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Studies on the antigenic properties of serum albumins

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SUMMARY

Bovine serum albumin (BSA) is one of the most widely studied proteins; its structure is well known and its antigenic properties have been described in animal models. The aim of our work was to evaluate the role of conformation on antigenicity of serum albumins. This study was performed using electrophoresis associated with the immunoblotting technique, where sera from children allergic to BSA were used. Data obtained in this research indicate that serum albumin antigenicity is only partially correlated to its native three-dimensional structure. Heat treatment and chemical denaturation (SDS treatment) are not able to significantly decrease its capability to bind circulating IgEs. Only the reducing treatment is able to modify the antigenicity of this protein. Moreover, a direct correlation between the cross-reactivity observed in immunoblotting between different serum albumins and the percentage of their sequence identity (phylogenetic similarity of the species) was shown.

INTRODUCTION

Bovine serum albumin is one of the most widely studied proteins; its complete amino acid sequence [1] and its three-dimensional conformation [2,3] have been identified. The molecule shows three homologous repeated units (1–190, 191–382, and 383–581), consisting of nine disulfide-bonded loops [4].

The antigenic activity of serum albumins is well known and their involvement in different food allergies and bird egg allergy has already been reported [5–9]. Different authors have shown a reduction of antigenicity by heat denaturation of whey proteins, including BSA [10,11]. The presence of disulfide bonds in BSA has also been indicated as an important

factor in maintaining the antigenic activity of this molecule; Habeeb and Borella showed that the reduction of disulfide bonds, which is associated with important conformational changes, completely abolishes the reaction of BSA with anti-BSA antibodies [12].

In this paper, we correlate the effect of conformation of serum albumins with their capability to bind circulating human IgEs; cross-reactivity between serum albumins from different animal species was also investigated.

MATERIALS AND METHODS

Serum albumins

BSA (bovine serum albumin), CSA (chicken SA), HSA (human SA), HoSA (horse SA), OSA (ovine, sheep SA), PSA (pork SA), RSA (rabbit

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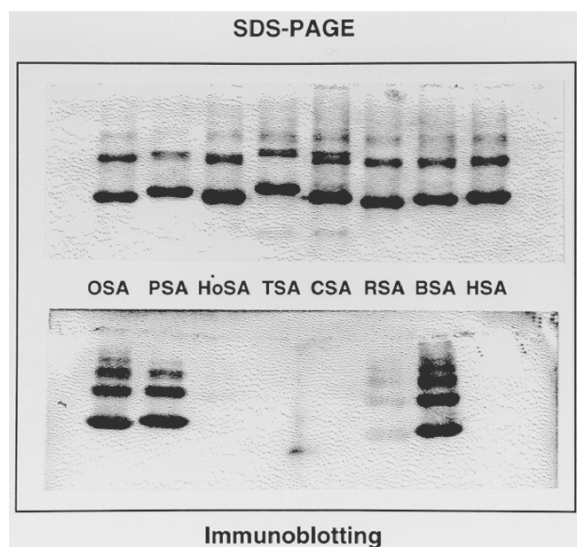


Fig. 1. SDS-PAGE and immunoblotting of native serum albumins (SA): OSA = sheep SA, PSA = pig SA, HoSA = horse SA, TSA = turkey SA, CSA = chicken SA, RSA = rabbit SA, BSA = beef SA, HSA = human SA.

SA) and TSA (turkey SA) were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.) at the highest available purification level. The albumins were solubilized in the sample buffer (0.25 M Tris (hydroxymethyl) aminomethane (TRIS)-HCl buffer, pH=6.8, containing 7.5% glycerol, 2% SDS, with or without 5% 2-ME) at a final concentration of 1 mg/ml and heated for 10 min at 100 °C. The native sample buffer (used for native and heated proteins) had the same composition reported above, but SDS and 2-ME were not included. Moreover, under native conditions no heating was applied to the sample.

Heated samples were obtained by heating solutions of serum albumins (2 mg/ml in water) for 10 min at 100 °C.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

All chemicals and instruments used in the electrophoretic analyses were purchased from BioRad (Richmond, CA, U.S.A.). The electrophoretic run was performed on a gradient (9–19%) polyacrylamide gel as previously described [6]. After the electrophoretic run (90 V at room temperature, for approximately 6 h), gels were dyed with Coomassie Brilliant Blue G-250 according to the method of

Neuhoff et al. [13] or used to transfer the proteins onto membranes by Western blotting. When gels were run under native conditions (native and heated samples), SDS was not included in gels and running buffer.

Immunoblotting

After SDS-PAGE, immunoblotting was performed as described previously [6]; when gels were run under native conditions, methanol was not included in the transfer buffer. 'Inhibition immunoblotting' was carried out in a similar way as the usual immunoblotting, apart from an incubation of sera with BSA (3.75 µg/ml) before its contact with proteins transferred onto the membrane.

Circulating IgEs

Sera were from children allergic to BSA, as shown by *in vitro* and *in vivo* tests (skin prick test, RAST, double blind placebo controlled food challenge).

RESULTS

The electrophoretic distribution and immunoblotting of serum albumins, from different animal species and under native conditions, are shown in Fig. 1. Electrophoretic mobilities of serum albumins were very similar, as expected from their similar MW. In the immunoblotting membrane, IgEs from the serum of the child shown here recognized BSA, OSA and PSA with the same avidity, while the reactivity with RSA was very weak.

All children tested showed circulating IgEs against native BSA, as expected, since the BSA sensitization was our criterion for inclusion in the study. On the other hand, no positive response was observed for chicken and turkey serum albumins.

In Fig. 2 we report, in parallel, the number of positive responses obtained in immunoblotting for every native serum albumin and the percentage of their sequence identity with BSA. The percentage of sequence identity between BSA and other serum albumins (when the sequence was known), and the number of positive responses were directly correlated. It is important to underline that the small difference observed in the percentage of identity

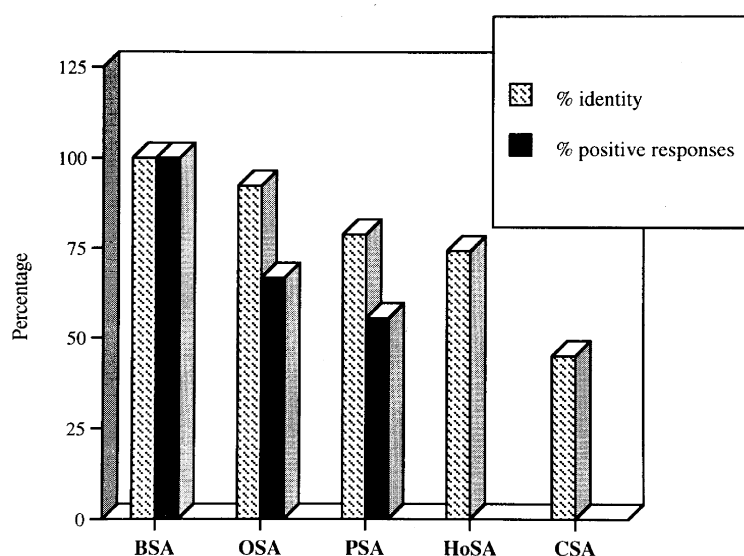


Fig. 2. Relationship between sequence identity of different serum albumins and positive responses obtained under native conditions in immunoblotting (no. of subjects = 9).

between PSA and HoSA (78.84 and 74.14%, respectively) and BSA produced a remarkable reduction of positive responses.

To evaluate if the observed phenomenon was a real cross-reactivity or a co-sensitization of the subjects, inhibition immunoblotting was performed. In this experiment, BSA was pre-incubated with the serum, before its introduction in the incubation chamber, where the binding between standard proteins and IgEs occurs. The pre-incubation undid all positive responses (binding to IgEs).

In Fig. 3, electrophoretic and immunoblotting patterns of serum albumin from four animal species are shown. Native proteins are illustrated on the left and heat-treated serum albumins on the right. Heat-treated serum albumin patterns showed a higher number of bands compared to the same untreated proteins; between them the component having the highest electrophoretic mobility corresponds to the protein in its monomeric form.

In Fig. 4, IgE-mediated positive responses against serum albumins under the four studied conformational conditions are summarized. IgEs from subjects allergic to BSA showed the same reactivity for native and heated serum albumins apart from one child who showed negative response against

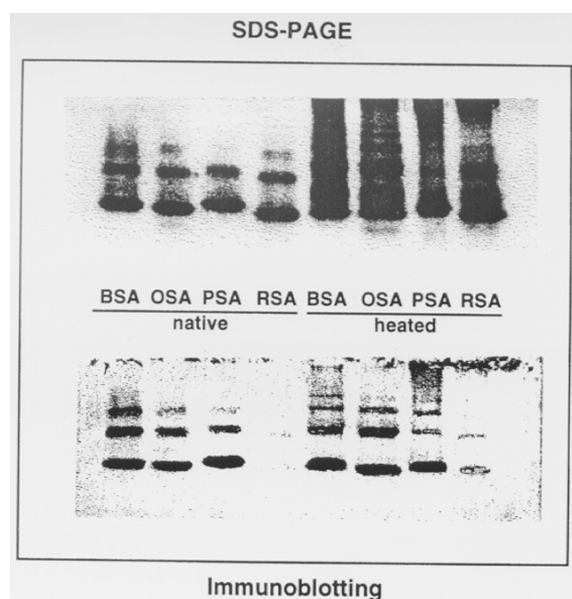


Fig. 3. SDS-PAGE and immunoblotting of native and heated serum albumins. The serum was from the same child as the one described in Fig. 1. BSA = beef SA, OSA = sheep SA, PSA = pig SA, RSA = rabbit SA.

heated PSA. When chemical denaturation of serum albumins was obtained by SDS treatment, a weak reduction of responses was observed only with PSA. Finally, as a consequence of the reduction of the

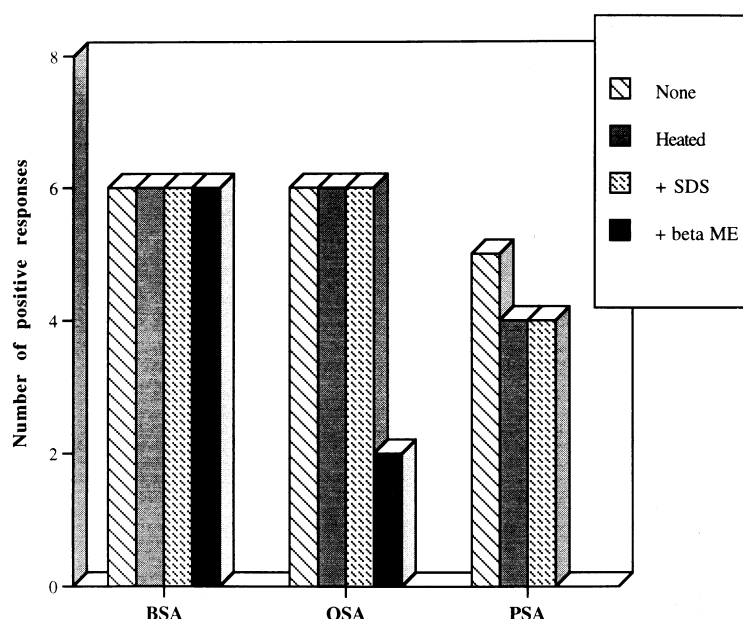


Fig. 4. Number of positive responses obtained in immunoblotting after thermic and chemical denaturation of serum albumins (no. of subjects = 6).

disulfide bridges by 2-ME treatment, only BSA (and partially OSA) was still capable of binding IgEs.

DISCUSSION AND CONCLUSIONS

Serum albumins are important proteins not only for their presence in blood, where they act as carriers of different substances, but also for their presence in some foods. Among these, milk is one of the most widely investigated and BSA has been reported as one of the major milk allergens [5,6]. Whey proteins are considered to be heat labile and it has been reported that their antigenicity can be reduced by heat treatment [14]. The reason for this behavior is the structural modification observed when the temperature is increased. It has also been suggested that the conservation of disulfide bridges is important for maintaining BSA antigenicity [12]. These have the effect of stabilizing the tertiary structure of the native protein, characterized by the presence of loops with repeated amino acid sequences [15].

Although data exist on the reduction of antigenicity of total whey proteins after heating in *in vitro* and animal models [11,12], little is known about the antigenicity of heated BSA in humans. It would also be

interesting to investigate the binding between human IgEs and serum albumins after different structural modifications, since such data could supply new information on IgE-mediated reactions associated with BSA.

Sera used in this study were from children clinically allergic to BSA. In immunoblotting performed with protein under native conditions, the number of positive responses correlated well with the percentage of sequence identity (at least for sequences reported in the literature). The remarkable reduction of positive responses, observed when PSA and HoSA (only 4% difference in the percentage of sequence identity with BSA) are considered, can be justified by a specific change in the sequence of the epitopic area.

The direct correlation between structural identity and antibody binding suggested the occurrence of cross-reactivity between different serum albumins. This cross-reactivity was experimentally demonstrated by using the competitive immunoblotting technique, where a preincubation of sera with BSA (but not with CSA or TSA) totally abolished the subsequent binding of the IgEs to the BSA on the membrane.

As far as the role of the conformation on the antigenicity of serum albumins is concerned, heat denaturation only rarely modified the antigenicity of serum albumins. Only the chemical reduction of disulfide bridges, obtained after 2-ME treatment, was able to reduce the antigenic activity of serum albumins significantly in our experimental model. However, even under reducing conditions the antigenicity of BSA is well preserved.

Since some of the beef-allergic children (allergic to BSA) can tolerate sheep and pork, we hypothesized that the differences found in amino acid sequences between the different serum albumins could be important for their susceptibility to proteolysis, as shown for OSA in one of our previous papers [9]. Further studies are therefore in progress in order to verify serum albumin antigenicity after controlled proteolysis. This could be important not only to improve our knowledge of the chemistry of these proteins (epitope identification), but also to better evaluate their role in food allergy.

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