A Novel Acidic Allergen, Hev b 5, in Latex
PURIFICATION, CLONING AND CHARACTERIZATION*

(Received for publication, April 5, 1996, and in revised form, July 24, 1996)

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Latex allergy is recognized as a serious health problem among health care workers and children with spina bifida. A number of IgE-reactive proteins have been identified in natural and processed latex products. One of the most acidic proteins in the cytoplasm of laticifer cells of rubber trees (Hevea brasiliensis) is demonstrated to be a potent allergen in eliciting allergic reactions in humans. This protein, with pI = 3.5, has a molecular mass of 16 kDa with a blocked N terminus and an unusual amino acid composition. This acidic protein was found in extracts prepared from latex gloves, which were shown to be allergenic. The purified protein elicits histamine release from human basophils passively sensitized with serum from latex-allergic individuals in a dose-dependent manner.

From a latex cDNA library, the cDNA coding for this protein was isolated and sequenced. The deduced amino acid sequence shows a high degree of homology to another acidic protein identified in kiwifruit (Actinidia deliciosa var. deliciosa). The sequence homology (47% sequence identity) between these two acidic proteins suggests a molecular explanation for the high frequency of fruit hypersensitivity in latex-allergic patients.

Allergy to natural rubber products has been recognized as a serious medical problem especially among health care workers and children with spina bifida (1, 2). Five to 10% of surgeons and operating room nurses were reported to be allergic to latex gloves (3, 4), and as high as 34% of children with spina bifida were allergic to latex gloves or other rubber products (5). Agents responsible for eliciting the type I hypersensitivity have been demonstrated in native latex proteins with molecular sizes ranging from 5 to 200 kDa (6–11).

Latex of the rubber tree (Hevea brasiliensis) is produced by a group of specialized cells, laticifers. The cytosol of these cells contain rubber particles and a large number of proteins. Upon wounding, the cytoplasmic content of these cells is expelled in the form of latex. For the manufacturing of rubber goods, the fresh milky latex is collected in strong ammonia solution (20% concentration of 50% (v/v) to prevent degradation and the growth of microorganisms. The mixture was stored at 4°C until use.

The C-serum was desalted in a 20 mM citrate buffer, pH 3.0, by passing through a PD-10 column (Sephadex G-25M, Pharmacia Biotech Inc.). The fraction collected in the void volume was subjected to ion-exchange chromatography on a Pharmacia cation exchange column (HiTrap™ SP) using a FPLC system: buffer A, 20 mM sodium citrate, 0.5 M NaCl, pH 7.5; buffer B, 0.5 M NaCl, pH 7.5; linear gradient of 0 to 1 M NaCl.

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) U51631.

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The abbreviations used are: PAGE, polyacrylamide gel electrophoresis; IEF, isoelectric focusing; HPLC, high performance liquid chromatography; LB, Luria-Bertani medium; PIPES, piperazine-N,N'-bis(2-ethanesulfonic acid); PCR, polymerase chain reaction; MALDI, matrix-assisted laser desorption ionization.
buffer, pH 3.8; buffer B, 20 mM sodium citrate buffer, pH 3.8, and 1 mM NaCl, pH 8.0, and heated for 15 min at 60 °C. 5% buffer B containing the pl 3.5 protein. This fraction was further purified by a C-4 reverse phase column (Vydac) with a gradient of 0.12% trifluoroacetic acid in water, 0.1% trifluoroacetic acid in acetone.

Trypsin and Endoproteinase Asp-N Digestions and Separation of Digested Fragments—For trypsin digestion, 50 μg of purified pl 3.5 protein was dissolved in 10 ml of 4 mM urea in 0.1 mM Tris-HCl, 2.0 mM CaCl2, pH 8.0, and heated for 15 min at 60 °C. 5% buffer B containing the pl 3.5 protein was digested at an approximately 5% buffer B containing the pl 3.5 protein. This fraction was further purified by a C-4 reverse phase column (Vydac) with a gradient of 0.12% trifluoroacetic acid in water, 0.1% trifluoroacetic acid in acetone.

Amino Acid Analysis and Amino Acid Sequence Analysis—Amino acid analysis was carried out after hydrolysis in 6 N HCl for 90 min at 150 °C by using a Beckman model 6300 amino acid analyzer (16, 17). The amino acid sequences of several peaks from the trypsin digestion were sequenced by automated Edman degradation and analyzed on a model 473A microsequencer (Applied Biosystems) connected to an on-line model 120A phenylthiohydantoin analyzer. The endoproteinase Asp-N peptides were sequenced on a model LP 3000 Beckman protein sequencer with on-line phenylthiohydantoin analyzer.

Latex Glove Extract, Ammoniated Latex Extract, and Heat-treated Latex Extract—Glove extracts were generous gifts from Dr. Robert Hamilton (The Johns Hopkins University, Baltimore, MD) and Dr. John Yunginger (Mayo Clinic, Rochester, MN). The extracts were prepared and concentrated as described previously (10, 18, 19). Ammoniated latex extract was from Dr. Robert Hamilton. To test the stability of the pI 3.5 protein at high temperature, a condition used in the manufacture of latex gloves, a preparation of the latex extract was heat-treated in an autoclave (250 °F, 18 p.s.i.) for 20 min (19).

SDS-PAGE, IEF Gel Analysis, and Immunoblotting—SDS-PAGE was performed with a 16% gel under non-reducing conditions and proteins were visualized with silver stain as described previously (10). IEF gel electrophoresis was performed with Ampholine® PAG plates, pH 3.5–9.5 (Pharmacia), at 500 V for 90 min. After completion of the electrophoresis, immunoblotting was carried out with passive capillary blotting by pressing a nitrocellulose membrane directly against the gel for 45 min. The membrane was reacted with a serum (31 years old, female) that reacts strongly to the pI 3.5 protein followed by peroxidase-labeled anti-human IgE antibody. The IgE-reactive bands were detected by the chemiluminescence horseradish peroxidase system (Amersham Life Science) according to the manufacturer's instructions.

Mass Spectroscopy—To accurately determine the molecular weight of the pl 3.5 protein, HPLC-purified pl 3.5 protein was analyzed on a Kratos (Shimadzu) MALDI III mass spectrometer using matrix-assisted laser desorption ionization (MALDI). Approximately 5 pmol (0.5 μl) of protein was dissolved in 0.1% trifluoroacetic acid and applied to the target with 0.5 μl of sinapinic acid (Sigma) matrix solution on top. The sample/matrix mixture was dried under vacuum and then analyzed by MALDI.

Histamine Release from Human Basophils—For passive sensitization of human basophils (20), venous blood was collected from a volunteer who is not allergic to latex. Twenty ml of heparinized blood was mixed with 25 ml of 154 mM NaCl solution containing 6% dextran for 1 h. After sedimentation of red cells, the polynuclear leukocytes and basophil-containing supernatant was centrifuged at 150 × g for 8 min at 4 °C. The cell pellet was washed twice with PAG solution (25 mM Pipes, pH 7.4, 110 mM NaCl, 5 mM KCl containing containing 0.1% dextran and 0.003% human serum albumin) and once with saline. To strip IgE antibody from basophils, 5 ml of 10 mM lactic acid, pH 3.9, 140 mM NaCl, 5 mM KCl was added and incubated for 3.5 min at room temperature. Cells were washed once with PAG solution by centrifugation. To sensitize the cells, the cell pellet was incubated with 100 ml of patient’s serum, 300 ml of PAG solution, and 100 ml of 20 mM EDTA containing 0.05% heparin for 60 min at 37 °C. Following this incubation, the cells were washed twice with PAG solution. The cell pellet was resuspended in 5 ml of PAG-CM solution (PAG solution with 1 mM MgCl2 and 1 mM CaCl2) and aliquoted into 14 tubes (7 sets in duplicate). Tubs were prewarmed at 37 °C for 2 min, and serially diluted pl 3.5 protein (1000, 100, 10, and 1 ng/ml) and 1% pericholine (to determine total histamine in cells) were added and incubated for 45 min at 37 °C. After incubation, tubes were centrifuged at 150 × g for 8 min at 4 °C and supernatants were subjected to histamine measurement by an automatic histamine analyzer (Toosh Manufacturing Co. Ltd., Tokyo, Ja-
Most of the glove extracts contained this protein in varying concentrations. The pl 3.5 protein appeared to be unaffected by autoclaving, while most of other proteins formed aggregates that remained near the origin of the sample loading zone (Fig. 2, lane 3). To demonstrate that the acidic proteins from the glove extracts were the pI 3.5 protein, an inhibition immunoblot experiment was performed. The serum was treated with the purified pI 3.5 protein prior to immunoblotting. Once the IgE specific for this acidic protein was removed, the band at pI 3.5 was no longer detectable (data not shown).

Amino Acid Analysis and Amino Acid Sequence—The amino acid composition derived from the amino acid analysis of the purified protein is comparable with that from the sequence deduced from the cDNA. The protein contains unusually high amounts of glutamic acid (46 residues), threonine (21 residues), alanine (29 residues), and proline (22 residues) and no aromatic amino acids, methionine or cysteine/cystine.

Amino acid sequence analysis indicated that the N terminus of the pl 3.5 protein was blocked. Several amino acid sequences were obtained from HPLC-purified, trypsin-generated, and endopeptidase Asp-N-generated peptides of the purified protein (Fig. 3). The deduced amino acid sequence from the cDNA was nearly identical to that obtained by amino acid sequencing, with the exception of two amino acids at positions 67 and 103 (Fig. 3). At position 81, both Ser and Pro were found in different cDNA clones while amino acid sequence indicated a Pro at this position.

Molecular Mass Determination by Mass Spectroscopy—The molecular mass of the purified pl 3.5 protein as determined by mass spectroscopy was 16001.2 Da. The molecular mass calculated from the deduced amino acid sequence was 15957.5 Da. The discrepancy of 43 Da is mostly likely from an acetyl group (43 Da) attached to the N-terminal alanine residue.

Complementary cDNA Coding for the pl 3.5 Protein—Fig. 3 is the result of DNA sequence analysis of five independent clones of cDNAs coding for the pI 3.5 protein. The DNA sequences for the coding region of all clones were identical with the exception of position 312, which is either a C or a T. This change gave rise to a different amino acid, either proline or serine. The noncoding regions at the 3′-end adjacent to the poly(A) tail were quite variable. Fig. 3 depicts the sequence of a cDNA clone with the shortest sequence at the 3′-end.

Sequence Homology Analysis—The computer search identified an acidic protein (pI 3.5) from kiwifruit that has a substantial sequence homology to the latex acidic protein (22). Fig. 4 is the sequence comparison of the latex pl 3.5 protein with a kiwifruit acidic protein, which has a molecular mass of 18.9 kDa with a pI of 3.5. The latex acidic protein has a truncated middle region when the sequences of these proteins are aligned with maximal sequence homology. The homology is most striking in the segments around the N and C termini.

Histamine Release Induced by Purified Latex pl 3.5 Protein—To analyze the allergenic properties of the pl 3.5 protein, histamine release was performed using passively sensitized human basophils. Fig. 5 demonstrates that the purified pl 3.5 protein induced a dose-dependent histamine release when cells were first incubated with sera from allergic patients. The amount of histamine release also correlated with the amount of pl 3.5-specific IgE present in the individual serum. (The higher the level of pl 3.5-specific IgE, the better the responses of dosage of pl 3.5 proteins.) Patient 6 was allergic to latex but with a very low level of pl 3.5-specific IgE in the serum. When this serum was used for sensitization of basophils, no detecta-
its presence in most of the glove extracts tested demonstrate the potential importance of this protein in allergy to latex products. The fact that the purified pI 3.5 protein induced histamine release in IgE sensitized-basophils confirms that it is an allergen, Hev b 5. Histamine release could be detected at an allergen concentration as low as 10 ng when cells were sensitized with serum from patients who had a high IgE titer to this specific protein.

In a previous report (23), rubber elongation factor (molecular mass, 14.5 kDa), Hev b 1, was shown to react with sera from all 13 latex-allergic patients in the study. In our experience with 50 latex-allergic patient sera, only 22% reacted to the rubber elongation factor, while 52% of latex-allergic sera reacted with the pI 3.5 protein (10). Due to the large number of allergens in latex, it does not seem feasible to use a single allergen for immunotherapy or for diagnostic purposes.

The pI 3.5 protein in latex is composed of only 14 of the 21 naturally occurring amino acids, with unusually high numbers of glutamic acid and proline residues in the repeated motif of XEEX or XEEX(X) can be any amino acid, but most frequently Lys or Ala residues). The molecular mass determined by MALDI mass spectrometry (16001.2 Da) agrees well with that calculated from the amino acid composition deduced from the cDNA (15957.5 Da). The difference can be explained by the presence of an acetyl group, which blocked the N terminus. There were sequence heterogeneities among the five cDNA clones, suggesting the possible existence of a family of genes coding for the proteins.

Recently a high percentage of latex allergy patients have been reported to also have food allergy (13). Fruits frequently have been reported to have cross-reactivities with latex including chestnut, banana, papaya, avocado, and kiwifruit (14). A health care worker who developed allergy to latex gloves after working in the hospital later also developed allergy to avocado and kiwifruit. The most likely explanation for the cross-reactivities is the existence of constituents with common antigenic determinants in latex and various fruits. In our study, the acidic latex protein shares a 47% sequence identity with the kiwifruit pI 3.5 protein. The homology is most striking in the N- and C-terminal segments. The pI 3.5 protein in kiwifruit is one of the proteins that appears in the early stage of fruit development (21), but its biological function is not known. The highest level of mRNA coding for the acidic protein in kiwifruit appears on day 6 after anthesis. The biological role of the pI 3.5 protein in latex is also unknown and needs further investigation. The molecular bases of allergenic cross-reactivity between latex and other fruits are currently under investigation.

Acknowledgments—We thank Dr. Robert Hamilton (The Johns Hopkins University) and Dr. Yunginger (Mayo Clinic) for their extracts of gloves, ammoniated latex, and latex-allergic serum. We are grateful for the generous gift of the latex cDNA library from Dr. Anil Kush, University of Singapore. We are also grateful to Dr. Esah Yip from the Rubber Research Institute of Malaysia for providing us with the latex for our study. We thank Dr. Lewis K. Pannell (NIDDK, NIH) for performing the Mass Spectroscopy, Robert A. Boykins for the amino acid analysis, and John B. Ewell for some of the protein sequence determination and oligonucleotide synthesis. We also thank Leo Vieira, Debra Spagnola, and Amy Doherty for technical assistance.

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Acidic Latex Allergen

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doi: 10.1074/jbc.271.41.25389

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