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IMMUNOLOGICAL SIMILARITY OF OAT PROTEINS WITH PEANUT ARA h1 ALLERGEN

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Oat is considered as one of food raw materials of high nutritional value. Its proteins are characterised by very high content of globulins rich in exogenous amino acids as compared to other cereal proteins. In spite of the positive nutritional aspects, oat consumption can result in some human health threats, as oat proteins have been demonstrated to be able to cross react with wheat anti-gliadin polyclonal antibodies. The aim of this work was to determine the cross reactivity between Ara h1 allergen present in peanuts (*Arachis hypogaea*) and proteins of oat grains. The cross reactivity of oat proteins with Ara h1 allergen was determined with the use of RIDASCREEN Peanut test. In our study, it was shown that within oat protein structures there are hidden similar determinants immunologically comparable to those found in Ara h1 allergen, and temperature appears to be a very important factor which can affect their revealing and thus increase their availability to anti–Ara h1 allergen antibodies.

INTRODUCTION

Oat due to its high nutritional value is considered as one of food raw materials of high importance and is grown world-wide. In Poland only 3% of total oat crops (about 2.2 mln tons per annum) have been intended for food production [Bartnikowska *et al.*, 2000], however its dietetic significance is on increase, as its biological value is more and more recognised.

Oat proteins are those nutritional components which gained special attention as they are characterised by very high content of globulins rich in exogenous amino acids as compared to other cereal proteins [Gąsiorowski, 1998]. On average the higher content of lysine (4.2%), treonine (3.3%), phenyloalanine and tyrosine (above 8.8%), tryptophane (1.3%), methionine (above 3%) as well branched amino acids was found. The amino acid composition of oat proteins contributes to its high biological value.

In spite of the positive nutritional aspects, oat consumption can pose some threats to human health. A number of studies [Picarelli *et al.*, 2001; Hollen *et al.*, 2003; Janatuinen *et al.*, 2000; Kumar & Farthing, 1995; Varjonen, 1995; Hoffenberg *et al.*, 2000; Rocher *et al.*, 1992] have been devoted to oat proteins and their possibility to induce coeliac disease in humans. In the study of Rocher *et al.* [1992], it has been demonstrated that high molecular avenins present in oat can cross react with wheat anti-gliadin polyclonal antibodies, which implies the possibility of the similar immunogenic reactivity of oat proteins and leads to a conclusion that oat prolamin shows immunoreactive properties in coeliac disease. The other threat, as it was previously found, can result from the presence of similar structures of Ara h1 antigen in oat.

The aim of this work was to determine the cross reactivity between Ara h1 allergen present in peanuts (*Arachis hypogaea*) and proteins of oat grains.

MATERIAL AND METHODS

Material. Oat grains (cul. var. Deresz) were obtained from the Seed Central Company in Olsztyn, Poland.

Protein extraction. The following procedures of protein extraction were applied in the study:

A. The protein extract was prepared from the grains using 0.02 mol/L phosphate buffer, pH 8.0 at a 1:3 (w/v) ratio. Extraction was performed at a temperature range of 0–4°C for 60 min with occasional stirring. The mixture was then centrifuged at 3000 × g for 20 min. Pellet was discarded and supernatant (further referred to as protein extract A) was subjected to subsequent analyses. **B**. The protein extract was prepared from the grains using 0.02 mol/L phosphate buffer, pH 8.0 at a 1:3 (w/v) ratio. Extraction was performed at a temperature of 60°C for 60 min with occasional stirring. The mixture was then centrifuged at 3000 × g for 20 min. Pellet was discarded and supernatant (further referred to as protein extract B) was subjected to subsequent analyses. **C.** The protein extract was prepared with the use of the extraction buffer included in the RIDASCREEN Peanut test kit at a temperature of 60°C for 20 min according to the test recommendations (further referred to as protein extract C).

Chromatographic separation of oat protein extracts. The protein extracts A and B were subjected to the chromatographic separations. The crude extract was loaded

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onto the chromatography column (HR 10/30) filled with ion-exchanger (DEAE-Sepharose, Fast Flow) and then separated in the linear gradient of two buffers: A: 0.025 mol/L phosphate buffer, pH 8.0 and B: 0.025 mol/L phosphate buffer, pH 8.0 containing 1 mol/L NaCl at a flow rate of 0.6 mL/min. The gradient was programmed by the use Programmer GP-250 PLUS (Pharmacia LKB). Elution profile was monitored by absorbance at 280 nm.

Protein content determination. Protein content was determined by Bradford's method [Bradford, 1976]. Bovine serum albumin – BSA was used as a standard protein.

Cross reactivity of oat proteins with Ara h1 allergen. Cross reactivity of oat proteins in crude extracts as well their purified fractions with Ara h1 allergen was determined with the use of RIDASCREEN Peanut test with detection limit found to be <2.5 mg/kg (ppm) according to the test procedures.

RESULTS AND DISCUSSION

The applied extraction procedures yielded three different protein extracts which were characterised by different immunoreactive properties towards antibodies raised against peanut Ara h1 allergen, thus extract A did not show any immunologic similarity with Ara h1 allergen (Table 1). Its ion-exchange chromatographic separation resulted in seven protein fractions (Figure 1) none of them was proved to be immunoreactive towards anti-Ara h1 allergen antibodies. When the extraction was carried out at the temperature of 60°C, both extracts B and C were characterised by strong immunoreactivity towards anti-Ara h1 allergen antibodies (Table 1). The protein extract B was loaded into the ion-exchange column and separated into particular protein fractions (Figure 2). Fractions III, IV and V showed the immunologic similarity with peanut allergen Ara h1.

TABLE 1. Content of Ara h 1 allergen in selected samples by Ridascreen® Peanut test (firm R-Biopharm GmbH).

Sample	Absorbance at 450 nm	Ara h1 allergen concentration (ppm)
Protein extract A	0.118	
[1]Fraction I	0.049	Below
[1]Fraction II	0.046	detection
[1]Fraction III	0.050	limit
[1]Fraction IV	0.047	
[1]Fraction V	0.044	
[1]Fraction VI	0.054	
[1]Fraction VII	0.071	
[2]Fraction I	0.065	
[2]Fraction II	0.064	
[2]Fraction III	0.190	7200
[2]Fraction IV	0.740	2900
[2]Fraction V	1.708	330
Protein extract B[3]	2.849	Above
Protein extract C	2.658	detection limit

[1] Protein fractions obtained in the result of ion-exchange chromatographic separation of oat protein extract A; [2] Protein fractions obtained in the result of ion-exchange chromatographic separation of oat protein extract B; [3] Protein extract B diluted at the ratio of 1:100 (v/v).

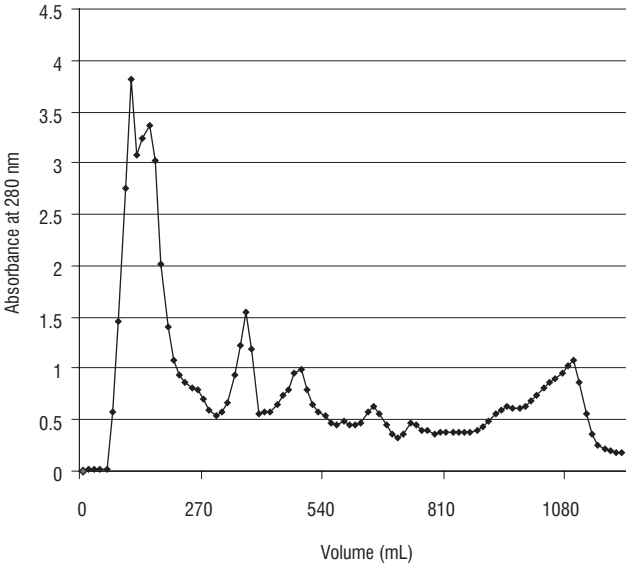

FIGURE 1. Chromatographic separation of oat protein extract A.

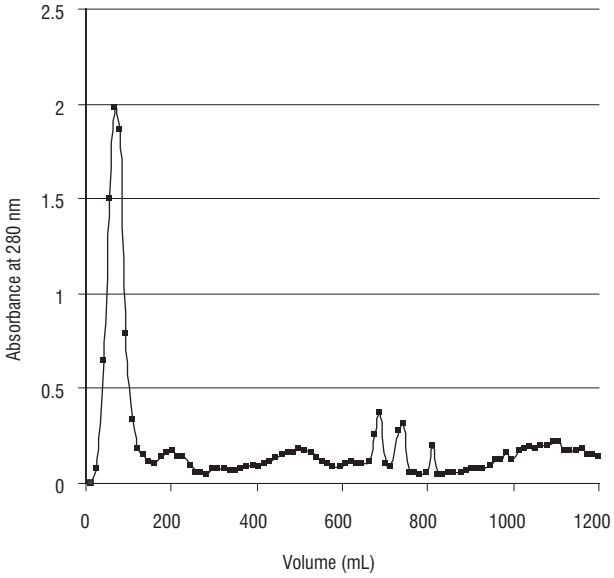

FIGURE 2. Chromatographic separation of oat protein extract B.

As the number of elements forming epitopes regions in proteins is limited it can be possible that the proteins from different species can have the similar immunoreactive regions within their structures. It was shown that oat 12S globulin is homologous with rice globulin (in 70%) and globulin characteristic for the papilinoaceous family (in 30–40%) [Mikola, 2001], thus there can exist some structural similarity between oat and peanut proteins. Peanuts contain highly allergenic proteins and Ara h1 allergen is responsible for anaphylaxis reaction in humans even at trace concentrations [Wróblewska & Jędrychowski, 2000; Wüthrich, 2000]. According to Stanley [2003] in oat there is protein of molecular mass of 66 kD showing strong tendency to bind IgE of children sensitive to wheat proteins, and Ara h1 allergen is protein of molecular mass 65 kD, so it can be supposed – concluding – that oat and peanut proteins can show similar immunogenic proteins. In our study it was shown that within oat protein structures there are hidden similar determinants immunologically comparable to those found in Ara h1 allergen, and temperature appears to be

very important factor, which can affect their revealing and thus increase their availability to anti – Ara h1 allergen antibodies.

CONCLUSION

The result of our study is of high importance since Ara h1 allergen is responsible for anaphylaxis reaction in humans and oat grains products are consumed after heat processing, thus with allergenic determinants exposed, and so the human health threats can be increased.

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