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Stability of recombinant 2 S albumin allergens *in vitro*

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Abstract

Two well known 2 S albumins, Ber e 1 from brazil nut and sunflower 2 S albumin 8 (SFA-8), have been expressed in a eukaryotic system and purified. Analysis of recombinant versions of Ber e 1 and SFA-8 revealed them to be significantly more resistant to digestion by pepsin than BSA, and to be stable for up to 30 min in simulated gastric fluid. Unfolding monitored by CD indicated that both proteins were also very resistant to denaturation induced by heat and low pH. These results suggest that, although the ability of 2 S albumins to reach the circulatory system may be a prerequisite for the allergenicity of this group of proteins, stability is just one of a number of characteristics that provoke a selective immune response.

Introduction

In light of the growing number of genetically engineered crops and the observation that the horizontal transfer of allergens can occur unintentionally during transgenic studies [1], a decision tree has been recommended to ensure the safety of foods derived from biotechnology [2,3]. The stability of proteins, as measured by resistance to proteolysis by simulated gastric fluid (SGF), is an important factor in the assessment of the allergenicity of proteins [4]. Many allergens have been found to be consistently more resistant to digestion by pepsin than other proteins, and the ability of an intact protein to reach the circulatory system maybe a prerequisite for allergenicity [3]. 2 S seed albumins have been associated with both allergy, in the oil milling and baking industries [5], and the genetic enhancement of nutrient-poor crops [6].

Key words: allergenicity, Ber e 1, SFA-8, simulated gastric fluid, unfolding.
Abbreviations used: Ber e 1 and rBer e 1, native and recombinant brazil nut 2 S albumin respectively; SFA-8 and rSFA-8, native and recombinant sunflower 2 S albumin 8 respectively; SGF, simulated gastric fluid.
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Several proteins from this group, including Ber e 1 from brazil nut, have been characterized as clinically important allergens [7]. In contrast with Ber e 1, and despite a high level of consumption, allergy to sunflower 2 S albumin (SFA-8) is less well described [8,9]. Purification of allergens is often problematic, and the production of recombinant versions may enable standardization of allergens for mechanistic studies, diagnostics and antigen-based therapies [10].

Materials and methods

Extraction and purification of native and recombinant proteins

Recombinant Ber e 1 and SFA-8 (rBer e 1 and rSFA-8 respectively) were produced in the yeast *Pichia pastoris* and purified by FPLC using a heparin–Sepharose column [11]. BSA was obtained from Sigma.

SGF digests

SGF contained 0.32 % (w/v) pepsin (Sigma) in 30 mM NaCl/0.7 % (v/v) HCl, pH 1.2 [12,13]. Digestions contained 120 μl of SGF and 23 μg of purified protein. Incubations were maintained at 37 °C and samples were removed at each time point and quenched with Na_2CO_3 (final concentration 70 mM), before freezing until electrophoresis. Proteins were separated by discontinuous SDS/PAGE under reducing conditions using the NuPAGE® Novex system (Invitrogen) as per the manufacturer's instructions.

CD measurements

Far-UV CD spectra for the analysis of unfolding induced by heat and acidic conditions were generated as described in [11]. Protein concentration was determined using a BCA protein assay kit (Pierce) and a concentration of 0.146 mg · ml^{-1} (rBer e 1) or 0.318 mg · ml^{-1} (rSFA-8) in 10 mM phosphate buffer, pH 6.8, or 0.1 M glycine buffer, pH 2.2, used in experiments.

Results

Digestion in SGF

In previous studies of plant-food allergens, native 2 S albumins were found to be among the proteins that are most stable to digestion by SGF [13]. In the current study, purified recombinant versions of the 2 S albumins Ber e 1 and SFA-8 were also found to be resistant to hydrolysis by SGF. BSA, included as a non-plant albumin representative, was found to be very unstable in the presence of SGF, with the 62 kDa band disappearing after approx. 5 s. (Figure 1). Investigations of purified rBer e 1 revealed it to be stable to digestion in SGF for over 15 min, with no fragments visible after 30 min. Experiments with rSFA-8 protein revealed a pattern of digestibility similar to that observed with rBer e 1, with bands and fragments persisting for up to 30 min after the addition of SGF (Figure 1).

CD analysis

The stability of 2 S albumins was further investigated by far-UV CD spectroscopy of rBer e 1 and rSFA-8 during heating and at low pH. At pH 6.8, the secondary structure of rBer e 1 was highly resistant to thermal unfolding (Figure 2A). Temperatures higher than 75 °C were achieved before rBer e 1 started to unfold, while at 95 °C these proteins still retained most of their secondary structure (results not shown). Spectra obtained at pH 2.2 and 25 °C were superimposable with those recorded at pH 6.8, confirming that rBer e 1 was also highly resistant to acidic pH (Figures 2A and 2B). When heated at pH 2.2, rBer e 1 started to unfold at approx. 65 °C. At 95 °C, although the protein was significantly destabilized, the transition was not complete (results not shown). Upon cooling, the denaturation was largely reversible (Figure 2).

At temperatures up to 95 °C at pH 6.8, rSFA-8 also retained most of its native structure, similar to both rBer e 1 and the behaviour described for the corresponding plant protein [14]. rSFA-8 was slightly less resistant to unfolding induced by acidic pH than was rBer e 1 (results not shown).

Discussion

We have demonstrated that the recombinant 2 S albumins Ber e 1 and SFA-8 have considerable

Figure 1

In vitro digestibility in SGF

C represents a sample without the addition of SGF.

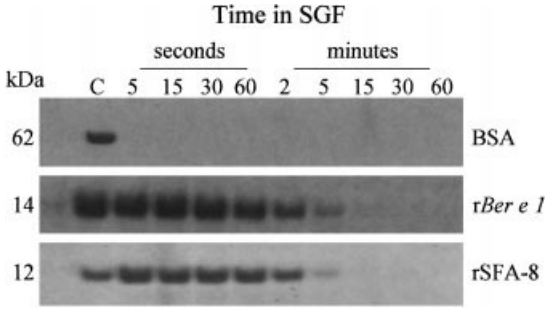

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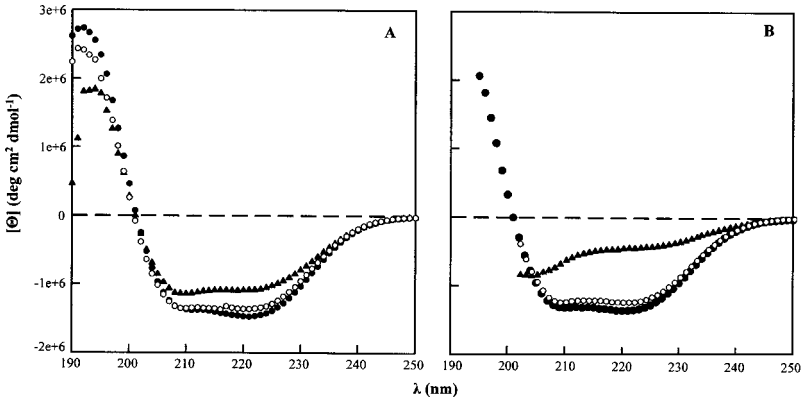
Figure 2

CD spectra of Ber e 1 (0.146 mg · ml^{-1}) under various conditions

Conditions were (**A**) 10 mM phosphate buffer, pH 6.8, and (**B**) 10 mM glycine buffer, pH 2.2. Temperatures: ●, 25 °C upon heating; ▲, 95 °C; ○, 25 °C upon cooling.



stability to hydrolysis by SGF, whereas BSA was hydrolysed after a few seconds. Further, this proteolytic stability was found to be reflected by a high resistance to unfolding induced by heat and acidity. As the 2 S albumins are non-glycosylated proteins, the high stability observed may be explained by their compact three-dimensional structure. Similar structural characteristics are shared by other members of the prolamin superfamily, which accounts for 35% of the allergenic or allergenic-like sequences currently known (www.sanger.ac.uk/software/pfam).

It is of considerable interest that allergenicity of SFA-8 has not been well described, despite common use and consumption. Although IgE binding to sunflower albumins has been observed, it is unclear whether or not SFA-8 is the major allergen [9], and reports of adverse reactions are very rare [8]. This may suggest that stability is only one of a number of characteristics that confer on proteins the ability to provoke selective immune responses.

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