

THE POTENTIAL OF NARROW-LEAFED LUPIN PROTEINS FOR BINDING SPECIFIC AND UNSPECIFIC IGEs FROM HUMAN SERA

Patrycja Kłos¹, Elżbieta Poręba², Ewa Springer³, Eleonora Lampart-Szczapa¹ and Anna Goździcka-Józefiak²

¹Department of Food Biochemistry and Analysis, August Cieszkowski Agricultural University, Poznań, Poland

²Department of Molecular Virology, Adam Mickiewicz University, Poznań, Poland

³SNZOZ Alergologia Plus, Center of Diagnostics and Treatment of Allergy, Poznań, Poland

Corresponding author's email: paattison@wp.pl

ABSTRACT

Since lupin has been introduced as a food ingredient on the market there are more and more reports concerning the potential of its proteins to bind human IgEs. However, it is yet to be proved if lupin proteins can bind only specific IgEs from the sera of people who suffer from lupin allergy. The aim of the research has been to analyse the potential of lupin globulins for binding IgEs from the sera of people who are not allergic to any legume species. Isolated lupin globulins have been subjected to immunoblotting with the sera from non-legume allergic individuals and the control sera from lupin allergic people, respectively. The immunoblotting experiment has been doubled, with a use of methyl α -D-galactopyranoside, to eliminate possible unspecific binding of IgEs to blotted proteins. Western blot analysis performed with the sera of non-legume allergic patients has revealed protein signals only in the blots that had not been incubated with galactopyranoside. When dealing with the sera of lupin allergic patients, the protein bands have been detected irrespective of the use of galactopyranoside. The results have proved that lupin globulins not only have the potential for binding specific IgEs from the sera of lupin allergic people, but also they can bind unspecific IgEs from the sera of non-allergic people.

KEYWORDS

Narrow-leaved lupin, allergy, immunoblotting, IgE

INTRODUCTION

Since lupin has been introduced as a food ingredient on the market there are more and more reports stating that it may be a cause of IgE-mediated allergic reactions. It has been well established that ingestion of lupin-containing food products may cause life-threatening allergic reactions in people who are sensitive to other legumes (peanuts and soybean among them), because of the cross-reactivity between the proteins from legume plants (Faeste *et al.* 2004).

Lupin flour also has been recognised as a cause of occupational airborne allergy (Crespo *et al.* 2001). Some of lupin globulins has been identified as IgE-binding allergens (Rojas-Hijazo *et al.* 2006). However, it is yet to be established if lupin proteins can bind only specific IgEs from the sera of people who suffer from lupin allergy, or if they also bind unspecific IgE molecules. Therefore the aim of the research has been to analyse the potential of lupin globulins to bind IgEs from the sera of people who are not allergic to lupin nor to other legume species.

MATERIALS AND METHODS

ISOLATION AND FRACTIONATION OF LUPIN GLOBULINS

Lupin globulins were isolated (Freitas *et al.* 2000) from narrow-leaved lupin flour (*Lupinus angustifolius*, var. Baron), supplied by the Institute of Food Technology of Plant Origin (Poznań University of Life Sciences), and fractionated by means of gel filtration chromatography (glass column packed with Sephadex G 200, Labart). Three globulin fractions with the protein concentration of 676 μ g/mL, 177 μ g/mL and 144 μ g/mL (Bradford, 1976), respectively, were numbered from 1 to 3 and subjected to some further analysis.

SDS-PAGE AND IMMUNOBLOTTING

The sera from four non-atopic, non-legume allergic individuals and the control sera from four people with the positive history of lupin allergy and positive skin prick tests (SPTs) with lupin protein extracts were used. The research protocol was approved by the Bioethical Commission of the University of Medical Sciences in Poznań.

Globulin fractions 1 to 3, with protein concentration adjusted to 144 μ g/ml, by use of 50 mM Tris-HCl buffer (pH 7,5), were resolved by means of SDS-PAGE (Laemmli, 1970), with 10% acrylamide concentration. Protein weight marker (Page Ruler™ Prestained Protein Ladder, Fermentas) with protein molecular weights ranging from 10 to 170 kD was used. Separated electrophoretically globulins were transferred onto PVDF membranes (Immobilon-P, Millipore) by means

of electroblotting (Fastblot, Biometra), with 30V voltage, or stained with Coomassie Brilliant Blue R-250. The membranes with transferred proteins were blocked with 1X PBS-T containing 3% BSA as a blocking agent (4°C, overnight) and then incubated for 1h, at room temperature, with the patients' sera diluted in the ratio of 1:100 with 1X PBS-T containing 1% BSA. After that, the blots were incubated for 1h, at room temperature, with polyclonal goat anti-human IgE-HRP antibodies (Bethyl Laboratories) diluted in the ratio of 1:1000 with 1X PBS-T containing 1% BSA. Three washes (for 10 min each) with 1X PBS-T were done before and after each incubation step. Some of the blots were washed with 1X PBS-T with the addition of 10% methyl α -methyl-D-galactopyranoside (Fluka, Sigma). A galactopyranoside was used to eliminate possible unspecific binding of IgEs to blotted proteins. All the blots were developed with ECL Plus (Amersham Biosciences) and the image of lupin globulins binding human IgEs was visualised on the X-ray membranes, with the time of exposure -1 min.

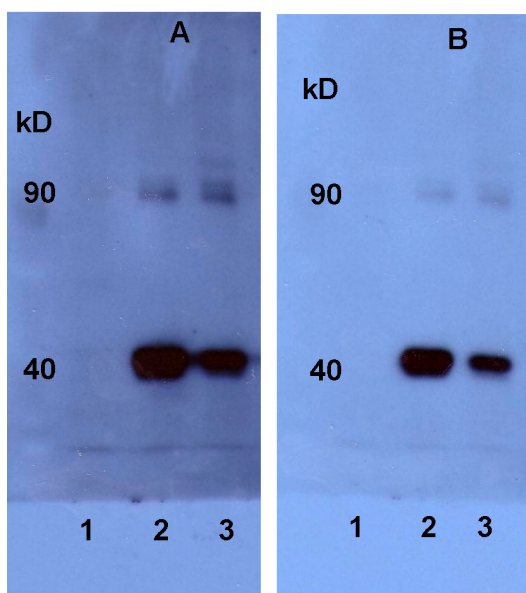


Fig. 1. Blotted lupin globulins binding IgEs from the one of the investigated sera (A), and the one of the control sera (B). Incubation **without** α -methyl-D-galactopyranoside. 1, 2, 3, - analysed globulin fractions.

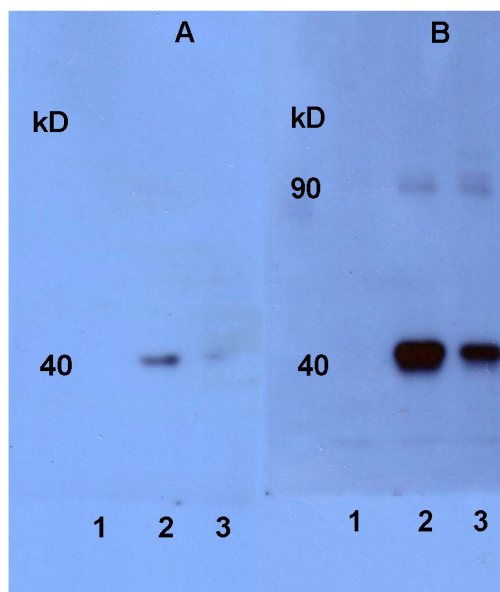


Fig. 2. Blotted lupin globulins binding IgEs from the one of the investigated sera (A), and the one of the control sera (B). Incubation **with** α -methyl-D-galactopyranoside. 1, 2, 3, - analysed globulin fractions.

RESULTS AND DISCUSSION

MOLECULAR WEIGHTS OF INVESTIGATED GLOBULINS

The analysis of molecular weights of lupin globulins from the three investigated globulin fractions was performed by means of SDS-PAGE along with Coomassie Brilliant Blue staining. The results showed the presence of proteins of around 35-55 kD in the fraction no. 1 and the proteins around 35, 40, 55 and 90 kD in the fraction no. 2 and 3 (data not shown).

THE POTENTIAL FOR BINDING UNSPECIFIC IGEs

The potential of lupin globulins for binding unspecific IgEs was analysed by using immunoblotting method with the sera of people who are not allergic to lupin (investigated sera). The results achieved showed that globulins from fraction no. 2 and 3, with molecular weights of around 40 kD and 90 kD, bind IgEs from the investigated sera of non-legume-allergic patients (Fig. 1A). The incubation of globulins with the control sera of lupin-allergic people resulted in the same protein binding pattern (Fig. 1B). However, the addition of α -methyl-D-galactopyranoside during the washing steps caused almost complete elimination of binding of IgEs from the investigated sera by lupin globulins (Fig. 2A). In spite of using the galactopyranoside, the binding pattern of globulins incubated with the control sera remained unchanged (Fig. 2B). The binding of IgEs by globulins from the fraction no. 1 was not observed in any of the experiment variants.

The results of the study proved that not only can lupin globulins bind specific IgEs from lupin-allergic patients' sera, but also they can bind IgEs from the sera

of people who are not allergic to any legume species. The majority of lupin globulins have the affinity for carbohydrates, mainly galactose (Falcón *et al.* 2000). This may lead to unspecific binding of IgEs, which Fc fragments are highly glycosylated (Robertson & Liu, 1991), by the globulins. The use of an appropriate carbohydrate, such as α -methyl-D-galactopyranoside, may block the binding of unspecific IgEs through their Fc regions by the globulins. Simultaneously, this may visualise the paratope-epitope reaction of specific IgEs and allergens. The affinity of lupin globulins for Fc fragments of IgEs does not, probably, play a role in inducing the IgE-mediated allergic reaction.

ACKNOWLEDGMENTS

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