Biochimica et Biophysica Acta 1834 (2013) 2539–2545

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbapap

The peel and pulp of mango fruit: A proteomic samba

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ARTICLE INFO

Article history:
Received 1 August 2013
Received in revised form 29 August 2013
Accepted 10 September 2013
Available online 18 September 2013

Keywords:
Mango peel
Mango pulp
Low abundance proteome
Combinatorial peptide ligand libraries
Mass spectrometry

ABSTRACT

Combinatorial peptide ligand libraries (CPLLs) have been adopted for investigating the proteomes of mango peel and pulp as well their peptidome content (the latter as captured with a C_{18} resin). The aim of this study was not only to perform the deepest investigation so far of the mango proteome, but also to assess the potential presence of allergens and of peptides endowed with biological activities. The proteins of peel and pulp have been captured under both native and denaturing extraction techniques. A total of 334 unique protein species have been identified in the peel vs. 2855 in the pulp, via capture with CPLLs at different pH values (2.2 and 7.2).

1. Introduction

The mango is a fleshy stone fruit belonging to the genus *Mangifera*, consisting of numerous tropical fruiting trees in the flowering plant family Anacardiaceae. Mango is native to South Asia, wherefrom it has spread worldwide to become one of the most cultivated fruits in the tropics. While other *Mangifera* species (e.g. horse mango, *Mangifera foetida*) are also grown on a more localized basis, *Mangifera indica* – the 'common mango' or 'Indian mango' – is the only mango tree commonly cultivated in many tropical and subtropical regions. It is the national fruit of India, Pakistan and the Philippines, and the national tree of Bangladesh. The ripe fruit varies in size and color. Cultivars are typically yellow, orange, red or green, and carry a single flat, oblong pit that can be fibrous or hairy on the surface, and which does not separate easily from the pulp. Ripe, unpeeled mangoes give off a distinctive resinous, sweet smell. Inside the pit is a thin (1–2 mm thick) lining covering a single seed, 4–7 cm long. The seed contains the plant embryo [1].

Mango is used to make juices, smoothies, ice cream, fruit bars, *raspados*, *aguas frescas*, pies and sweet chili sauce, or mixed with *chamoy*, a sweet and spicy chili paste. It is popular on a stick dipped in hot chili powder and salt or as a main ingredient in fresh fruit combinations. In Central America, mango is either eaten green mixed with salt, vinegar, black pepper and hot sauce, or ripe in various forms.

When reading the literature on this tropical fruit, one has the impression that mango is almost a thaumaturgic foodstuff. In mango fruit pulp, the antioxidant vitamins A and C, vitamin B_6 (pyridoxine), folate, other B vitamins and essential nutrients, such as potassium, copper and amino acids, are present. Mango peel and pulp contain other compounds, such as pigment carotenoids and polyphenols, and omega-3 and -6 polyunsaturated fatty acids [2,3]. Additionally, mango peel pigments seem to have important biological effects [4], including carotenoids, such as the provitamin A compound, beta-carotene, lutein and alpha-carotene [5], polyphenols [6,7] such as quercetin, kaempferol, gallic acid, caffeic acid, catechins, tannins, and the unique mango xanthonoid, mangiferin [8], any of which may counteract free radicals in various disease processes as described in some reports [9,10]. Phytochemical and nutrient content appears to vary across mango species [11]. Up to 25 different carotenoids have been isolated from mango pulp, the most abundant of which was beta-carotene, which accounts for the yellow–orange pigmentation of most mango species [12]. Peel and leaves also have significant polyphenol content, including xanthonoids, mangiferin and gallic acid [13]. The mango triterpene, lupeol [14], is an effective inhibitor in laboratory models of prostate and skin cancers [15,16]. An extract of mango branch bark called Vimang, isolated by Cuban scientists, contains numerous polyphenols with antioxidant properties in vitro [17] and on blood parameters of elderly humans [18].

Despite the large consumption of mangoes, especially in India, hypersensitivity reactions are distinctly rare. Allergy to mango can manifest in two forms: the immediate hypersensitivity reaction, presenting as anaphylaxis, angioedema, erythema, urticaria, wheezing dyspnoea, and the late reaction, presenting as contact dermatitis and periorbital edema [11]. Mango allergy, like that of poison ivy, is a version called also delayed hypersensitivity because the symptoms usually appear 48 or 72 h after exposure to the offending substance that triggers the response [19]. The fruit mango can cause immediate or delayed hypersensitivity reactions and allergic reactions without

Abbreviations: CPLLs, combinatorial peptide ligand libraries; PM, ProteoMiner

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http://dx.doi.org/10.1016/j.bbapap.2013.09.004

prior exposure, owing to cross reactivity and it may also manifest as "oral allergy syndrome". It has been shown that pollens from trees (especially birch), grasses and weeds contain proteins of similar structure to those present in certain fruits, vegetables, nuts and spices. These proteins are recognized by the immune system and the ingestion of a foodstuff, which shares the same protein as the pollen, can result in triggering an allergic reaction in a susceptible individual. Mango allergens have been shown to cross react with *Artemisia* pollen, birch pollen, poison ivy, poison oak, mugwort, celery, carrot, pistachio nut, tomato, papaya and banana [20].

Notwithstanding all data reported above on mango metabolites, not much is known on this fruit proteome. Only recently a two-dimensional (2D) mango pulp analysis has been reported, aiming at identifying modulation of protein expression associated with ripening [21]. A total of 373 spots could be visualized in the 2D map, leading to the identification of 51 unique gene products. In another report, via database searches of mango-derived ESTs and proteins along with proteins from six other closely related plant species, Renuse et al. [22] could identify 1001 peptides that matched to 538 proteins. However, this set of proteins applied to mango leaves, not to pulp nor peel. Given these scanty proteomics data, we have decided to perform an in depth exploration of both peel and pulp proteomes of mango via the well-ingrained combinatorial peptide ligand library (CPLL) methodology, a well-established tool for searching low- to very-low abundance proteins in any proteome, as amply illustrated in a number of reviews [23–27]. This project is aimed not only at offering an ample coverage of these proteomes, but also at detecting unreported allergens (if any) and to see if any of these species might have important nutraceutical effects. Additionally, in these two same biological compartments, we have performed a capture of peptides present in the intact fruit, via a C_{18} resin, in future search of potential bioactive compounds.

2. Materials and methods

2.1. Chemicals and biologicals

ProteoMiner™ (combinatorial hexapeptide ligand library beads, PM), Laemmli buffer, 40% acrylamide/Bis solution, N,N,N′,N′-tetramethylethylenediamine (TEMED), molecular mass standards and electrophoresis apparatus for one-dimensional electrophoresis were from Bio-Rad Laboratories, Hercules CA. β-Mercaptoethanol, dithiothreitol (DTT), ammonium persulfate, 3-[3-cholamidopropyl dimethylammonio]-1-propanosulfonate (CHAPS), acetonitrile (ACN), trifluoroacetic acid (TFA), sodium dodecyl sulfate (SDS), iodoacetamide (IAA), formic acid (FA) and all other chemicals used all along the experimental work were current pure analytical grade products and purchased from Sigma-Aldrich, St Louis, MO. C_{18} resins were purchased from Pierce Chemical Company, Rockford, IL, USA. Complete protease inhibitor cocktail tablets and sequencing grade trypsin were from Roche Diagnostics (Basel, CH).

2.2. Plant materials and protein extraction protocols

Three mango fruits were bought in local supermarkets. After washing the surface in lukewarm 1% SDS solution, so as to eliminate bacterial and surface contamination from human hands, the peel was carefully excised and homogenized for 15 min in a fruit blender. For exploring the proteomes of both, peels and pulp, two extraction protocols were devised after full homogenizations of these two tissues. The native extraction buffer contained 50 mM Tris–HCl (pH 7.2), 50 mM NaCl and 2% (m/v) CHAPS, whereas the denaturing buffer contained additionally 1% (m/v) SDS and 25 mM DTT dissolved into the same buffer. Protease inhibitor cocktails were added to both extraction buffers in order to prevent protein degradation by protease action. In detail 5 g of minced peel was mixed with 10 mL of each extraction buffer and they were gently shaken for 3 h at room temperature for native buffer and at boiling condition for denaturing one. As regards the pulp, 100 g of pulp was solubilized in 20 mL of each extraction buffer and treated in the same conditions reported for peel samples. Prior to capture with CPLLs, the homogenates were centrifuged at 18,000 rpm for 10 min to separate the insoluble debris from the clarified protein solution: the recovered volumes were 8 mL of each extraction for the peel and 18 mL for the pulp. While the native samples were immediately incubated with CPLLs, the denatured ones were diluted 1:10 (v/v) with a buffer containing 50 mM Tris–HCl (pH 7.2), 50 mM NaCl, 25 mM DTT and protease inhibitor cocktail in order to reduce the original 1% SDS amount to 0.1% (m/v) so as to allow an effective protein capture. Two technical replicas on the three different mango fruits were performed.

2.3. ProteoMiner and C_{18} resins treatment

Each sample (both peel and pulp extracts) was titrated at pH 7.2 [28] and, subsequently, individually added with 100 μL of ProteoMiner beads overnight at room temperature under gentle shaking. The last CPLL incubation was performed by reducing the pH at a value of 2.2 with the addition of 0.1% TFA and formic acid and was always done overnight at room temperature under gentle shaking, in order to mimic reverse phase conditions for the capture of hydrophobic proteins. Additionally, for both peel and pulp, a third treatment with C_{18} beads was performed, to harvest any peptide potentially present in these two tissues. After centrifuging at 4000 rpm for 5 min, the beads were washed with their respective buffers and the captured proteins were then desorbed (twice, with 100 μL each time) with a solution composed of 4% SDS and 20 mM DTT for 5 min, under boiling conditions [29]. Prior to SDS electrophoresis, the eluted proteins were alkylated with 50 mM iodoacetamide. The C_{18} beads, conversely, were treated with pure acetonitrile and the eluted peptides, after solvent evaporation, sent directly to MS analysis. SDS-polyacrylamide gel electrophoresis and two-dimensional map analyses were performed as previously described [30].

2.4. Mass spectrometry and data analysis

Ten microliter of tryptic digested sample was injected on a reversed-phase trap column (Acclaim PepMap100, C18, 100 Å, 5 μm, 100 μm ID × 2 cm length, Thermo Scientific) for peptide clean-up and preconcentration. After clean-up, the trap column was placed in series with a fused silica reverse-phase column (picoFrit column, C18 HALO, 90 Å, 75 μm ID, 2.7 μm, 10.5 cm length, New Objective). A nano chromatographic system (UltiMate 3000 RSLCnano System, Thermo Scientific) delivered a constant flow rate of 300 μL/min. The separating gradient ramped linearly from 1% acetonitrile to 40% acetonitrile in 30 min. The eluting peptides were on-line sprayed in a LTQ XL mass spectrometer (Thermo Scientific). Full scan mass spectra were acquired in the Orbitrap cell in the mass range of 350 to 1800 *m/z*. The nine most intense ions (minimum charge state 2+) were automatically selected and fragmented in the ion trap by collision-induced dissociation (CID). Target ions already selected for fragmentation were dynamically excluded for 60 s. The MS data were analyzed by Mascot search engine (Version 2.3.01), using Proteome Discoverer software (v. 1.2.0 Thermo) and consulting Uniprot_viridiplantae database. We set cysteine carbamidomethylation and oxidation of methionine residues as variable modifications; peptide mass tolerance was set to 1 Da, fragment mass tolerance to 0.8 Da and ion source cut-off of 20. The false discovery rate obtained consulting the decoy database was less than 0.05.

3. Results

Fig. 1 shows the SDS-PAGE profiles, in the case of mango peel, of the control vs. the eluates from various captures with CPLLs. PD (denaturated peel) and PN (native peel) represent, respectively,