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PROTEOMIC ANALYSIS OF ALBUMIN AND GLOBULIN FRACTIONS OF PEA (*PISUM SATIVUM* L.) SEEDS*

Jerzy Dziuba✉, Iwona Szerszunowicz, Dorota Nałęcz, Marta Dziuba

Department of Food Biochemistry, University of Warmia and Mazury in Olsztyn
Plac Cieszyński 1, 10-957 Olsztyn, **Poland**

ABSTRACT

Background. Proteomic analysis is emerging as a highly useful tool in food research, including studies of food allergies. Two-dimensional gel electrophoresis involving isoelectric focusing and sodium dodecyl sulfate polyacrylamide gel electrophoresis is the most effective method of separating hundreds or even thousands of proteins. In this study, albumin and globulin fractions of pea seeds cv. Ramrod were subjected to proteomic analysis. Selected potentially alergenic proteins were identified based on their molecular weights and isoelectric points.

Material and methods. Pea seeds (*Pisum sativum* L.) cv. Ramrod harvested over a period of two years (Plant Breeding Station in Piaski-Szelejewo) were used in the experiment. The isolated albumins, globulins and legumin and vicilin fractions of globulins were separated by two-dimensional gel electrophoresis. Proteomic images were analysed in the ImageMaster 2D Platinum program with the use of algorithms from the Melanie application. The relative content, isoelectric points and molecular weights were computed for all identified proteins. Electrophoregrams were analysed by matching spot positions from three independent replications.

Results. The proteomes of albumins, globulins and legumin and vicilin fractions of globulins produced up to several hundred spots (proteins). Spots most characteristic of a given fraction were identified by computer analysis and spot matching. The albumin proteome accumulated spots of relatively high intensity over a broad range of pI values of ~4.2-8.1 in 3 molecular weight (MW) ranges: I – high molecular-weight albumins with MW of ~50-110 kDa, II – average molecular-weight albumins with MW of ~20-35 kDa, and III – low molecular-weight albumins with MW of ~13-17 kDa. 2D gel electrophoregrams revealed the presence of 81 characteristic spots, including 24 characteristic of legumin and 14 – of vicilin.

Conclusions. Two-dimensional gel electrophoresis proved to be a useful tool for identifying pea proteins. Patterns of spots with similar isoelectric points and different molecular weights or spots with different isoelectric points and similar molecular weights play an important role in proteome analysis. The regions characteristic of albumin, globulin and legumin and vicilin fractions of globulin with typical MW and pI values were identified as the results of performed 2D electrophoretic separations of pea proteins. 2D gel electrophoresis of albumins and the vicilin fraction of globulins revealed the presence of 4 and 2 spots, respectively, representing potentially allergenic proteins. They probably corresponded to vicilin fragments synthesized during post-translational modification of the analysed protein.

Key words: pea seed proteins, 2D-PAGE, allergenic proteins

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✉dziuba@uwm.edu.pl

INTRODUCTION

Globulins and albumins are the major proteins of pea and other legumes. Pea proteins are characterised by high levels of genetic polymorphism [Baringer et al. 2004]. The UniProtKB database [Wu et al. 2006] available online at http://www.expasy.org contains 638 amino acid sequences of proteins and polypeptides found in different pea varieties, including seed storage proteins. The main components of the globulin fraction are globulin 11S – legumin and globulin 7S – vicilin [Lampart-Szczapa 2001, Kędzior 2004]. The sequence and structure of pea legumin are similar to those noted in soybean legumin [Mills et al. 2004]. Legumin is composed of an α-chain with molecular weight of approximately 40 kDa, which contains significant quantities of acidic amino acids, and a β--chain with molecular weight of approximately 20 kDa, which contains basic amino acids. Those chains are joined by disulfide bonds into a complex with molecular weight of 330 kDa. Pea vicilin can be separated into several fractions by enzymatic hydrolysis. Fractions α, β and γ with molecular weight of 19 kDa, 13.5 kDa and 16 kDa, respectively, have been identified [Lycett et al. 1983]. The amino acid sequences of vicilin listed in the UniProtKB database are nearly completely devoid of tryptophan, and they can be differentiated from other pea proteins based on their derivative UV absorption spectra [Minkiewicz et al. 2006]. Albumin proteins of legumes, including pea, have been less extensively researched than globulins [Mills et al. 2004, Kędzior 2004]. This fraction comprises proteins with molecular weight of 6 to 100 kDa, including enzymes, enzyme inhibitors and lectins. The latter have a smaller share of pea proteins in comparison with other legumes [Park et al. 2010]. Pea albumins are a rich source of sulfur-containing amino acids.

Studies into the allergenic and immunoreactive properties of food ingredients have revealed that pea proteins have allergenic potential [Salgado et al. 2002, Sanchez-Monge et al. 2004, Breiteneder and Radauer 2004, Sathe et al. 2005, Szymkiewicz and Jędrychowski 2006]. IgE-mediated cross-reactivity has been observed between selected plant proteins (soybean, lentil, pea, bean). Due to similarities in the tertiary structure and amino acid sequences of proteins (mainly their epitopes responsible for allergic reactions) from various sources, IgE specific antibodies do not distinguish between those proteins, which can lead to cross-reactivity. The major allergenic pea proteins are globulin 7S (both vicilin and convicilin) [Sanchez-Monge et al. 2004] and globulin 11S [Quillien et al. 1995]. Vicilin subunits with molecular weight of 50 kDa and 70 kDa, corresponding to vicilin (α + β + γ) and convicilin subunits, have been found to possess allergenic properties [Szymkiewicz and Jędrychowski 2006]. The allergenic potential of proteolytic vicilin fragments with MW of 36, 32, 16 and 13 kDa [Sanchez-Monge et al. 2004] has also been demonstrated.

Proteomic analysis is emerging as a highly useful tool in food research, including in studies of food allergies. Two-dimensional gel electrophoresis involving isoelectric focusing and sodium dodecyl sulfate polyacrylamide gel electrophoresis is the most effective method of separating hundreds or even thousands of proteins [Konopka et al. 2007]. In this study, albumin and globulin fractions of pea seeds cv. Ramrod were subjected to proteomic analysis. Selected potentially alergenic proteins were identified based on their molecular weight and isoelectric points.

MATERIAL AND METHODS

Pea seeds

Seeds of pea (*Pisum sativum* L.) cv. Ramrod were supplied by the Plant Breeding Station in Piaski-Szelejewo (Poland). Seeds were dehulled and milled into flour using a WZ-1 laboratory mill (Spomasz, Żnin, Poland) and a Fack S-2601 tecator (Höganas, Sweden) with a grid diameter < 150 μm. Flour was stored in hermetically closed plastic containers at a temperature of around 5°C.

Chemicals

Chemicals for 2-DE analysis like ethanol, methanol, acetic acid and o-phosphoric acid 85% were purchased from Polish Chemical Reagents (Gliwice, Poland). Staining solution Roti®-Blue (5 × concentrated Colloidal Coomassie G-250) was purchased from Roth (Karlsruhe, Germany). All other chemicals were purchased from Sigma-Aldrich (Seelze, Germany). Only electrophoresis-purity reagents were used.

Isolation and fractionation of pea proteins

Isolation of albumins and globulins from pea seeds and fractionation of globulins into legumin and vicilin was carried out according to Freitas et al. [2000], with small modifications. For isolating proteins from pea, defatted flour was used (0.5 g of flour and 17 ml of hexane were stirred for 4 h at 20°C and hexane was decanted and evaporated). Defatted flour was extracted with extraction buffer I (deionized water pH 8.0 with 10 mM $CaCl_2$, 10 mM $MgCl_2$ and 1 mM phenylmethylsulphonyl fluoride (PMSF)) and stirred for 4 h at 20°C. Next, the extraction mixture was centrifuged (Sigma 3K30 Centrifuge, Sigma Laboratory Centrifuges, Osterode am Harz, Germany) at 30 000 × g for 1 h at 20°C. The supernatant containing albumins was dialyzed with the use of Spectrum Float-A-Lyzers with sample volume 10 ml and molecular weight cut off (MWCO) 3.5 kDa (for 5 days at 4°C, vol. ratio of deionized water:sample 200:1). Samples were than centrifuged at 30 000 × g for 1 h at 20°C, lyophilized (Freeze Dry System 4.5, Labconco Corporation, Kansas City, USA) and stored at –80°C. For globulin isolation the obtained pellet after centrifugation of defatted flour with extraction buffer I was used. The extraction buffer II (0.1 M Tris-HCl buffer pH 8.0 containing 10% (w/v) NaCl, 0.05% (w/v) NaN_3, 1 mM PMSF and 10 mM ethylenediaminetetraacetic acid (EDTA) was added to pellet and than it was stirred for 12 h at 20°C and centrifuged at 30 000 × g for 1 h at 20°C. After centrifugation the obtained pellet was rejected. One part of supernatant containing globulins was dialysed, centrifuged and lyophilized (in the same conditions as albumins) and the second was used for fractionation of globulins into legumin and vicilin.

Legumin fraction was received by protein precipitation with 75% ammonium sulfate and further centrifugation of the solution at 30 000 × g for 0.5 h at 4°C. The obtained precipitate was next suspended in deionized water (1:1 ratio) and dialyzed (for 5 days at 4°C, with vol. ratio of deionized water:sample 200:1). To obtain vicilin fraction the received after legumin precipitation with 75% ammonium sulfate and centrifugation supernatant was additionally precipitated with 99% ammonium sulfate and than centrifuged at the same parameters as above and dialyzed in the same conditions as legumin fraction. To prevent microbial growth during dialysis of albumin and globulin fractions 0.02% (w/v) NaN_3/streptomycin was used. After dialysis the pure protein (legumin and vicilin) solutions were lyophilised and storaged at –80°C.

Measurement of protein concentration

Protein concentration of all samples was measured before electrophoretic analysis according to Bradford [1976] using the Bio-Rad Protein Assay (Bio-Rad, USA, cat. No. 500-0006) and bovine serum albumin (Bio-Rad, USA cat. No. 500-0007) as a standard protein.

Two-dimensional electrophoresis of pea seeds proteins

2D-PAGE was carried out according to Görg [2004]. Lyophilisates weights in the amount of 1.0 mg, 0.3 mg and 0.1 mg appropriately for albumins, globulins and the legumin/vicilin fractions obtained as a result of the pea seed protein extraction or fractionation were dissolved in 500 µl of the rehydration buffer (7 M urea, 2 M thiourea, 1.8 mM DL-dithiothreitol (DTT), 4% (w/v) 3-[(3-cholamidopropyl)dimethylammonio]--1-propanesulfonate (CHAPS), 0.5% (v/v) IPG buffer pH 3-10, 0.002% (w/v) bromophenol blue). Than 350 µl of such prepared solution was loaded onto IPG Dry Strips; 18 cm; pH 3-10 linear gradient (Bio-Rad, USA, cat. No. 163-2032).

Isoelectric focusing (IEF) was carried out using Ettan™ IPGphor™ (GE Healthcare) under the following conditions: 1 h at 100 V, 1 h at 300 V, 1 h at 600 V, 0.5 h at 1000 V, 0.5 h gradient up to 8000 V and 8 h at 8000 V. Rehydration of gel strips was conducted for 13 h at 30 V. The strips were then transferred to 12.5% polyacrylamide gels (215 × 276 × 1 mm) and covered with a 0.5% agarose solution in the Ettan™ DALTsix (GE Healthcare, Uppsala, Sweden) apparatus. 2D SDS-PAGE Standards (Bio-Rad, USA, cat. No. 161-0310) were used. 2D-PAGE gels were stabilized and stained using Roti®-Blue solution (Colloidal Coomassie G-250, Roth, Karlsruhe, Germany). After staining gels were washed to destain the background. Gels were finally rinsed with deionized water and scanned. The analysis was performed in three replications [Dziuba et al. 2009].

Computer aided image analysis of gels and statistical analysis

Computer aided image analysis of gels was performed using the Image Master™ 2D Platinum 6.0 program (GE Healthcare, Uppsala, Sweden). Isoelectric points (pI) and observed molecular weights (MW) were calculated for all spots. Values of pI and MW were expressed as the mean of three independent replications ±standard error of the mean (mean ±SEM). Statistical analysis was performed using Statistica 10.0 PL software (StatSoft, Tulsa, OK, USA).

In order to match protein spots an algorithm recommended by Kjærsgaard et al. [2006] was used. The spots corresponding to the standards of molecular weight and points corresponding to the beginning and the end of strip were marked on gels. After matching spots shared for the standard (reference) and for examined (analysed) gels were being marked and a procedure was being repeated. Matching was repeated until no further improvement was observed.

RESULTS AND DISCUSSION

Images of 2D electrophoresis (2DE) gels of pea albumins, globulins, legumin (globulin 11 S) and vicilin (globulin 7S) are presented in Figure 1, 2, 3 and 4, respectively. Theoretical molecular weights and isoelectric points of the analysed proteins were determined in the Compute pI/MW application [Gasteiger et al. 2005] to compare and identify protein spots. Spots

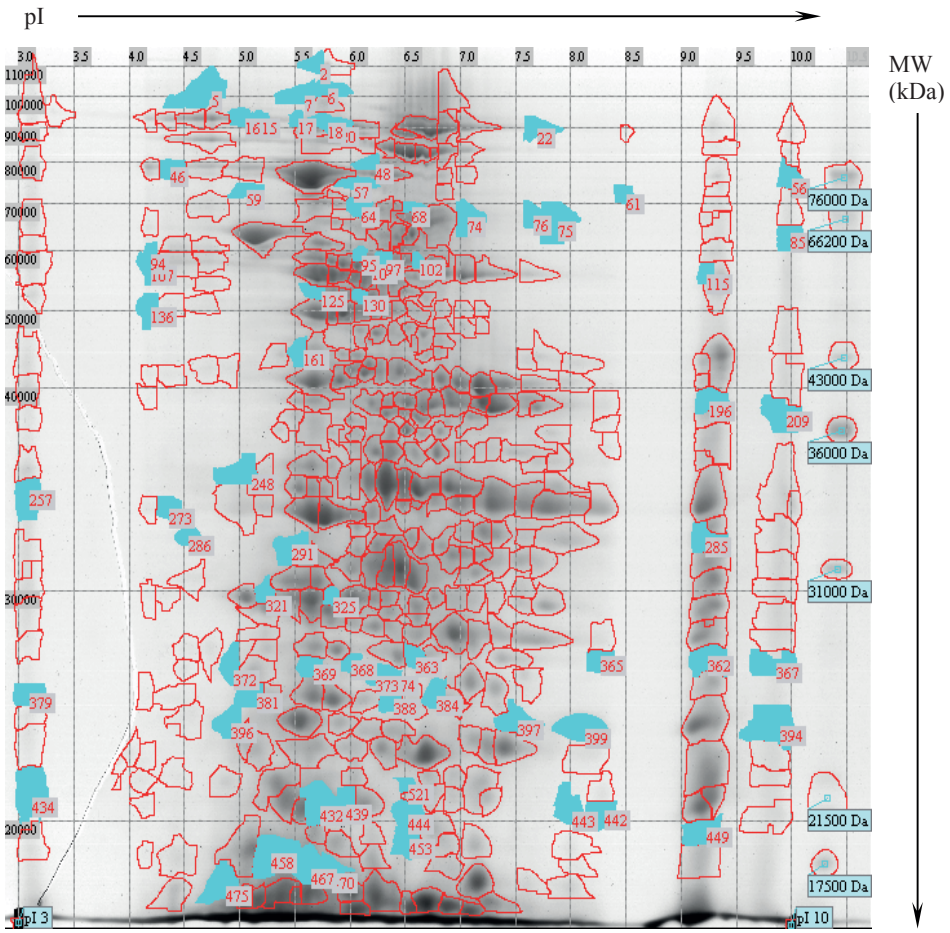

Fig. 1. 2D gel electrophoregram of pea albumins. Characteristic spots for albumin are marked

classified as characteristic of the analysed fractions are shown in 2DE images.

Pea albumins are characterized by significant heterogeneity, and they comprise several groups of proteins with molecular weight of 6-100 kDa, including all biologically active proteins – enzymes as well as enzyme inhibitors and lectins [Kędzior 2004, Park et al. 2010]. Characteristic for 2DE images of pea albumins, containing 73 spots (Fig. 1), was accumulation of most of them and with relatively high intensity over a broad range of isoelectric points pI ~4.2-8.1 in three MW ranges: I – high molecular-weight albumins with MW of ~50-110 kDa, II – average molecular-weight albumins with MW of ~20-35 kDa, and III – low molecular-weight albumins with MW of ~13-17 kDa. Spots characterised by a broad range of MW values at ~20-75 kDa were also present in the pI range of ~9-10. Intensive spots in the MW range of 32-35 kDa and pI range of 5.5-8.0 were also determined in 2DE images of pea globulins (Fig. 2), therefore, they were not identified as albumin fractions. Selected albumins that were not completely dissolved during extraction can be present in the globulin fraction, and matched spots in gels of different fractions can constitute artefacts [Camposstrini et al. 2005, Hunsucker and Duncan 2006].

Numerous research attempts have been made to isolate, separate and identify pea albumins and determine their allergenic potential [Croy et al. 1984, Higgins et al. 1987]. The cited authors isolated, separated and identified two main pea albumin fractions

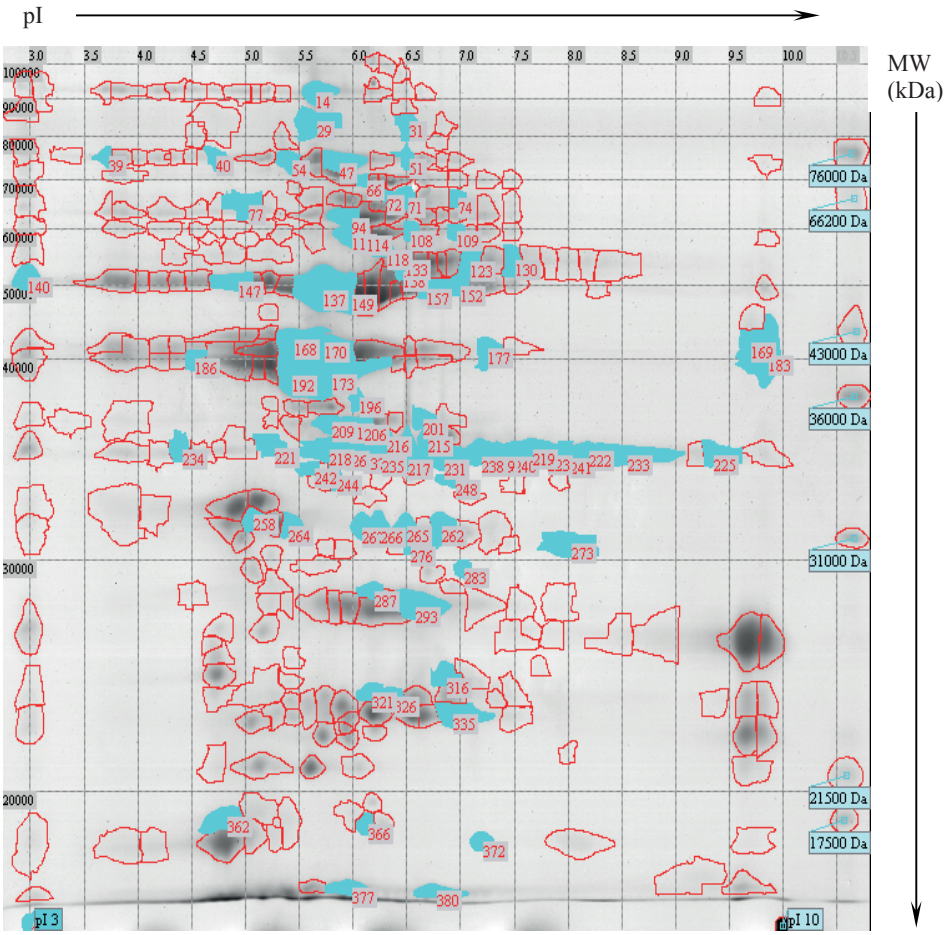

Fig. 2. 2D gel electrophoregram of pea globulins. Characteristic spots for globulin are marked

responsible for allergenic reactions: albumin PMA-L with MW of ~53 kDa, containing two subunits with MW of 25 kDa (pI ~5.0-5.5), and albumin PMA-S with MW of ~48 kDa, containing two subunits with MW of 24 kDa (pI ~5.0-5.5). The analysed 2DE image of albumins (Fig. 1) features 4 spots that could correspond to the above subunits. The molecular weights and isoelectric points of the identified proteins were determined at 26.6 (±0.90), 26.3 (±0.56), 25.4 (±0.94) and 24.2 kDa (±0.51) and 5.7 (±0.07), 4.9 (±0.10), 5.1 (±0.08) and 4.9 (±0.02), respectively.

In view of their sedimentation coefficient, globulins are classified as proteins 11S (legumin) and 7S (vicilin). Legumin is a hexamer comprising three acidic (α-subunit, MW ~40 kDa) and three basic subunits (β-subunit, MW ~20 kDa) [Tzitizkas et al. 2006]. α- and β-subunits are produced by post-translational hydrolysis of their precursors, and their composition is balanced [Müntz et al. 1985]. Vicilin comprises a major polypeptide with MW of 50 kDa that is polymerized to a major trimer with MW of 150 kDa [Tzitizkas et al. 2006]. Three polypeptides can be separated by controlled enzymatic hydrolysis from one molecule of pea vicilin: α-chain (19-20 kDa), β-chain (13 kDa) and γ-chain (12-16 kDa). α+β-chains create a subunit with MW of 30-36 kDa that, together with the γ-chain, forms globulin 7S [Lycett et al. 1983, O'Kane et al. 2004, Tzitizkas et al. 2006]. Convicilin, which is often identified as a vicilin fraction due to significant similarities in their amino acid sequences, is a polypeptide with MW

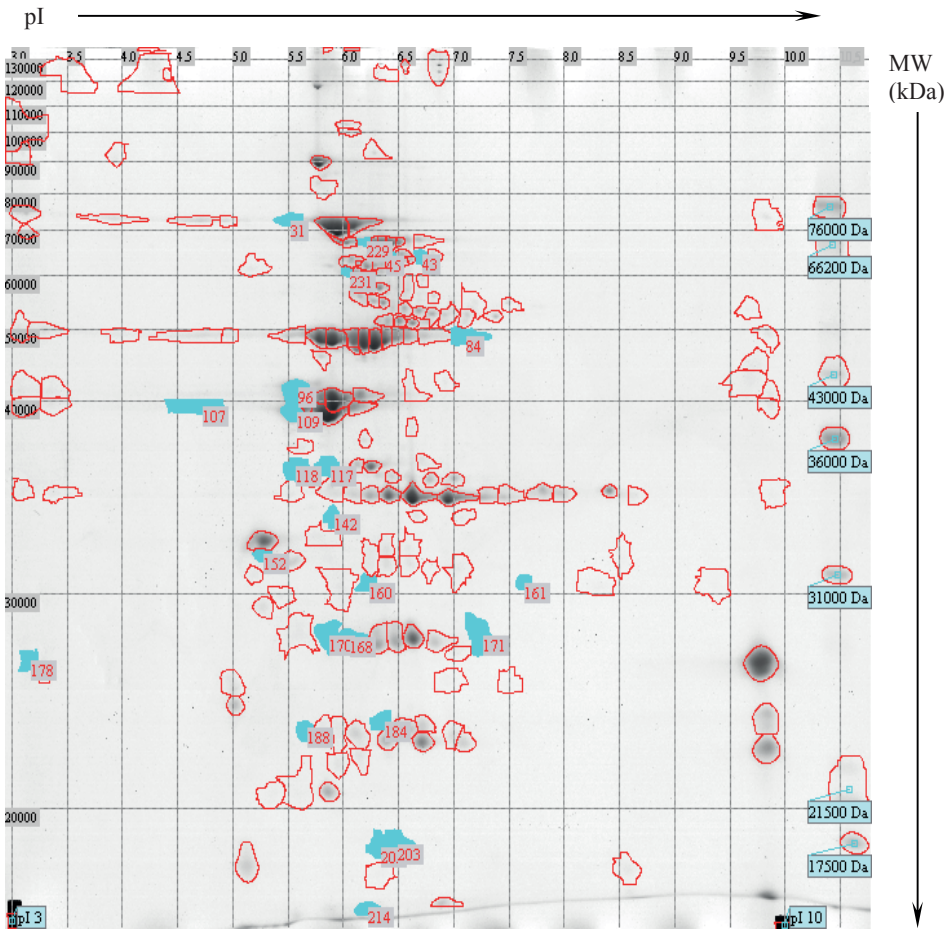

Fig. 3. 2D gel electrophoregram of pea legumin. Characteristic spots for legumin are marked

of ~70 kDa, and it can create trimers with MW of ~210 kDa [O'Kane et al. 2004, Tzitizkas et al. 2006].

A total of 81 characteristic spots were identified in the 2DE image (Fig. 2). Some of them could represent acidic subunits of globulin 11S (legumin A2, A, J) with MW of 36.8, 36.4 and 34.4 kDa and pI of 5.3, 5.2 and 5.0, respectively (calculated based on amino acid sequences available in the Swiss-Prot/TrEMBL database). The presence of spots corresponding to fragments of α+β-vicilin (30 do 36 kDa) cannot be exclude in this range of MW values [Lycett et al. 1983, O'Kane et al. 2004, Tzitizkas et al. 2006]. The presence of spots in both legumin and vicilin images can be attributed to the high heterogeneity of subunits forming globulin 11S and 7S. Those globulins are not well defined on account of the heterogeneity of their subunits. Coincidental association is sometimes observed [Akazawa and Hara-Nishimura 1985, Gatehouse et al. 1981], leading to a significant increase in the number of proteins present in every fraction. Many proteins (in particular globulin 7S) are unevenly glycosylated, which additionally inhibits the identification of globulin subunits. For this reason, vicilin is a more heterogeneous protein than globulin 11S [Scholz et al. 1983]. Vicilin does not contain cysteine residues, and it does not form disulfide bonds [O'Kane et al. 2004]. It is more susceptible to proteolytic change than legumin, which results in a large number of polypeptide fragments [Monsalve et al. 1990]. In 2DE gels, proteolytic products create spots corresponding to lower MW

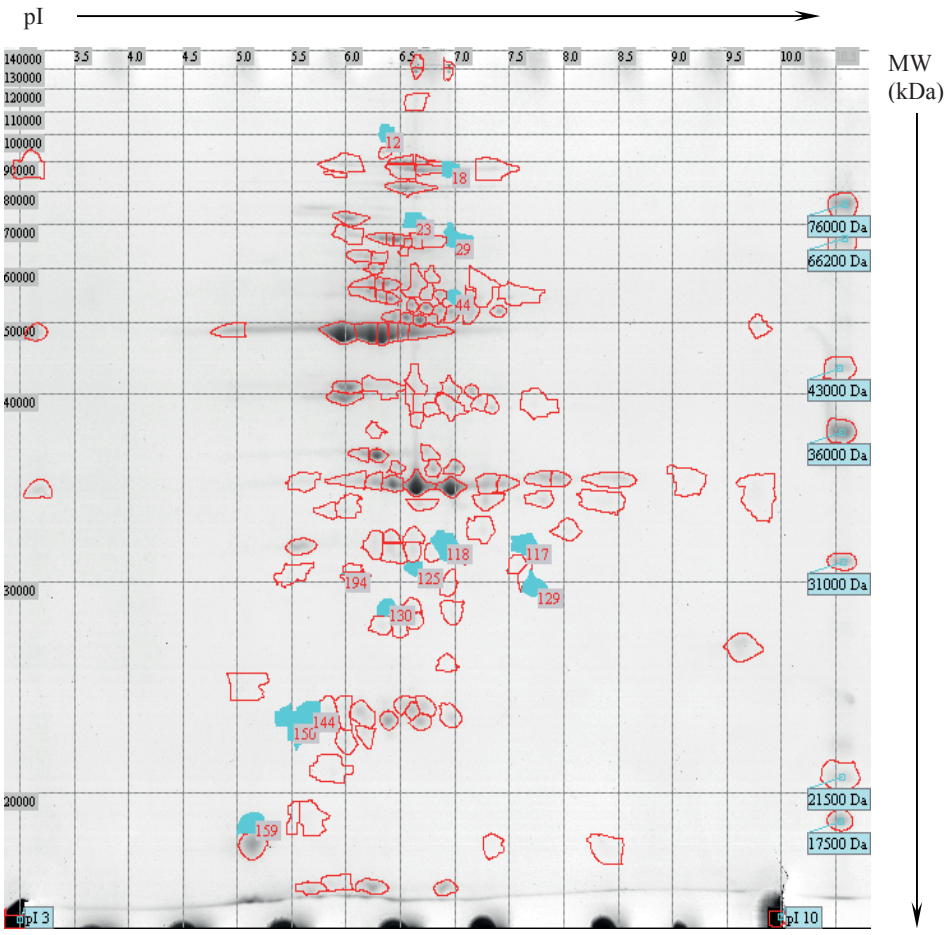

Fig. 4. 2D gel electrophoregram of pea vicilin. Characteristic spots for vicilin are marked

values than the native protein. The isoelectric points of peptide fragments can also differ significantly from those of the native protein.

The 2D gel electrophoregram of pea legumin (Fig. 3) features 24 spots characteristic of globulin 11S that were also identified in 2DE images of globulins (Fig. 2). The legumin electrophoregram contains 5 spots with MW of 60.5-72.4 kDa and pI of ~5.5-6.7. Some of them could correspond to convicilin subunits characteristic of globulin 7S that could be present in globulin 11S during fractionation [Kędzior 2004, Park et al. 2010, Tzitizkas et al. 2006]. Spots with MW of 31.7-41.6 kDa (pI 4.7-5.9) could constitute the acidic subunits of globulin 11S (legumin A2, A, J) with MW of 36.8, 36.4 and 34.4 kDa and pI of 5.3, 5.2 and 5.0, respectively. The 2D gel electrophoregram of legumin also features spots with MW of 23.8-30.6 kDa (pI ~3.2--7.6) and 17.2-17.5 kDa (pI ~6.3-6.5) and one spot with MW of 13.7 (±0.39) kDa (pI 6.21 ±0.07). Protein fractions corresponding to MW of ~17 kDa could constitute fragments of α-legumin subunits. They do not represent β-legumin subunits whose MW approximates 20 kDa (pI 9.7). Highly intense spots (MW of 38-42 kDa, pI of 5.9-6.1) in 2DE images of legumin (Fig. 3) and vicilin (Fig. 4) could correspond to acidic α-subunits of legumin. Those spots were more intensive in 2D gel electrophoregrams of legumin than vicilin.

2DE images of pea vicilin (Fig. 4) revealed 14 spots corresponding to globulin 7S, which were present in globulin images (Fig. 2) but were not identified in legumin electrophoregrams (Fig. 3). Vicilin fraction proteins were characterized by MW of 12-50 kDa and pI of 5.0 to 7.0. Spots with MW of 66 (±0.07) kDa (pI of 7.0 ±0.02) and 71 (±0.25) kDa (pI of 6.6 ±0.04) in vicilin images could be convicilin subunits. Protein fractions with MW of 23.6 to 31.6 kDa (pI 5.5-7.6) could constitute β+γ-subunits (25 to 30 kDa) or α+β--subunits of vicilin (30 to 36 kDa), whereas spots with MW of 54.6 (±0.38) kDa (pI of 7.0 ±0.05) and 17.4 (±0.08) kDa (pI of 5.2 ±0.13) could correspond to the basic vicilin subunit (α+β+γ) and the γ-subunit. In the analysed range of MW values, two spots (MW 15.6 ±0.88 kDa, pI 5.1 ±0.05 and 17.4 ±0.08 kDa, pI 5.1 ±0.13) with allergenic potential were found [Sanchez-Monge et al. 2004]. They were probably products of partial hydrolysis of vicilin that were synthesized during post-translational modification.

CONCLUSIONS

Two-dimensional gel electrophoresis proved to be a useful tool for identifying pea proteins. Patterns of spots with similar isoelectric points and different molecular weights or spots with different isoelectric points and similar molecular weights play an important role in proteome analysis. The regions characteristic of albumin, globulin and legumin and vicilin fractions of globulin with typical MW and pI values were identified as the results of performed 2D electrophoretic separations of pea proteins. The presence of 81, 24 and 14 spots characteristic for globulins, legumin and vicilin, respectively, was noted in 2DE images. 2D gel electrophoresis of albumins and the vicilin fraction of globulins revealed the presence of 4 and 2 spots, respectively, representing proteins with allergenic potential. They probably corresponded to vicilin fragments synthesized during post-translational modification of the analysed protein.

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PROTEOMICZNE BADANIA FRAKCJI ALBUMINOWEJ I GLOBULINOWEJ ZIAREN GROCHU SIEWNEGO (*PISUM SATIVUM* L.)

STRESZCZENIE

Wprowadzenie. Badania proteomiczne zyskują coraz większe znaczenie zarówno w nauce o żywności, jak i w badaniach nad alergiami. Elektroforeza dwukierunkowa, która obejmuje ogniskowanie izoelektryczne oraz elektroforezę w żelu poliakryloamidowym w obecności siarczanu dodecylu sodu, jest obecnie najbardziej efektywną metodą rozdzielania białek. Liczba tak rozdzielonych białek może sięgać setek, a nawet tysięcy. W pracy przeprowadzono badania proteomiczne frakcji albuminowej i globulinowej ziaren grochu odmiany Ramrod. Istotą badań była identyfikacja określonych białek, głównie mogących wykazywać właściwości alergenne na podstawie ich mas cząsteczkowych i punktów izoelektrycznych.

Materiał i metody. Do badań wykorzystano ziarno grochu siewnego, gładkiego (*Pisum sativum* L.), odmiany Ramrod pochodzące z dwuletnich zbiorów (Stacja Hodowli Roślin, Piaski-Szelejewo).Wyizolowane albuminy, globuliny oraz frakcje globuliny, leguminę i wicylinę, rozdzielano metodą elektroforezy dwukierunkowej. Uzyskane obrazy proteomiczne analizowano komputerowo za pomocą Image Master™ 2D Platinum. Program wykorzystuje algorytmy programu Melanie. Obliczano względną zawartość, punkty izoelektryczne oraz obserwowane masy cząsteczkowe wszystkich wykrytych białek. Wyniki były obliczane poprzez nakładanie spotów pochodzących z obrazów elektroforegramów, otrzymanych w wyniku trzech niezależnych powtórzeń.

Wyniki. Proteomy albumin, globulin i frakcji globulinowych, leguminy i wicyliny, charakteryzowały się obecnością do kilkuset spotów (białek). Na podstawie komputerowej analizy obrazu oraz dopasowania spotów wyznaczono uznane za charakterystyczne dla danej frakcji. Charakterystyczną cechą proteomu albumin grochu było nagromadzenie spotów o stosunkowo dużej intensywności w rozległym zakresie punktów izoelektrycznych pI ~4.2-8.1 w trzech zakresach mas cząsteczkowych MW: I obszar to albuminy wysokocząsteczkowe o MW ~50-110 kDa, II obszar to albuminy średniocząsteczkowe, których MW mieszczą się w zakresie ~20-35 kDa i III obszar to albuminy niskocząsteczkowe o masach cząsteczkowych ~13-17 kDa. W obrazie żelu 2D globulin stwierdzono obecność 81 charakterystycznych spotów, z których 24 odpowiadało leguminie, a 14 wicylinie.

Wnioski. Metoda elektroforezy dwukierunkowej okazała się narzędziem przydatnym do charakterystyki białek pochodzących z nasion grochu. W ocenie badanych proteomów istotne znaczenie mają uporządkowane serie spotów o zbliżonych punktach izoelektrycznych i różnych masach cząsteczkowych lub zbliżonych masach cząsteczkowych i różnych punktach izoelektrycznych. W wyniku przeprowadzonej analizy elektroforetycznej 2D białek grochu zidentyfikowano obszary występowania charakterystycznych spotów albumin i globulin (w tym leguminy i wicyliny) o określonych wartościach MW i pI, typowych dla tych frakcji. Na podstawie analizy elektroforetycznej 2D albumin i wicylinowej frakcji globulin stwierdzono obecność odpowiednio czterech i dwóch spotów reprezentujących białka, którym są przypisywane właściwości alergenne. Prawdopodobnie odpowiadają one fragmentom wicyliny, które powstają w wyniku posttranslacyjnej hydrolizy tego białka.

Słowa kluczowe: białka nasion grochu, 2D-PAGE, białka alergenne

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