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Protocol for simultaneous isolation of three important banana allergens

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ABSTRACT

Banana fruit (*Musa acuminata*) has become an important food allergen source in recent years. So far, 5 IgE reactive banana proteins have been identified, and the major allergens are: Mus a 2 (a class I chitinase, 31 kDa), Mus a 4 (thaumatin-like protein, 21 kDa), and Mus a 5 (β-1,3-glucanase, 33 kDa). Due to variations in allergen expression levels, diagnostic reagents for food allergy can be improved by using individual allergen components instead of banana allergen extracts. The purpose of this study was to optimize the purification protocol of the three major allergens present in banana fruit: Mus a 2, Mus a 4 and Mus a 5. By employing a three-step purification protocol (a combination of anion-exchange, cation-exchange and reversed-phase chromatography) three important banana allergens were obtained in sufficient yield and high purity. Characterization of the purified proteins was performed by both biochemical (2-D PAGE, mass fingerprint and *N*-terminal sequencing) and immunochemical (immunoblot) methods. IgE reactivity to the purified allergens was tested by employing sera of five allergic patients. The purified allergens displayed higher sensitivity in IgE detection than the routinely used extracts. The three purified allergens are good candidates for reagents in component-based diagnosis of banana allergy.

1. Introduction

Based on clinical studies it has been estimated that 11–26 million people [1] in Europe suffer from food allergies, while on the global scale this number is between 220 and 520 million. Progress in the prevention and treatment of IgE mediated food hypersensitivity is essential and current research is focused on the development of novel diagnostic and therapeutic approaches for food allergy treatment [1].

Banana (*Musa acuminata*) is very present in the human diet because of its high nutritive value and pleasant taste. Consumption of banana has been associated with reduced risk of chronic diseases (cardiovascular diseases, cancer) due to the antioxidant and cell antiproliferative activities of phytochemicals present in this fruit [2]. However, banana fruit is also recognized as an allergen source, and five proteins from this fruit have been identified as food allergens. These allergens are: profilin (denoted as Mus a 1), class I chitinase (denoted as Mus a 2), non-specific lipid transfer protein (LTP, denoted as Mus a 3), thaumatin-like protein (TLP, denoted as Mus a 4), and β-1,3-glucanase (denoted as Mus a 5) (http://www.allergen.org/).

Banana allergy is often associated with allergies to latex and/or pollen, indicating the involvement of banana allergens in latex–fruit, pollen–fruit and latex–pollen–fruit syndrome [3–11]. However, there are also reports that show banana allergy occurs in patients without latex [12–14] or pollen allergy [12]. Cross-reactivity has been reported between banana allergens and allergens from other food sources, such as avocado [7,12,15], kiwi fruit [8,15], peach [4,15], and apple [16], as well as with allergens from palm pollen [15] and olive pollen [9,10].

Fruit extracts employed for allergy diagnosis represent mixtures of allergens and non-allergenic material and, therefore, may contribute to misdiagnosing of food allergy [7]. The quality of

Abbreviations: Mus a 1, banana fruit profilin; Mus a 2, banana fruit chitinase; LTP, lipid-transfer protein; Mus a 3, banana fruit lipid-transfer protein; TLP, thaumatin-like protein; Mus a 4, banana fruit thaumatin-like protein; Mus a 5, banana fruit glucanase; SPT, skin prick test; FEIA, fluorenzymeimmunoassay; M, missed cleavages.

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Table 1
Synopsis of allergy diagnostic tests of five patients with manifest banana allergy.

Patient-ID	Gender	Age	History for banana allergy		SPT[b]	IgE to banana extract [kU/L]	Total serum IgE [kU/L]	Birch pollen allergy	IgE to Bet v 1 [kU/L]	IgE to Bet v 2[d] (profilin) [kU/L]	IgE to Pru p 3[e] (LTP) [kU/L]
1[f]	F	35	Negative		+	0.24	154	+	53.1	–	–
2	M	65	+	Angioedema	n.d.[c]	0.09	65	–	–	–	n.d.[c]
3	F	33	+	OAS[a], laryngeal edema	+	0.91	142	+	16.9	0.65	–
4	M	26	+	Anaphylaxis grade III	+	77.6	>5000	+	2.14	28.0	>100
5	F	29	+	Anaphylaxis grade II	+	–	16.3	–	–	–	12.9

[a] OAS: oral allergy syndrome.
[b] SPT: skin prick test.
[c] n.d.: not done.
[d] Bet v 2: marker allergen for profilins.
[e] Pru p 3: marker allergen for LTPs.
[f] Sensitized but clinically tolerant to banana ingestion.

Fig. 1. SDS-PAGE analysis of purified banana proteins: (1) raw banana extract; (2) protein fraction after QAE-Sephadex; (3) protein fraction after Hi Trap SP; (4) 31 kDa protein; (5) 21 kDa protein; (6) 33 kDa protein; (7) molecular mass marker.

allergen extracts from fruits and other plant-derived foods vary due to both the inherent presence of proteolytic enzymes, and to the ripening stage and/or storage conditions of the allergenic source materials [17–19]. Ciardiello and coworkers (2009) observed a significant influence of the ripening stage and the extraction method on the composition of green and gold kiwifruit extracts. Their results indicated that in the green kiwi species, ripe fruits may have different concentrations of total proteins and different amounts of single components when ripeness is reached by different means of postharvest handling (ethylene exposure with or without previous cold storage). Also, it was previously demonstrated that the transcript accumulation pattern of beta-1,3-glucanase during ripening in banana fruit depends on various endogenous (ethylene, auxin, abscisic acid) and exogenous (wounding, cold and light–dark cycles) factors [20]. Employment of non-standardized food extracts in allergy diagnosis can be misleading, and, indeed, reports on systemic anaphylaxis to banana in a patient with negative SPT results to commercial banana fruit extract exist [21]. Therefore, replacement of allergen extracts with a panel of IgE-reactive molecules from a particular allergen source is a promising strategy for the improvement of allergy diagnosis. A panel of three recombinant cherry allergens was shown to be superior to diagnostic methods based on cherry extract [22], while the employment of single kiwifruit allergens in ImmunoCAP increased the quantitative test performance and diagnostic sensitivity compared with the commercial extract [23]. Understanding the molecular basis of food allergy, including the structural and functional features of allergens, will contribute to the development of novel therapeutic approaches [24].

The purpose of this research was to establish a fast, simple, efficient, and low-cost process for the isolation of three important banana proteins relevant for banana allergy diagnosis.

2. Materials and methods

2.1. Preparation of protein extract

For the preparation of protein extract banana (*Musa acuminata*) was purchased in a local store. Protein extraction was performed