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Structure and antigenicity changes in 7S soyabean allergen by enzymic deglycosylation

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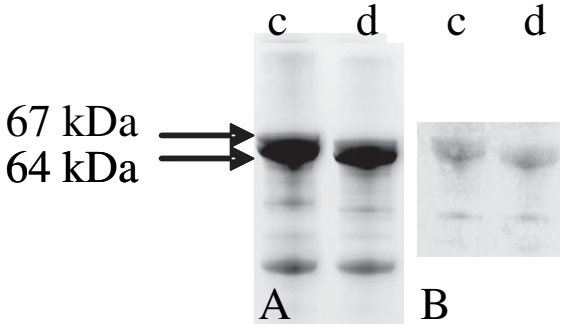
Soyabean allergy is one of the most common food allergies. The marked rise in allergenic reactions to soyabean is related to the increasing use of soya products in processed foods, which convert it in a major public health and soyabean industry concern. Different processes have been used to mask soyabean allergenicity, including heat treatment, fermentation, enzymic proteolysis, carbohydrate conjugation, genetic modification, extrusion and agronomic practices[1]. It is still unclear which of the components of soyabean cause allergenic reactions. Some authors have identified 7S as a major soybean allergen[2]; 7S is a N-glycoprotein that constitutes about 25% of the total soyabean proteins[3]. Glycans forming glycoproteins have been described as being responsible for the allergenicity of some plants and insects[4] and their removal has been proposed to reduce allergenicity[5]. PNGase F is an enzyme used to deglycosylate different N-glycans such as those present in ribonuclease B, among others. No studies focused on deglycosylation of 7S by PNGase F activity for reducing its allergenicity have been found in the literature. The aim of the present research was to assess enzymic deglycosylation as a strategy for reducing soyabean protein allergenicity.

7S was isolated by isoelectric precipitation and the protein purity checked by SDS–PAGE. Enzymic deglycosylation was carried out on the isolated 7S (1 mg/ml) with PNGase F at pH 8 and 37°C for 24 h. Deglycosylation was followed by reversed-phase HPLC, SDS–PAGE and capillary zone electrophoresis analyses, which provided information relating to changes in protein structure. Antigenicity was assayed by immunoblotting and indirect ELISA with polyclonal rabbit anti-soyabean sera and horseradish peroxidase-labelled goat anti-rabbit IgG.

The Figure shows SDS–PAGE (A) and immunoblotting (B), corresponding to the 7S control (c) and its deglycosylated form (d). As can be observed, the electrophoretic mobility of 7S changed as a result of deglycosylation, indicating a decrease in protein molecular mass. Immunoblotting and ELISA data suggest that the carbohydrate moiety forming 7S does not have a key role in its *in vitro* antigenic response as assayed in the present study. Further studies should be conducted in order to confirm the results by employing digestion and absorption assays *in vitro* and *in vivo* as well as an immunological test using serum from patients allergic to soyabean.



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