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Resistance of purified seed storage proteins from sesame (*Sesamum indicum* L.) to proteolytic digestive enzymes

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ABSTRACT

Sesame has been increasingly associated with food allergy. The main seed storage proteins of sesame (the 2S albumin and the 7S and 11S globulins) were purified and subjected to proteolysis with pepsin, trypsin and chymotrypsin. The degree of proteolysis obtained was monitored by SDS–PAGE, followed by densitometry of the main bands. The 2S albumin was found to be stable to proteolysis, being extremely resistant to pepsin, and relatively resistant to trypsin and chymotrypsin. The 7S and 11S proteins were relatively labile to pepsin. Acidic polypeptides from the 11S protein were more susceptible to proteolysis than the basic polypeptides. Both 7S and 11S proteins generated what appeared to be stable polypeptides after proteolysis with trypsin and chymotrypsin. The results are discussed in relation to similar studies on related seed storage proteins, available structural information, and the potential allergenicity of the sesame proteins.

1. Introduction

Sesame (*Sesamum indicum* L.) is a valuable seed crop owing to its high content of oil and protein (the latter usually found between 19% and 25%). The majority of the proteins present in sesame seeds are storage proteins classed as albumins (9%), globulins (67%), prolamins (1%) and glutelins (7%) on the basis of their solubility (Rivas, Dench, & Caygill, 1981). The water-insoluble 11S globulin and the soluble 2S albumin are the two major storage proteins of sesame.

The sesame 11S globulin has the typical oligomeric structure of the 11S seed proteins, being composed of six subunits that interact non-covalently (Okubo, Nishimura, & Shibasaki, 1979a). The 11S hexamers contain a random combination of different subunits, with each of these subunits consisting of an acidic polypeptide (30–34 kDa) and a basic polypeptide (20–25 kDa) linked by a single disulphide bond (Okubo, Nishimura, & Shibasaki, 1979b). The sedimentation coefficient of the sesame 11S globulin varies between 11.0 and 13.0, depending on the ionic strength of the buffer in which the measurements are taken. The cDNA sequences encoding four sesame 11S globulin isoforms have been cloned successfully (Hsiao, Lin, Wang, Liao, & Tzen, 2006; Tai, Lee, Tsai, Yiu, & Tzen, 2001; Tai, Wu, Chen, & Tzen, 1999).

The molecular weight of the sesame 2S albumin has been estimated to be in the range of 13–15 kDa (Rajendran & Prakash, 1988; Tai et al., 1999). Rajendran and Prakash (1988) showed that the 2S albumin was composed of a single polypeptide chain. However, it has been reported that the sesame 2S albumin can be cleaved into a large subunit of around 9 kDa and a smaller subunit of around 4 kDa in the presence of a disulphide reducing agent (Tzen, personal communication), which is in accordance with the typical subunit structure of most 2S albumins. The amino acid sequences of three isoforms of the sesame 2S albumin have been deduced, indicating that not all the 2S albumin isoforms are sulphur-rich (Hsiao et al., 2006; Tai et al., 1999, 2001).

In contrast to the sesame 11S globulin and 2S albumin, information about the 7S globulin, the third major storage protein of sesame, is scarce. The existence of a 7S globulin as a minor constituent of the total storage proteins in sesame was reported briefly by Prakash and Nandi (1978). Tai et al. (2001) obtained a cDNA sequence encoding the sesame 7S globulin and identified its corresponding polypeptide as a minor constituent in sesame seeds via immunodetection. The purification of the 7S globulin from sesame seeds has been previously described showing that the protein is composed of at least eight polypeptide chains ranging from 12.4 to 65.5 kDa judged by SDS–PAGE analysis and does not contain disulphide bonds (Orruño & Morgan, 2007).

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Food allergy is of increasing concern to individuals suffering from the syndrome, to food producers and to regulators. Many of the most important allergenic foods (such as peanut, tree nuts, wheat, soybean, mustard, celeriac and sesame) are of plant origin and are consumed as seed or nut products; seed storage proteins have often been associated with specific reactivity towards IgE from patient serum. Understanding more about the properties of the seed storage proteins has been perceived to be important in identifying key molecular characteristics predisposing to allergenicity. During the last few years, the number of sesame allergic individuals seems to have increased significantly in a number of countries (Gangur, Kelly, & Navuluri, 2005; Sicherer & Leung, 2009). Furthermore, the increasing and widespread consumption of sesame-containing foods and the frequent presence of sesame as a hidden allergen are likely to make sesame allergy become even more common in future. Severe reactions to sesame, such as anaphylactic shock, have been reported in the literature (Asero, Mistrello, Roncarolo, Antonotti, & Falagiani, 1999; Kolopp-Sarda et al., 1997).

It is generally believed that a protein, which is resistant to proteolytic digestion in the digestive tract, retains sufficient structural integrity to have an increased probability of stimulating immune reactions. Small amounts of intact or partially digested proteins are absorbed through the intestine and enter the circulatory system under normal circumstances as a physiological process. Hence, the ability of food allergens to reach the intestinal mucosa intact seems to be a likely requirement for allergenicity (Fuchs & Astwood, 1996). Astwood, Leach, and Fuchs (1996) evaluated the digestive stability of a group of known allergens and a group of non-allergenic enzymes in simulated gastric fluid (SGF). The authors concluded that food allergens were more stable than non-allergenic proteins in SGF and that the digestibility parameter can be used to distinguish allergens from non-allergenic proteins. A critical review of assessment of allergenicity has been published (Orruño & Morgan, 2006).

The sesame seed storage proteins have not been investigated previously with regard to their susceptibility to enzymic digestion. In the present study the digestibility of the three main sesame storage proteins, i.e. 2S albumin, 7S globulin and 11S globulin, was investigated and characterised using single digestive enzymes. Protein degradation was monitored by SDS–PAGE and the percentage of undigested protein bands was quantified using densitometry.

2. Materials and methods

2.1. Chemicals

Acrylogel-3-solution, ethanol and hydrochloric acid were purchased from BDH Ltd., Poole, UK. Glacial acetic acid, glycerol, *n*-hexane and sodium dodecyl sulphate (SDS) were purchased from Fisons/Fisher, Loughborough, UK. Dithiothreitol (DTT) was purchased from Lancaster, Morecambe, UK. Ammonium persulphate, ammonium sulphate, bromophenol blue, *n*-butanol, calcium chloride, disodium hydrogen orthophosphate, electrophoresis molecular weight markers (wide-range and low-range), glycine, sodium carbonate, sodium chloride, sodium dihydrogen orthophosphate, *N*,*N*,*N*′,*N*′-tetramethyl-ethylene diamine (TEMED), and Trizma™ base were purchased from Sigma–Aldrich Chemicals Co., Poole, UK.

2.2. Purification of sesame proteins

The seed storage proteins from sesame were purified following the procedures described by Orruño and Morgan (2007). White sesame seeds (purchased from local retailers) were ground and defatted with hexane for 16 h using a Soxhlet apparatus. Sesame proteins were extracted from the defatted flour with phosphate buffer (20 mM, pH 7.5) containing 1 M sodium chloride (1:10, w:v, sesame flour to solvent ratio) under constant stirring for 2 h at room temperature. The slurry was centrifuged at 19,800*g* for 1 h at 4 °C. The supernatant obtained after centrifugation was filtered through Watman No. 1 filter paper. Following a number of ammonium sulphate fractionation steps, the sesame 2S albumin was purified by gel filtration chromatography on Sephadex G-100 followed by re-chromatography on Sephadex G-50, and the 11S globulin by using Sephacryl S-200 HR followed by re-chromatography on Sephacryl S-300 HR. The sesame 7S globulin was purified by anion exchange chromatography on Q Sepharose Fast Flow. Chromatographic materials were purchased from Sigma–Aldrich Chemical Co., Poole, UK. Each of the three protein preparations gave single symmetrical peaks on column chromatography, the expected bands and subunit composition by SDS–PAGE and single bands by native PAGE.

2.3. Protein determination

Estimation of protein concentration was performed with Coomassie Plus Protein Assay reagent (Perbio Science UK Ltd., Cramlington, UK) according to the method of Bradford (1976).

2.4. Enzymic hydrolysis

The enzyme digestions of sesame 11S and 7S globulins and 2S albumin were performed for 30 min at 37 °C with pepsin, chymotrypsin and trypsin (TLCK-treated α-chymotrypsin type VII from bovine pancreas, pepsin A from porcine stomach mucosa, and TPCK-treated trypsin from bovine pancreas, all obtained from Sigma–Aldrich Chemical Co., Poole, UK). The pH, temperature and reaction time were kept constant and a range of enzyme to sesame protein ratios (1:5, 1:10, 1:50 and 1:100 (w:w)) were tested. Pepsin digestions were carried out in saline (0.03 M) adjusted with hydrochloric acid to pH 1.2; trypsin digestions in Tris–HCl buffer (0.1 M, pH 8.0) containing 20 mM calcium chloride; and chymotrypsin digestions in Tris–HCl buffer (0.1 M, pH 8.0). Each of the sesame protein solutions (70 μl) containing 100 μg of protein were added to eppendorf tubes (Camlab, Cambridge, UK) and pre-incubated for 10 min in a water bath set at 37 °C. The appropriate enzyme solutions (30 μl) were added at 1 min intervals to each of the tubes, the reaction mixture was vortex mixed and placed into a water bath for 30 min at 37 °C. Enzymatic digestions were stopped by the addition of 100 μl of electrophoresis sample buffer containing DTT and boiling of the samples for 5 min. Sodium carbonate solution (1 M, 10 μl) was added to the pepsin containing samples prior to the addition of sample buffer. Sesame 11S, 7S and 2S protein blanks were prepared by replacing the enzyme solutions with an equal volume of appropriate buffer. Each enzyme stock solution (1 mg/ml; 100 μl) was used as the enzyme blank. All blanks were incubated in the same conditions as the samples.

2.5. SDS–PAGE analysis

The digested samples were analysed by SDS–PAGE on a Mini-Protean® II electrophoresis cell (Bio-Rad, Hemel Hempstead, UK) according to a modification of the method of Laemmli (1970). The samples and blanks containing 15 μg of protein (30 μl) were loaded in the gel wells and electrophoresis was carried out at constant current of 75 mA on 15% T acrylamide mini gels. The protein bands were stained with Coomassie Brilliant Blue R-250 (Sigma–Aldrich Chemical Co., Poole, UK). Gels were photographed digitally (University of Leeds Media Services).