

Original article

Characterization of bovine serum albumin epitopes and their role in allergic reactions

Objective: This review provides updated information on conformational and sequential epitopes identified in bovine serum albumin (BSA) and summarizes available data about the role of structural modifications on BSA antigenicity/allergenicity.

Data sources: Data on beef allergy and BSA antigenicity are reported, with reference both to the basic literature and to clinical results obtained by our group.

Results and discussion: BSA is an important allergen involved in milk and beef allergy. The presence of conformational epitopes has been suggested by indirect evidence, while at least one sequential epitope has been experimentally identified. The role of structural modifications on BSA antigenicity is discussed as well as the increased tolerance observed in allergic subjects consuming beef as strained (homogenized) and freeze-dried derivatives.

Conclusion: Study of the molecular characteristics of a known major allergen allows the identification of technological processes that may be capable of improving the tolerance of allergic subjects to a specific food. Even though any hoped for reduced allergenicity must be verified under medical supervision, the use of new products could obviate the need to avoid important foods such as meat in childhood.

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Immediate allergic reactions (type 1) are associated with the binding between circulating immunoglobulin E (IgE) and the corresponding allergens, which are mainly proteins. IgE recognizes specific conformational and linear molecular structures on allergenic proteins. An epitope is defined as *linear* if IgE binds a series of adjacent amino acids with no requirement for a particular secondary or tertiary structure, whereas a *conformational* epitope is strictly dependent on the folding of the protein chain.

Conformational epitopes are involved mainly in inducing clinical reactions when an allergic subject is exposed to aeroallergens, but they can also mediate reactions to food allergens (oral syndrome, lip edema, etc.). However, most food allergens involve sequential epitopes that maintain their binding capacity even after the structural modifications caused by strong acidic conditions in the stomach or the proteolytic activity of gastrointestinal enzymes. Conformational and linear epitopes are differently affected by technological treatments.

Any antigenic molecule may present several different epitopes, but in most allergens only a few are immunodominant. The characterization of such immuno-

dominant epitopes clarifies the reactions in which they are involved, and in some cases the technological treatments capable of reducing the antigenic potential of a food protein can then be identified.

The three-dimensional structure of most antigenic proteins is unknown even where the amino acid sequence has been precisely identified, because their conformation is not immutable but is influenced by the surrounding environment. However, in bovine serum albumin (BSA), as in some other allergens, covalent bonds maintain the tertiary structure under denaturing conditions (e.g. low pH or heating), and its conformation is therefore well known. BSA is involved both in milk (5–8) and in beef (9–11) allergy. Its tertiary structure is stable, and its three-dimensional conformation is therefore well known.

The protein is organized in three homologous domains (I–III) and consists of nine loops connected by 17 covalent disulfide bridges (Fig. 1) (1–4). Most of the disulfide bonds are well protected in the *core* of the protein and are not readily accessible to the solvent. We have examined the effect of denaturing conditions on the structure and allergenicity of BSA.

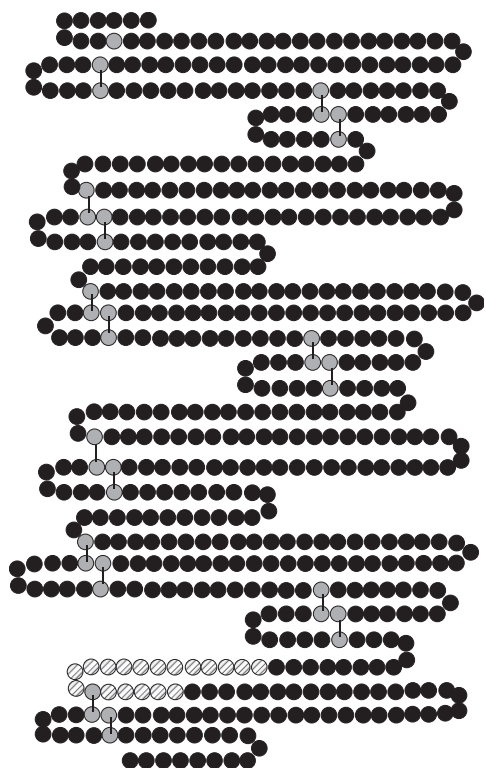


Figure 1. BSA amino acid sequence: the molecule is made up of three homologous domains brought into nine loops by 17 disulfide bonds. Gray units represent cysteine residues, all involved in S-S bonds apart from the 10th amino acid of the sequence (residue 34 of the precursor). The major sequential epitope is indicated by the sequence of barred units.

BSA conformation and antigenicity

BSA induced immediate allergic symptoms (lip edema, urticaria, cough and rhinitis) in four children allergic to beef who received the protein in a double-blind, placebo-controlled food challenge (9). The effects of heating, chemical denaturation, or reduction of disulfide bonds in BSA on its binding to specific circulating IgEs from the same four subjects have been studied (11).

BSA was examined by gradient polyacrylamide gel electrophoresis both under native conditions and after different denaturing treatments (Fig. 2). Lane A in Fig. 2 shows that under native conditions BSA exists in dimeric and trimeric forms; this is the result of the strong reactivity of the sole free -SH group (10th amino acid of the sequence illustrated in Fig. 1). After being at 100°C for 10 min, BSA shows several bands (Lane B); the component with the highest electrophoretic mobility (at the bottom of the gel) corresponds to the protein in its monomeric form. The other bands derive from the polymerization brought about by heat. Different types of chemical bonds (intermolecular bonds and hydrophobic interactions) are responsible for the complex electrophoretic pattern. When native or heat-treated BSA

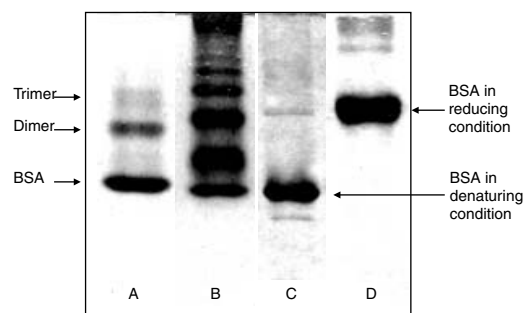


Figure 2. Polyacrylamide gel electrophoresis of BSA in different conditions; for details of methods see ref. (11). A = native BSA, B = heated (100°C 10 min) BSA in native gel, C = BSA in denaturing gel (containing sodium dodecyl sulfate), D = BSA after reduction by β -mercaptoethanol.

is denatured by treatment with sodium dodecyl sulfate, the molecule loses all noncovalent bindings and its electrophoretic run is similar to that obtained in native conditions (Lane C). When the protein was treated with a reducing agent (β -mercaptoethanol), the electrophoretic pattern (Lane D) contained only one band corresponding to a monomeric BSA with a lower mobility than the native form because the three-dimensional structure has been drastically modified by the reduction of disulfide bridges and the breaking of nine loops. In this new linear condition, BSA is larger overall and consequently has lower electrophoretic mobility.

Immunoblotting after incubation of the gels in Fig. 2 with the serum of one of the allergic children is shown in Fig. 3. The circulating IgEs reacted with BSA under all conditions, although positivity was lower after the reducing treatment (Fig. 3, Lane D). This reduction was less sizeable than that reported in previous studies, where the conservation of the disulfide bridges has been indicated as a critical factor for maintaining the BSA

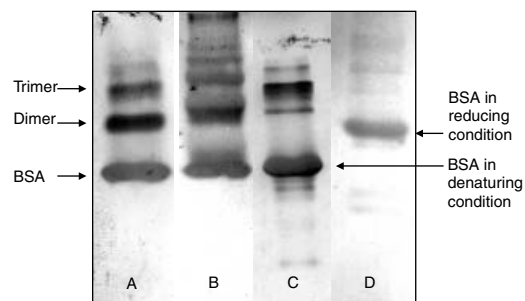


Figure 3. Immunoblotting of BSA in different conditions; the incubation was performed with the serum of a child who had a severe allergy to beef. Methods used are reported in ref. (11). A = BSA in native condition, B = heated (100°C 10 min) BSA, in native gel, C = BSA in denaturing gel (containing sodium dodecyl sulfate), D = BSA in reducing condition (use of β -mercaptoethanol).

antigenicity (12). The presence of the loops is critical for maintaining the conformational epitopes of BSA but, as shown in our previous papers (13), BSA contains at least one sequential epitope corresponding to the amino acids 524–542 (Fig. 1).

Bovine serum albumin digestibility and antigenicity

Although very soluble in water and rich in amino acids recognized by gastrointestinal enzymes, BSA is relatively resistant to digestion. Sequential epitopes were able to resist at least for 60 min when BSA was digested *in vitro* with pepsin at the ratio of enzyme to substrate of 1 : 120 (w/w) (13). The presence of the nine loops maintained by disulfide bonds that are not easily reduced under physiological conditions slows the fragmentation of BSA into short peptides with decreased antigenic activity. The suggested long-lasting allergenicity of different serum albumins was demonstrated in our previous work (9), where one child presented a delayed reaction 3 h after the protein administration in the double-blind, placebo-controlled food challenge.

Technological treatments and BSA antigenicity

It is well known that some food treatments can modify allergenic properties. Twelve children allergic to beef, and reactive to BSA showed almost no positive responses in either a Skin Prick Test or a RadioAllergoSorbent Test when beef was used as strained or freeze-dried products (Table 1). The lower BSA content and/or immuno-reactivity in these products could be responsible. The

antigenicity of BSA to monoclonal anti-BSA antibodies shows (Fig. 4B) a critical decrease of reactivity in strained and freeze-dried products, even when the overall protein concentration in the different samples is similar (Fig. 4A). The reduced allergenicity after technological treatments could also be due to the greater digestibility of strained or freeze-dried meat; we have shown previously (14) that the enhanced proteolysis reduces protein antigenicity.

In conclusion, strained and freeze-dried beef seem better tolerated by allergic subjects. Even though any hoped-for hypoallergenicity of processed beef must be verified under medical supervision, the use of new technologies may be able to avoid the need to remove important foods, such as meat, from the diet of allergic children.

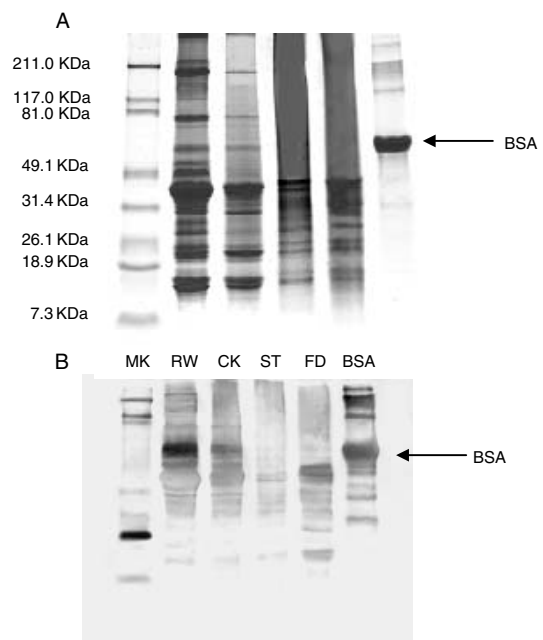


Figure 4. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (A) and immunoblotting (B) of beef samples, obtained loading the same protein amounts. Membrane was incubated with anti-BSA monoclonal antibodies. Methods are reported in ref. (14). MK = marker solution, RW = raw beef; CK = cooked beef; ST = strained beef; FD = freeze-dried beef.

Table 1. Positive responses observed in 12 children allergic to beef and reactive to BSA

Product	SPT	RAST
Raw beef	12	12
Cooked beef	12	12
Strained beef	0	0
Freeze-dried beef	1	1

SPT, skin prick test; RAST, radioallergosorbent test.

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