

Short communication

2S methionine-rich protein (SSA) from sunflower seed is an IgE-binding protein*

Background: Sunflower seed contains 2S albumins that in other crops have been associated with allergenicity. The sunflower seed methionine-rich 2S albumin (SSA) may be an IgE-binding protein responsible for anaphylactic reactions in some sunflower seed-sensitive subjects. The objective was to demonstrate that SSA is an IgE-binding protein.

Methods: SSA was purified and the amino-acid sequence determined. The degree of purity of SSA was evaluated by silver staining, and its IgE-binding capacity by immunoblotting with serum from a subject with a convincing clinical history of anaphylaxis to sunflower seed.

Results: The amino-acid sequence confirmed that the purified protein was the mature form of the methionine-rich storage protein SSA from sunflower seed (*Helianthus annuus*). The SSA was specifically recognized by IgE from the serum of the sunflower seed-allergic subject.

Conclusions: SSA is an IgE-binding protein, and subjects allergic to sunflower seed whose IgE binds to SSA are at risk of developing allergic reactions if they consume SSA.

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Food-allergic reactions occur when allergens in the food react and cross-link with specific IgE on the surface of sensitized mast cells and basophils. The cross-linking triggers the release of histamine and other mediators that elicit the symptoms of immediate hypersensitivity reactions. Identification and characterization of allergenic proteins in foods is essential for the development of better diagnostic tools and immunotherapy for food allergy.

Sunflower seed is extensively used in oil form, in margarine, as a condiment, and in bread (1). However, few clinical reports have indicated that sunflower seed causes anaphylactic reactions in sunflower seed-allergic subjects (2–4). The nature of the sunflower seed allergen is not known, but this seed contains 2S albumin proteins that in other seeds, such as rapeseed, castor beans, cottonseed, Brazil nuts, and walnuts, has been associated with allergenicity (5–11). Consequently, the 2S albumin proteins in sunflower seed may also be allergens.

There are eight different albumins in the 2S fraction of sunflower seeds, and one of them is the mature methionine-rich protein designated as SSA (12). This albumin has a molecular mass of about 12 kDa, and consists of four intrachain disulfide bonds in a single polypeptide protein (12–14). One of the unique characteristics of SSA is that it possesses a significant amount of sulfur-containing amino acids, and, as such, can be used to improve the protein quality of other crops by genetic engineering (15, 16).

Previous studies in our laboratory have shown that sunflower seed-allergic subjects have IgE-binding sites to different proteins, including regions of the SSA protein (17). However, this 2S-sunflower seed protein has not been characterized for its IgE-binding capacity. Therefore, the objective of this study was to determine the IgE-binding potential of SSA.

Material and methods

SSA purification

Unsalted raw sunflower seeds were washed, dried, and then defatted with MeOH and acetone. Albumins from the defatted sunflower meal were extracted as previously described (18) and separated by HPLC by a published method (12). The SSA fraction was collected, dialyzed with water, and lyophilized. The fraction was

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resuspended in 10 mM phosphate-buffered saline (PBS) containing 0.02% sodium azide. Amino-acid sequencing was confirmed by Edman degradation at the protein structure core facility of the University of Nebraska Medical Center, Omaha, NE, USA.

SDS-PAGE

SSA protein and unsalted sunflower seed extract were loaded and separated in 10–20% gradient precast gels (Novex, San Diego, CA, USA). After electrophoresis, the gels were either stained by a silver stain kit (Pharmacia Biotech, Piscataway, NJ, USA) or transferred for immunoblotting purposes.

Immunoblotting

The gel was incubated in equilibrating buffer (25 mM Tris-HCl, 192 mM glycine containing 20% MeOH) for 15 min, and then proteins were transferred to a PVDF (BioRad Labs., Hercules, CA, USA) membrane with a semidry blotter (Labconco, Kansas City, MO, USA). The membrane was then blocked with 1% casein in PBS, 0.025% Tween 20. The membrane was cut into strips before incubation with either serum from a sunflower seed-allergic subject or a negative control pool (1:10 diluted in casein blocking solution) overnight at 4°C. The strips were then washed four times with PBS solution containing 0.025% Tween 20. Membranes were then incubated with goat antihuman IgE horseradish peroxidase conjugate (Bethyl Labs., Montgomery, TX, USA, 1:1000) for 4 h at 4°C. The membranes were washed again four times as before, and chemiluminescent reagents (Amersham, Arlington Heights, IL, USA) added for 1–2 min before exposing membranes to radiographic film (Amersham, Arlington Heights, IL, USA).

Results

SDS-PAGE

A single protein with a mass of 12 kDa was detected. When gel lanes were overloaded, other minor proteins appeared (Fig. 1). The apparent degree of purity was about 90% by amino-acid sequencing.

Immunoblotting

IgE from the sunflower seed-allergic individual recognized the purified SSA and also other proteins in the sunflower seed extract, including one with a mass of 12 kDa, similar to that of the SSA (Fig. 2). Negative control sera did not recognize either the purified protein SSA or the raw sunflower extract (data not shown).

Discussion

The mature form of the SSA protein, due to its high degree of sulfur-containing residues, is being used to improve the protein quality of other plants such as alfalfa and lupin for use in animal feed (15, 16). However, a complete evaluation of its allergenic potential is necessary if transgenic plants containing this protein are to be used for human consumption, since there are reports of anaphylactic reactions associated with sunflower seed. However, to date, no specific protein has been characterized as the major sunflower seed allergen. Previous work in our laboratory has demonstrated that different sunflower seed-allergic subjects recognize different IgE-binding proteins, including one that has a similar mass and pI to that of mature SSA protein (17). The current data conclusively demonstrate that the mature methionine-rich 2S albumin known as SSA in sunflower seed is an IgE-binding protein.

The immature form of the SSA is about 16 kDa (1–141 a.a. residues); during seed maturity, it is cleaved at two residues (25 and 39) to form the mature polypeptide (19). The mass of the mature SSA is about 12 kDa (residues 39–141), and the protocol to purify this protein has been described previously (12). The purity of the mature SSA fraction was confirmed

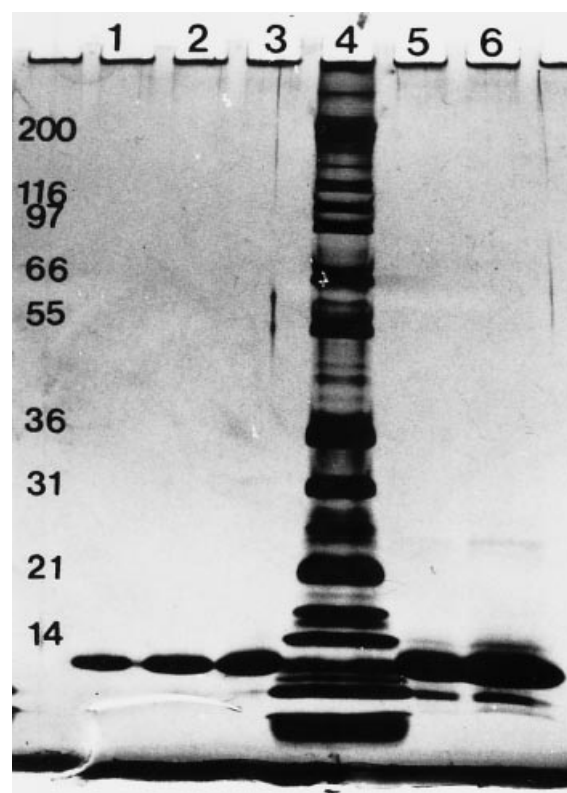


Figure 1. Silver-staining of SDS-PAGE of purified SSA. Lanes 1–3, 5, and 6 correspond to 0.8, 1.6, 3.3, 6.6, and 13.2 µg protein; lane 4 is molecular weight marker lane.

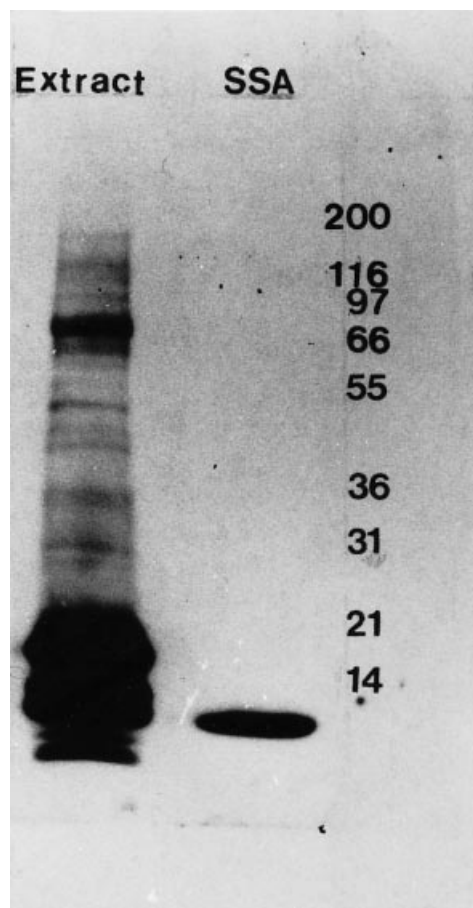


Figure 2. IgE immunoblotting of purified SSA (8.25 μ g) and raw sunflower seed extract (56 μ g) with sunflower seed-allergic serum.

by amino-acid N-terminal sequencing and silver staining. The sequence of the first 15 amino acids is PYGRGRTESG CYQQM, which matches 100% with residues 39–53 of the immature protein (accession number P23110 in the SwissProtTM database).

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The IgE-binding associated with SSA seems to be specific. Even though the protein was at least 90% pure by amino-acid sequencing analysis, IgE from the sunflower seed-allergic individual bound to only the 12-kDa SSA specifically (Fig. 2). No binding was observed to any of the other minor proteins detected by silver staining (Fig. 1).

IgE from the sunflower seed-allergic subject recognized a protein in the extract with the same mass as the mature purified SSA as well as other proteins. The IgE-binding proteins of 14–21 kDa may include the immature SSA (16 kDa). The recognition of the immature SSA is not surprising since any epitopes associated with the mature form will also be present in the immature protein.

Any potential allergen must have at least divalent epitopes, which may be either linear, conformational, or a combination of the two. Since sunflower seed-allergic IgE bound to denatured SSA in the immunoblotting study, it is suggestive that SSA has linear epitopes, but the number of linear epitopes and the contribution of conformational epitopes are not known. Research is in progress in our laboratory to identify the number of linear and conformational epitopes in SSA.

One of the limitations of this study is the lack of enough sunflower seed-sensitive subjects to determine more adequately whether SSA is the major allergen. In spite of that, these findings show that SSA is an IgE-binding protein, and any transgenic crops containing this protein may cause severe allergic reactions in some sunflower seed-allergic individuals.

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