5 and specific IgE antibodies could be induced or impaired depending on the structural changes of the allergen induced by the specific conditions of the test system. That is, different environmental conditions could induce conformational changes associated with a variation in the epitope exposition of the allergenic protein.

To deal with this hypothesis, a study of the Act d 5 behaviour in different experimental conditions and allergy test systems has been performed. In this paper, a screening of the kiwellin conformational features has been carried out by circular dichroism (CD) in different media, and the clinical and IgE-binding properties have

been investigated by SPT, immunoblotting experiments and ISAC system microarrayed allergens (VBC-Genomics, Vienna, Austria).

Materials and methods

Preparation of protein extract and solutions of purified Act d 5 for skin prick and in vitro tests

A total protein extract was obtained by homogenizing the pulp of ripe green kiwi fruit (*A. deliciosa*) after addition of 1.0 M NaCl in deionized water at a 1 : 1 (w : w) ratio, and collecting the supernatant obtained after centrifugation at 10 400 g for 20 min. The extract was dialyzed against water, aliquoted and stored at -20°C until required. The pH of the kiwi fruit homogenate and of the dialyzed solution used for SPT was 3.5 ± 0.02 . Protein concentration was determined by the BIO-RAD Protein Assay (Biorad, Milan, Italy), using calibration curves made with bovine serum albumin.

Kiwellin was purified following a previously developed protocol [9]. The purity of protein preparation was estimated to be >98% by SDS-PAGE electrophoresis, RP-HPLC and N-terminal amino acid sequencing. Purified protein was first dialyzed against NaCl 0.2 M and then against water. Then, the protein was lyophilized, aliquoted and stored at -20°C until required.

For SPT, (i) the extract solution was mixed with sterile glycerine in a ratio of 1 : 1 and the final protein concentration was 1 mg/mL; (ii) aliquots of lyophilized Act d 5 were solubilized in water brought to pH 7.4 by the addition of NaOH, and then mixed with sterile glycerine in a ratio of 1 : 1, and the final protein concentration was 0.5 mg/mL. Acidified solutions of kiwellin were obtained by solubilizing the protein in water brought to pH 4.5 by the addition of HCl, and the final protein concentration was 0.5 mg/mL. The value of pH 4.5 was chosen in order to test conditions as much similar to those of kiwi fruit as possible, trying to avoid too acidic pHs that could discomfort patients, whereas pH 7.4 was chosen as a reference condition, as commercial preparations for SPT have neutral pH values. Solutions for SPT were sterilized by membrane filtration through 0.22 μm filters (Millex, Millipore, Bedford, MA, USA), in a sterile horizontal laminar flow hood.

Study population, skin prick test and sera

The protocol of the present study was approved by the Institutional Review Board (IDI/CE 2006/66). All patients gave written informed consent before enrolment in the study.

Twenty-nine patients were selected on the basis of (i) reported tight relationship between kiwi fruit ingestion and symptoms onset, (ii) previous Emergency Room Intervention (documented by a paper report) or use of

rescue medication (steroids and antihistamine) following the symptoms onset, (iii) overall patient reliability and (iv) follow-up since years at our hospital.

All the patients included in the study were evaluated by SPT with (i) a kiwi commercial diagnostic extract (Staller-genes, Antony, France), (ii) an in-house produced solution of green kiwi extract [13], (iii) Act d 5 at neutral pH and (iv) Act d 5 in acidic solution.

SPT was performed on the volar surface of the distal forearm by using standardized lancets. Patients were asked to avoid antihistamine intake for at least 7 days before testing. Test evaluation was performed after 15 min. Weal areas, transferred on a record sheet by means of a transparent adhesive tape, were calculated by means of computer-aided design software (Autocad 22; Autodesk Inc., San Rafael, CA, USA). Only patients having a negative test with saline, and a positive result with histamine were tested. All SPT were performed in duplicate and the widest surface of the two test areas was considered [14].

IgE to kiwi extract was detected by using the CAP system (Phadia, Uppsala, Sweden). IgE values corresponding or higher than 0.35 kUA/L were considered positive.

The patients were also tested using an allergen micro-array system (ISAC CRD 89b, VBC-Genomics) [15, 16] to measure specific IgE to Act d 5 and to Act d 1 used as a control. IgE values > 0.03 kUA/L were considered positive.

Following the SPT, sera were obtained from patients who consented to blood sampling for *in vitro* diagnostic tests, and stored at -20°C until required.

Immunoglobulin E immunoblotting experiments

Purified Act d 5 was subjected to reducing or non-reducing (i.e. with or without β -mercaptoethanol, respectively) 15% SDS-PAGE, transferred onto PVDF membrane and stained with ponceau red.

Serum samples of patients that had shown a positive SPT were selected and used in immunoblotting experiments. IgE immunoblotting was carried out by using the blocking solution and goat anti-human IgE of the Enzallergy Specific E kit (Bio Genetix, Fiumicino, Italy). After washing with the blocking solution, membranes were incubated with primary antibodies (1 : 4 dilutions of sera) for 3 h. IgE-allergen complex was detected by incubation with secondary antibody, i.e. 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's instructions. Non-specific binding of the anti-IgE antibody conjugate was estimated in a similar blotting procedure omitting the incubation step with patient serum.

Circular dichroism studies

All CD spectra were recorded on a JASCO J-810 spectropolarimeter (Easton, MD, USA) using a quartz cell of 0.1 cm path length. Spectra were recorded over the wavelength

range 260–190 nm with a bandwidth of 2.0 nm and a time constant of 8.0 s. The temperature was 25°C . Spectra were baseline corrected for the contribution of the solvent. Unless otherwise indicated, a protein concentration of 0.15 mg/mL was used thoroughly. CD spectra were acquired in pure water and in mixtures containing two different fluorinated alcohols, hexafluoroisopropanol (HFIP) and 2,2,2-trifluoroethanol (TFE), corresponding to 20/80 and 80/20 (v/v) water/alcohol composition. To attain the same protein concentration in all samples, two stock protein solutions of equal concentration were prepared and adjusted at either pH 4.5 or pH 7.5 with HCl or NaOH, respectively. Identical aliquots of these stock solutions were diluted by the addition of the same volume of water or preformed mixtures of water with the appropriate amount of fluorinated alcohol.

Results

Skin testing with kiwifruit extracts and purified Act d 5

Twenty-nine patients were selected on the basis of a reliable history of allergic reactions to kiwi fruit. They were tested with both commercial and in-house green kiwi extract, and with Act d 5 in a neutral solution (pH 7.4), and in acidic water solution (pH 4.5) (Tables 1a and b).

Eleven subjects of 29 (38%) had a positive SPT to purified Act d 5. Among them, three patients (10%) showed a reaction only to Act d 5 at pH 4.5, three (10%) showed a reaction only to the allergen in neutral solutions and five (17%) showed a reaction to Act d 5 solubilized in both conditions. Some features of the 11 subjects that gave a positive SPT, in at least one condition, are listed in Table 1a. This population includes four males and seven females. The average age was 34.5 (from 13 to 57). Six patients reported oral symptoms (O), one patient oedema and oral symptoms (E, O) and one patient vomiting (V). Three patients had asthma (A): one together with atopic dermatitis (D), one with skin itching (P) and another with urticaria (U).

All the 11 subjects with a positive SPT to purified Act d 5, were tested with both commercial and in-house kiwi extract by SPT. Two of them (7%) had no reaction to the commercial extract, whereas all patients reacted to the in-house one.

Table 1b reports clinical and specific IgE data of the 18 subjects allergic to kiwi fruit but showing negative SPT to kiwellin.

Immunoglobulin E binding experiments

The sera of the 11 subjects with positive SPT were also tested on Act d 5 immobilized on the ISAC system. As shown in Table 1a, none of the tested sera recognized the

Table 1a. Clinical and specific IgE data of the study population showing positive SPT to kiwellin

Patient	Gender	Age	Symptoms with kiwi	Specific IgE (CAP)	Specific IgE (ISAC)	Specific IgE (ISAC)	SPT (mm ²)					Other food allergies (ISAC)
				Kiwi extract	Act d 1	Act d 5	Commercial GrK extract	In-house GrK extract	Prick by prick GrK	Act d 5 pH 7.4	Act d 5 pH 4.5	
1	M	15	A,D	2.05	1.37	0	+	++++	++++	NEG	+	Pru p 3
2	F	23	O	51.7	19.58	0	+	+++	++	NEG	+	Bet v 1
3	M	13	V	3.41	0.73	0	++	+++	+++	+	NEG	Bet v 1
4	F	53	O	0.05	0	0	++	+++	+++	NEG	+	NEG
5	M	25	A,P	0.61	0	0	++	+++	+++	++	+	Pru p 3
6	F	33	O	0.04	0	0	+	+	NEG	+/-	NEG	NEG
7	F	32	O	0.27	0	0	NEG	+	ND	+/-	NEG	NEG
8	F	55	A,U	0.96	0	0	++	+++	+++	+	+	NEG
9	F	34	O	3.34	1.13	0	+	+++	++	+	+	NEG
10	F	50	O	ND	0.26	0	NEG	+++	++	++	+	NEG
11	M	57	E,O	4.05	2.35	0	+++	+++	+++	+	+	NEG

A, Asthma; An, anaphylaxis; Ap, abdominal pain; D, atopic dermatitis; E, oedema; O, oral symptoms; P, skin itching; U, urticaria; V, vomiting; ND, not done; GrK, green kiwi extract; CAP, ImmunoCAP (Phadia, Uppsala, Sweden); ISAC, (VBC-Genomics, Vienna, Austria); NEG, negative.

Table 1b. Clinical and specific IgE data of the study population showing negative SPT to kiwellin

Patient	Gender	Age	Symptoms with kiwi	Specific IgE (CAP)	Specific IgE (ISAC)	Specific IgE (ISAC)	SPT (mm ²)					Other food allergies (ISAC)
				Kiwi extract	Act d 1	Act d 5	Commercial GrK extract	In-house GrK extract	Prick by prick GrK	Act d 5 pH 7.4	Act d 5 pH 4.5	
1	F	30	O	0,01	0	0	0	0	0	NEG	NEG	NEG
2	F	56	E,U	0,66	0	0	+	+	+	NEG	NEG	NEG
3	F	26	O	7	0	0	++	++	++	NEG	NEG	Profilin, Pru p3
4	F	31	O,P	0	0	0	++	++	++	NEG	NEG	Pru p 3
5	F	44	O	0,01	0	0	0	0	0	NEG	NEG	NEG
6	F	20	O	ND	0	0	0	0	0	NEG	NEG	NEG
7	F	35	O	0,01	0	0	0	0	0	NEG	NEG	Pru p 3
8	F	35	O	1,24	0	0	++	+++	+++	NEG	NEG	Pru p 3
9	M	28	O	0,59	0	0	0	0	0	NEG	NEG	Act d 2
10	F	24	O	ND	0	0	0	0	0	NEG	NEG	NEG
11	F	32	A,O	ND	0	0	0	0	++	NEG	NEG	Profilin
12	M	20	An	6,24	0	0	+	++	++	NEG	NEG	Act d 2
13	M	14	O	ND	0	0	0	0	0	NEG	NEG	NEG
14	F	29	Ap	1,02	0	0	0	+	++	NEG	NEG	Pru p 3
15	F	41	A,P	ND	0	0	0	0	+	NEG	NEG	NEG
16	M	27	O	ND	0	0	+	++	+	NEG	NEG	Pru p 3
17	F	25	O	ND	0	0	0	0	0	NEG	NEG	NEG
18	F	40	E,P	ND	0	0	+	++	+	NEG	NEG	Pru p 3

A, Asthma; An, anaphylaxis; Ap, abdominal pain (Ap); D, atopic dermatitis; E, oedema O, oral symptoms; P, skin itching; U, urticaria; V, vomiting; ND, not done; GrK, green kiwi extract; CAP, ImmunoCAP (Phadia, Uppsala, Sweden); ISAC, (VBC-Genomics, Vienna, Austria); NEG, negative.

immobilized Act d 5, whereas 6 of them reacted with immobilized actinidin, used as a control.

Ten patients out of 11 were tested for specific IgE to green kiwi extract using the CAP system, and seven of them showed elevated levels (Table 1a).

Nine of the 11 sera that had shown a positive reaction to SPT were used to test Act d 5 by IgE immunoblotting

experiments after reducing or non-reducing SDS-PAGE (Fig. 1). Eight of nine sera contained specific IgE recognizing Act d 5 from both reducing and non-reducing conditions. Nevertheless, a variation of the signal intensities was observed, and generally the signals shown by the non-reduced Act d 5 were weaker than those observed for reduced Act d 5.

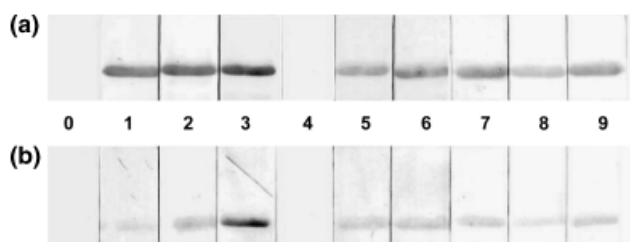


Fig. 1. IgE immunoblotting of Act d 5 after reducing (a) and non-reducing (b) SDS-PAGE. The allergen was probed with nine individual sera (lanes 1–9, Table 1a) and with buffer as a negative control for the anti-human IgE antibody (lane 0).

Circular dichroism studies

A structural analysis of Act d 5 has been performed by CD in several experimental conditions, to evaluate the effect of different environments on the protein conformation. Spectra have been acquired at pH 7.5, close to the human physiological value, and at pH 4.5, close to the acidic value of the kiwi fruit. In addition, we have also evaluated the influence of solvent polarity on the protein, trying to reproduce the different physico-chemical features of physiological environments experienced by the protein, before and during the interaction with its target cells. Water/fluorinated alcohol mixtures represent a handy medium to simulate biological environments with different dielectric constants [17], spanning from the fully aqueous transport fluids to the apolar lipid phase of the membrane interior and protein inner cavities. For these properties, they have been used successfully to characterize the conformation of several membrane-interacting peptides [18–23]. Figure 2 reports the CD spectra obtained at pH 7.5 (panel a) and pH 4.5 (panel b) in the different experimental conditions explored. The inspection of the CD curves shows that all of them display the typical shape of a mainly helical structure, characterized by two minima around 222 and 206 nm, although the not-fully canonical shape reflects the contribution of other secondary structures, i.e. β structures and/or random coils. Furthermore, the comparison among the two panels reveals that the curves registered at lower pH (panel b) are in general more intense than the curves obtained around a neutral pH (panel a), suggesting a higher degree of structure at acidic pH. Furthermore, at pH 4.5, the differences among the curves are well evident, and indicate that in the presence of both fluorinated alcohols the fraction of folded molecules increases, whereas in contrast at pH 7.5 all the curves are more or less superimposed.

For an easier comparison of the effect of the experimental conditions on Act d 5 structure, in Fig. 3 we have reported the molar ellipticity at 206 nm, which represents one of the hallmarks of helical structures, in the different experimental conditions explored. In this representation, it is well evident that in fully polar media (represented by

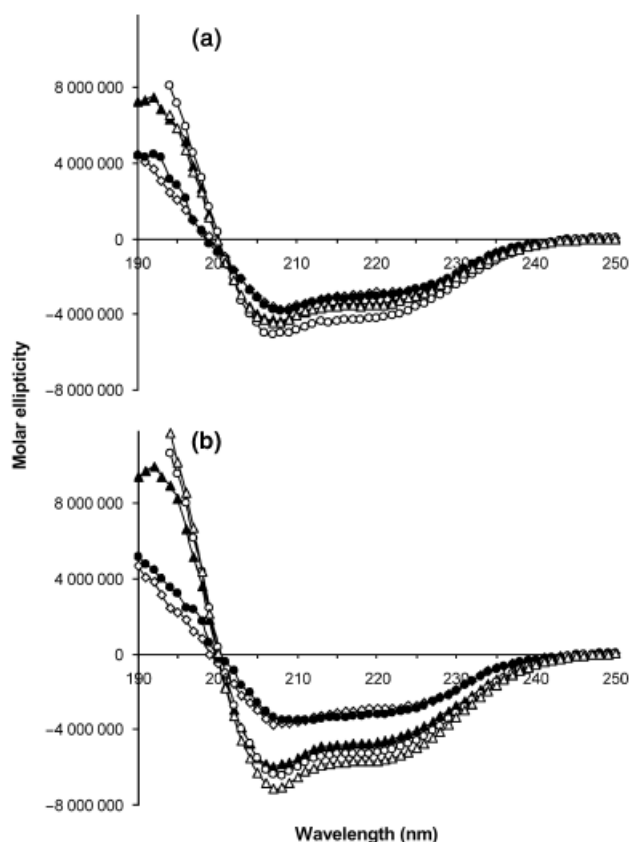


Fig. 2. Circular dichroism (CD) spectra of Act d 5 at pH 7.5 (a) and pH 4.5 (b). The following symbols have been used: ($\diamond$) water, ($\bullet$) TFE 20%, ($\blacktriangle$) TFE 80%, ($\circ$) HFIP 20% and ($\triangle$) HFIP 80%. TFE, 2,2,2-trifluoroethanol; HFIP, hexafluoroisopropanol.

bulk water) the protein is less structured and there is no pH effect. This behaviour is not affected by the addition of a low amount of TFE (20% by volume), but becomes more pronounced as far as the medium polarity decreases, i.e. in 80% TFE and in the presence of HFIP, both at 20% and 80% by volume. Overall, the data reported here indicate that the conformation of Act d 5 is strongly affected by the physico-chemical features of the environment, first of all by the solvent polarity, and that is more structured in low polarity media and at low pH.

Discussion

During the last decade, several efforts have been made to improve allergy diagnosis by integrating the traditional methods, based on protein extracts from natural raw materials, with components based on purified natural or recombinant allergens [10, 24–26].

Act d 5 was purified from the natural source and used to perform different tests. By testing, a population of subjects allergic to kiwifruit using a standard protocol for SPT, eight out of 29 (28%) had a positive reaction to Act d 5. However, the number of positive reactions to SPT

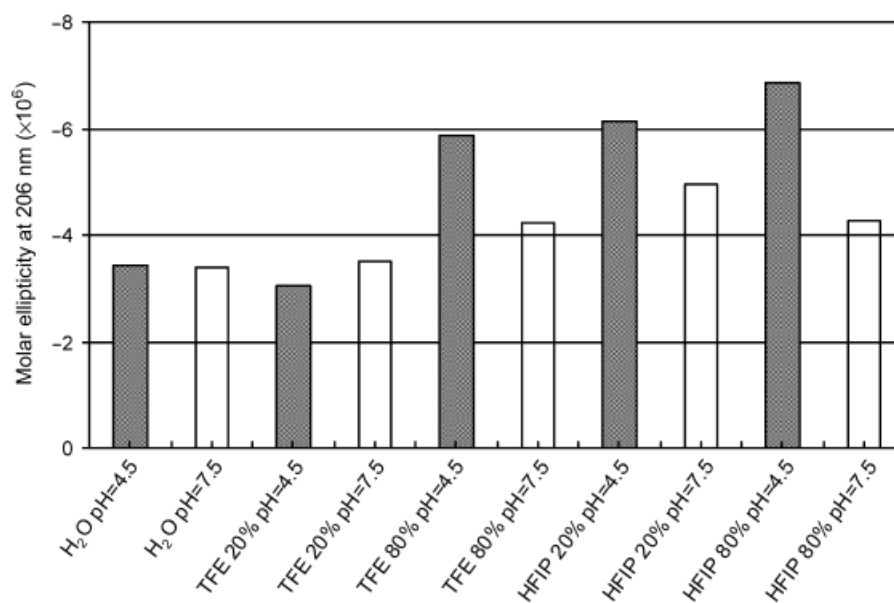


Fig. 3. Molar ellipticity of Act d 5 at 206 nm in the different experimental conditions was explored.

increased when an additional modified protocol was used. In fact, three additional positive reactions (10%) to SPT were observed using Act d 5 in acidic solution, which is an environment more similar to that of the natural source. The observation that some subjects had a positive reaction either at neutral or acidic pH values suggests that this allergen, depending on the experimental conditions, may expose different epitopes. Nevertheless, some subjects may have IgE antibodies recognizing epitopes exposed in each of the two examined experimental conditions.

These results are in line with the observations that kissper, the peptide originating from the *in vivo* proteolytic processing of Act d 5 [11], seems to produce ion channels in synthetic bilayers only after activation in acidic conditions [12]. This behaviour suggested that the active form of kissper has specific structural properties that are acquired in some physico-chemical environments and are lost in other conditions. It is noteworthy that kiwi fruit homogenate has an acidic pH value of 3.5, approximately. Therefore, it is not so surprising that an acidic environment induces a 'functional' structure in kissper, the N-terminal fragment of Act d 5.

The conformational analysis reported in this paper by CD measurements in different experimental conditions indicates that Act d 5 3D structure is modulated by the solvent pH and polarity. In fact, the protein, having a mainly helical conformation, is more structured in low polarity media and at acidic pH value, rather than in neutral and fully polar media. Therefore, similarly to kissper, its precursor (Act d 5) also displays pH-driven conformational changes but this effect is more evident in the presence of a low-dielectric constant medium, that is representative of several cellular environments [15], like

for instance the interior of biological membranes. During the transit in the gastrointestinal system, Act d 5 and/or fragments generated by the gastric and/or duodenal digestion can encounter environments characterized by different pH values, ranging from the very acidic one of the stomach to values close to neutrality, and can meet hydrophobic environments, for instance when approaching cell membranes or host proteins. Therefore, it may be hypothesized that, depending on the environments encountered, this allergen may undergo *in vivo* conformational changes and expose different epitopes, inducing the synthesis/interaction of different specific IgEs.

Unlike some other study populations (unpublished data), the sera of the subjects recruited for the investigation described here did not recognize Act d 5 immobilized on the ISAC biochip. These results suggest that the immobilization might make some epitopes unavailable for recognition by the antibodies. Nevertheless, it cannot be excluded that the experimental conditions of the ISAC procedure may affect the conformation and the IgE-binding capacity of the immobilized allergens.

IgE-immunoblotting experiments indicate that most of the subjects displaying reactivity to Act d 5 by SPT also recognize sequence epitopes. In fact, eight of nine subjects analysed by immunoblotting appear to have IgEs reacting with denatured Act d 5. One subject did not recognize sequence epitopes in the immunoblotting experiments and reacted to SPT only with Act d 5 at acidic pH, and not with Act d 5 in neutral condition. Therefore, this subject could have IgE antibodies recognizing only the conformational epitopes exposed in acidic conditions. These epitopes cannot be revealed by test systems using neutral pH conditions, such as those using the classical

PBS buffer, and cannot be revealed by IgE immunoblotting. Probably, the only way to detect the reactivity to Act d 5 of this subject is the prick by prick test, performed with the fresh kiwi fruit having its naturally acidic pH value. Otherwise, the SPT performed with the purified molecule in acidic conditions may represent a useful and more reliable alternative.

On the basis of the results described in this paper, it may be suggested that the environmental condition, such as the pH value of the allergen source, could be an additional factor affecting the sensitivity of allergy test performances. In fact, the structural features, and therefore, the ability of some allergens to interact with specific antibodies could be different in environments similar to those of the natural sources respect to other experimental conditions. This is an aspect to which usually very low or even no attention is paid. Similar to Act d 5, it can be imagined that, depending on the experimental conditions, other allergens may undergo conformational changes associated with the exposition of different combinations of epitopes recognized by the IgE antibodies of allergic subjects. This behaviour may affect in a very significant way the results of allergy testing, both *in vitro* and *in vivo*. Therefore, it appears to be an additional factor to be considered in the evaluation of allergy test system reliability.

Clinical relevance

The 3D structure of Act d 5 is sensitive to the physico-chemical characteristics of the environment. In fact, this allergen may undergo pH- and polarity-driven conformational changes that may affect the pattern of exposed antigenic epitopes. This behaviour may have clinical implications because it can affect the sensitivity of diagnostic tests, such as SPT. Therefore, a deep knowledge of the physico-chemical properties of allergenic proteins would be desirable and potentially useful for the improvement of diagnostic test systems.

References

- Mari A. When does a protein become an allergen? Searching for a dynamic definition based on most advanced technology tools. *Clin Exp Allergy* 2008; **38**:1089–94.
- Hamilton RG, Adkinson NF. Jr 23. Clinical laboratory assessment of IgE-dependent hypersensitivity. *J Allergy Clin Immunol* 2003; **111**:S687–701.
- Sharma HP, Wood RA, Bravo AR, Matsui EC. A comparison of skin prick tests, intradermal skin tests, and specific IgE in the diagnosis of mouse allergy. *J Allergy Clin Immunol* 2008; **121**:933–9.
- Nelson HS, Ikle D, Buchmeier A. Studies of allergen extract stability: the effects of dilution and mixing. *J Allergy Clin Immunol* 1996; **98**:382–8.
- Lucas JS, Lewis SA, Hourihane JO. Kiwi fruit allergy: a review. *Pediatr Allergy Immunol* 2003; **14**:420–8.
- Mari A, Scala E. Allergenic extracts for specific immunotherapy: to mix or not to mix? *Int Arch Allergy Immunol* 2006; **141**:57–60.
- Focke M, Marth K, Flicker S, Valenta R. Heterogeneity of commercial timothy grass pollen extracts. *Clin Exp Allergy* 2008; **38**:1400–8.
- Focke M, Marth K, Valenta R. Molecular composition and biological activity of commercial birch pollen allergen extracts. *Eur J Clin Invest* 2009; **39**:429–36.
- Tamburrini M, Cerasuolo I, Carratore V *et al.* Kiwellin, a novel protein from kiwi fruit. Purification, biochemical characterization and identification as an allergen*. *Protein J* 2005; **24**:423–9.
- Bublin M, Pfister M, Radauer C *et al.* Component-resolved diagnosis of kiwifruit allergy with purified natural and recombinant kiwifruit allergens. *J Allergy Clin Immunol* 2010; **125**:687–94.
- Tuppo L, Giangrieco I, Palazzo P *et al.* Kiwellin, a modular protein from green and gold kiwi fruits: evidence of *in vivo* and *in vitro* processing and IgE binding. *J Agric Food Chem* 2008; **56**:3812–7.
- Ciardiello MA, Meleleo D, Saviano G *et al.* Kissper, a kiwi fruit peptide with channel-like activity: structural and functional features. *J Pept Sci* 2008; **14**:742–54.
- Ciardiello MA, Giangrieco I, Tuppo L *et al.* Influence of the natural ripening stage, cold storage, and ethylene treatment on the protein and IgE-binding profiles of green and gold kiwi fruit extracts. *J Agric Food Chem* 2009; **57**:1565–71.
- Mari A, Di Felice G, Afferni C *et al.* Assessment of skin prick test and serum specific IgE detection in the diagnosis of Cupressaceae pollinosis. *J Allergy Clin Immunol* 1996; **98**:21–31.
- Hiller R, Laffer S, Harwanegg C *et al.* Microarrayed allergen molecules: diagnostic gatekeepers for allergy treatment. *FASEB J* 2002; **16**:414–6.
- Harwanegg C, Hutter S, Hiller R. Allergen microarrays for the diagnosis of specific IgE against components of cow's milk and hen's egg in a multiplex biochip-based immunoassay. *Methods Mol Biol* 2007; **385**:145–57.
- Tanizaki S, Clifford J, Connelly BD, Feig M. Conformational sampling of peptides in cellular environments. *Biophys J* 2008; **94**:747–59.
- Sticht H, Bayer P, Willbold D *et al.* Structure of amyloid A4-(1–40)-peptide of Alzheimer's disease. *Eur J Biochem* 1995; **233**:293–8.
- Saviano G, Crescenzi O, Picone D, Temussi P, Tancredi T. Solution structure of human beta-endorphin in helicogenic solvents: an NMR study. *J Pept Sci* 1999; **5**:410–22.
- Crescenzi O, Guerrini R, Picone D, Salvadori S, Tancredi T, Temussi PA. Solution structure of nocistatin, a new peptide analgesic. *Biopolymers* 2000; **53**:257–64.
- Crescenzi O, Tomaselli S, Guerrini R *et al.* Solution structure of the Alzheimer amyloid beta-peptide (1–42) in an apolar micro-environment. Similarity with a virus fusion domain. *Eur J Biochem* 2002; **269**:5642–8.
- Tomaselli S, Esposito V, Vangone P *et al.* The alpha-to-beta conformational transition of Alzheimer's Abeta-(1–42) peptide in aqueous media is reversible: a step by step conformational analysis suggests the location of beta conformation seeding. *Chembiochemistry* 2006; **7**:257–67.

- 23 Spadaccini R, D'Errico G, D'Alessio V *et al*. Structural characterization of the transmembrane proximal region of the hepatitis C virus E1 glycoprotein. *Biochim Biophys Acta* 2010; **1798**: 344–53.
- 24 Deinhofer K, Sevcik H, Balic N *et al*. Microarrayed allergens for IgE profiling. *Methods* 2004; **32**:249–54.
- 25 Lidholm J, Ballmer-Weber BK, Mari A, Vieths S. Component-resolved diagnostics in food allergy. *Curr Opin Allergy Clin Immunol* 2006; **6**:234–40.
- 26 Constantin C, Quirce S, Poorafshar M *et al*. Micro-arrayed wheat seed and grass pollen allergens for component-resolved diagnosis. *Allergy* 2009; **64**:1030–7.