

Characterization of Recombinant Per a 10 from Periplaneta americana

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TABLE 1 Intradermal test and ELISA results from cockroach-hypersensitive patients

				ELISA result (OD ₄₉₀) with:		
Serum no.	Age (yr)/sex ^a	Clinical history ^b	i.d. test grade ^c	Cockroach extract	nPer a 10	rPer a 10
Patients						
1	22/F	AR + BA	++	1.672	0.826	0.582
2	34/M	AR	++	1.520	0.799	0.527
3	29/F	AR	++	2.058	0.811	0.522
4	42/F	AR + BA	+++	2.017	0.879	0.604
5	38/M	AR	+++	1.666	0.925	0.432
6	25/F	AR + BA	+++	1.886	1.009	0.705
7	40/M	AR	++	1.774	0.838	0.501
8	29/F	AR + BA	++	1.791	1.083	0.608
9	25/M	AR	+++	1.783	0.816	0.541
10	32/F	AR + BA	+++	2.040	1.271	0.435
11	29/F	AR	++	2.060	0.819	0.621
12	19/F	AR + BA	+++	2.058	1.385	0.685
13	37/M	AR	++	1.302	0.872	0.514
14	21/M	AR + BA	+++	1.730	0.734	0.525
15	24/F	AR	++	1.833	0.927	0.570
16	36/M	AR+ BA	+++	1.917	0.926	0.566
Controls						
C1	25/F			0.114	0.109	0.108
C2	26/M			0.095	0.107	0.112
C3	35/F			0.108	0.118	0.113
C4	26/M			0.117	0.105	0.107
C5	36/M			0.113	0.115	0.103
C6	26/M			0.112	0.106	0.109

^a M, male; F, female.^b AR, allergic rhinitis; BA, bronchial asthma.^c ++, equivalent to histamine (positive control); +++, more than histamine (positive control). Histamine diphosphate (100 mg/ml) was used as a positive control. Sera showing an OD of ≥ 3 times the average value of control serum were considered positive (patients 1 to 16). The tests were also carried out on six nonallergic subjects (C1 to C6).

tration of 0.1 mg/ml. Spectra were measured from 260 to 190 nm, with a 0.5-nm resolution at a scanning speed of 50 nm/min, and results were expressed as the mean residue ellipticity (θ) at a given wavelength. The secondary structure content of rPer a 10 and nPer a 10 was calculated using the secondary structure estimation program K2D2 software as described previously (18).

Collection of allergic patients' blood or serum. Patients from the Outpatient Department, V.P. Chest Institute, Delhi, India, diagnosed with allergy were included in the present study (Table 1). The diagnosis of asthma was ascertained as per guidelines of the American Thoracic Society, and patients having any two symptoms, such as sneezing, rhinorrhea, nasal blockage, postnasal drip, etc., for the last 2 years were diagnosed as having rhinitis (17). Intradermal (i.d.) tests were carried out with a battery of allergen extracts, including *P. americana* extract (1:500 [wt/vol]), as the reactions were graded after 20 min on the basis of wheal size of the positive control (i.e., histamine diphosphate) (17). Blood was collected from patients showing a marked positive skin reaction (wheal size equal to or greater than that of the positive control) with cockroach extract. Blood was also collected from healthy subjects ($n = 6$) with negative skin tests to cockroach extract to use as the control. The study protocol was approved by the Human Ethics Committee of the institute, and the informed written consent of each subject was obtained for their participation.

ELISA. A specific IgE enzyme-linked immunosorbent assay (ELISA) was performed to determine the allergenicity of the rPer a 10 along with nPer a 10 and cockroach extract, as described previously (17). A microtiter plate (Nunc) was coated with purified rPer a 10 protein, nPer a 10 protein,

and cockroach extract (1 μ g/100 μ l per well) in carbonate buffer (pH 9.6) and incubated at 4°C overnight. The plate was washed and blocked with 3% defatted milk for 3 h at 37°C, washed, and incubated with 100 μ l each of 16 individual cockroach-hypersensitive patient's sera (1:10 [vol/vol]) at 4°C overnight. Sera from healthy subjects were used as control. After incubation, the plates were washed with PBS-T (PBS containing 0.05% Tween 20) twice followed by PBS twice. The plates were incubated with 1:1,000 (vol/vol) diluted anti-human IgE peroxidase (Sigma Chemicals, Co., St. Louis, MO) at 37°C for 3 h and washed with PBS-T (PBS containing 0.05% Tween 20) twice followed by PBS three times. The color was developed with *o*-phenylene diamine, the reaction was stopped by adding 50 μ l of 5 N H₂SO₄, and absorbance was read as the optical density at 492 nm (OD₄₉₂) in an ELISA reader (Benchmark Plus; Bio-Rad).

IgE immunoblot. The binding ability of rPer a 10 to human IgE antibodies from cockroach-hypersensitive patients' sera was studied by Western blotting. SDS-PAGE-resolved proteins were transferred electrophoretically onto a nitrocellulose membrane in buffer containing 25 mM Tris, 192 mM glycine, and 25% methanol at a constant 50 V overnight at 4°C, as described previously (17), using a mini-Trans Blot cell (Bio-Rad, CA). The bands were visualized with Ponceau-S (0.1% in 1% acetic acid) to ensure the transfer of the proteins. The membranes were cut into strips, blocked with 3% defatted milk in PBS, and incubated overnight with 1:10 (vol/vol)-diluted cockroach-hypersensitive patients' sera ($n = 16$) containing 0.05% defatted milk at 4°C; sera from healthy subjects were used as the control. After incubation, the strips were washed with PBS containing 0.05% Tween (PBS-T) followed by PBS, probed with 1:1,000 (vol/vol)-diluted anti-human IgE peroxidase (Sigma Chemicals Co., St. Louis, MO) for 3 h at 37°C, and washed with PBS and PBS-T. The IgE-reactive bands were visualized with diaminobenzidine (DAB) and hydrogen peroxide in acetate buffer. The reaction was stopped by washing the strips with deionized water.

ELISA inhibition. The specific IgE inhibition assay was carried out to determine the potency of nPer a 10 or rPer a 10. The microtiter plate was coated with antigen (1 μ g of *P. americana* extract) overnight at 4°C. Cockroach-hypersensitive pooled patient sera were preincubated at 4°C overnight with different concentrations (0.1 to 10,000 ng) of rPer a 10, nPer a 10, or cockroach extract separately, and sera from healthy subjects were used as control. The plates were blocked with 1% bovine serum albumin (BSA) and then incubated with 100 μ l of a preincubated mixture of 1:10 (vol/vol)-diluted cockroach-hypersensitive pooled patients' sera overnight at 4°C. Inhibition is presented as the percentage decrease in absorbance of the sample with inhibitor to that of sample without inhibitor, and allergenic potency was defined by establishing the concentration of protein required for 50% inhibition.

Immunoblot inhibition. Immunoblot inhibition was performed to establish the specificity of rPer a 10 and cockroach extract with other serine protease allergens. Cockroach-hypersensitive pooled patient sera were incubated with 500 μ g of cockroach extract, rPer a 10 for homologous inhibition, and *Curvularia* extract and 31-kDa purified serine protease from *Curvularia*. The SDS-PAGE-resolved protein was transferred onto nitrocellulose membrane. The bands were visualized with Ponceau-S to ensure the transfer of the proteins. The membranes were cut into strips and blocked with 3% defatted milk in PBS. After blocking, the strips were incubated with the inhibited pooled patients' sera. The rest of the procedure was similar to that described for the immunoblotting methods.

Zymography. The enzymatic activity of rPer a 10 and nPer a 10 was analyzed by gelatin zymography. The purified protein was fractionated on 12% polyacrylamide gel containing copolymerized 0.1% gelatin as the substrate. The gels were washed with 2.5% (vol/vol) Triton X-100 for 1 h on an orbital shaker to remove the SDS, as described previously (14). The gels were then rinsed twice with deionized water to remove the Triton X-100 and incubated overnight at 37°C in 100 mM Tris-glycine (pH 7.4), stained with CBB, and destained.

Histamine release assay. The histamine release assay was performed as described previously (19) for both active and heat-inactivated protein

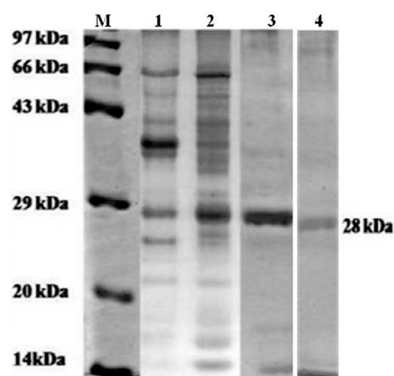


FIG 1 Affinity-purified rPer a 10 resolved by SDS-PAGE. Lane M, molecular mass marker; lane 1, uninduced; lane 2, induced; lane 3, purified protein (rPer a 10); lane 4, native protein (nPer a 10).

of rPer a 10 and nPer a 10, with cockroach-hypersensitive patients' sera. The proteins were inactivated by being heated at 95°C for 15 min. The leukocyte separation solution with 6% dextran in saline containing 0.01 M EDTA and 2% dextrose was mixed with the peripheral blood (1:5 [vol/vol]) drawn from healthy subjects, and the mixture was incubated for 90 min at room temperature to separate the leukocyte-rich layer. The separated layer was drawn off carefully without red blood cell (RBC) contamination, centrifuged at $300 \times g$ at 4°C, and then washed twice in saline. The cell count was performed, and the cell suspension containing 2.5×10^6 cells was taken for incubation. The bound IgE was stripped off from the basophil suspension by incubation in lactic acid buffer for 3.5 min at 37°C. The cells were washed in HEPES buffer containing 0.05% human serum albumin (HSA) (pH 7.4) and incubated with a sensitization mixture containing patient's sera against cockroach allergen ($n = 15$) at 37°C for 90 min. Cells sensitized with sera from healthy subjects were used the negative control. After passive sensitization with cockroach-hypersensitive patients' sera, the basophils were stimulated with 5 ng of cockroach extract or the nPer a 10 or rPer a 10 in HEPES buffer containing 0.05% HSA and 1 mM CaCl_2 at 37°C for 1 h, and simultaneously the same heat-inactivated proteins (95°C for 15 min) were also incubated in HEPES buffer under the conditions described above. After incubation, the reaction was stopped by the addition of 750 μl of ice-cold 0.9% NaCl (wt/vol). Then the cells were centrifuged at $12,000 \times g$ for 8 min, and the cell-free supernatant containing histamine was taken in a test tube and mixed with 200 μl of 12% perchloric acid. The histamine content was determined by the fluorometric method, using *o*-phthalaldehyde as the substrate. Spontaneous histamine release was measured in the supernatant of unstimulated cells. The total histamine content was determined by lysis of a separate aliquot of leukocytes with 3% perchloric acid. The allergen-induced histamine release was calculated as a percentage of the total histamine content after correction for spontaneous release.

Statistical analyses. Specific IgE and histamine releases in response to rPer a 10 and nPer a 10 were compared by a paired *t* test, and the correlation coefficients were determined by linear regression.

RESULTS

Expression and purification of Per a 10. rPer a 10 was expressed in *E. coli* and affinity purified. The yield of purified rPer a 10 was 3 mg/liter bacterial culture. The purified protein resolved at 28 kDa in 12% SDS-PAGE was shown to have more than 90% purity (Fig. 1). Furthermore, rPer a 10 was recognized by His tag polyclonal antibody. nPer a 10, purified by affinity chromatography from *P. americana* extract, yielded a single band at 28 kDa in SDS-PAGE (Fig. 1).

Homology. On the basis of the BLASTP homology searches,

the deduced amino acid sequences exhibited 41 to 52% similarity to insect trypsins. Per a 10 showed high similarity (71%) to the trypsin of *B. germanica* (Bla g trypsin) and was 27 to 38% similar to mite serine protease allergens like Blo t 3 (*Blomia tropicalis*), Der p 3 (*Dermatophagoides pteronyssinus*), Der p 9 (*D. pteronyssinus*), and Der f 3 (*Dermatophagoides farinae*) with the fully conserved catalytic domain GDSGG (Fig. 2).

CD. The far-UV CD spectra of purified nPer a 10 and rPer a 10 recorded at room temperature (25°C) showed two minima at 207 and 225 nm and a large maximum at 217 nm (Fig. 3). Calculation of the secondary structure using the program K2D2 resulted in 3.13% and 49.32% α -helix and β -sheets, respectively, for nPer a 10 and 19.92% and 27.52% α -helix and β -sheets, respectively, for rPer a 10 (Fig. 3).

Specific IgE binding of rPer a 10. The specific IgE binding of rPer a 10 or nPer a 10 was identified by ELISA and immunoblotting using individual cockroach-hypersensitive patient's sera ($n = 16$). For immunoblotting, patients' sera ($n = 16$) were probed against rPer a 10, and sera from healthy subjects were used as a negative control. All 16 patients' sera reacted with rPer a 10 protein, indicating it is a major allergen. Sera from healthy subjects showed no IgE binding in the immunoblot (Fig. 4). Cockroach-hypersensitive patients' sera showed raised IgE values against cockroach extract compared to nPer a 10 and rPer a 10. The specific IgE absorbance values for cockroach extract ranged from an optical density (OD) of 1.506 to 2.125, that of nPer a 10 ranged from OD of 0.812 to 1.3125, and that of rPer a 10 ranged from OD of 0.492 to 0.755. OD values ≥ 3 times that of the control sera (OD of 0.092) were taken as the cutoff for an ELISA-positive result (Table 1). IgE binding to rPer a 10 is positively correlated to nPer a 10 ($R = 0.71$).

Potency of purified protein. ELISA inhibition was performed to check the allergenic potency and specificity of IgE binding of rPer a 10 compared to that of nPer a 10. The dose-dependent inhibition of IgE binding to the solid phase of cockroach extract was observed. The pooled cockroach-hypersensitive patients' sera were incubated with different concentrations (0.1 to 10,000 ng) of cockroach extract, nPer a 10, or rPer a 10 protein. A maximum of 90% inhibition was achieved when 1 μg of cockroach extract was used, nPer a 10 showed 85% inhibition, and rPer a 10 protein showed 62% inhibition for solid-phase-bound crude cockroach allergen extract. For 50% inhibition (IC_{50}) of solid-phase-bound crude cockroach allergen extract, 96 ng of rPer a 10 protein was required, compared to 34 ng of nPer a 10 and 26 ng of cockroach extract (self) for the same level of inhibition (Fig. 5a).

Immunoblot inhibition was performed to find the specificity of rPer a 10. Preabsorption of pooled cockroach-hypersensitive patients' sera with cockroach extract and purified protein completely abolished IgE binding to cockroach extract and rPer a 10 protein. With *Curvularia* as an inhibitor, no cross-inhibition or cross-reactivity was observed in cockroach extract and rPer a 10 protein (Fig. 5b).

Proteolytic activity of recombinant protein. The qualitative analysis of rPer a 10 showed that the protein is not enzymatically active; this may be due to improper folding, or conformational changes in rPer a 10 may lead to an inactive form. nPer a 10 showed activity on the zymogram (Fig. 6).

Histamine release assay. The biological activity of nPer a 10 or rPer a 10 was assessed by its ability to release histamine from sensitized basophils. Basophils sensitized with cockroach-hyper-

Der f 3	-----MMIL--TIVVLLAAN-----ILATPILPSSPNATIVGGVKAQAGDCPYQIS	44
Eur m 3	-----MVICNAIVLLLAEN-----TLANPILPSSPNATIVGGQKAKAGECPYQIS	46
Der p 3	-----MI IYNILIVLLLAEN-----TLANPILPASPATIVGGKALAGECPYQIS	46
Blo t 3	-----MKVLVLECLVSLAAAGPLKDALNKAQVDAFYAEGYIVGGSNAADGDAPYQVS	52
Tyr p 3	-----MKILLFLCFLVSVAFAPK--PTIQLKSNTKSQNGFIVGGTEAVDGDAPHQVS	50
Per a 10	-----MLRYVLVASLIACS-----LSAVPKAKRPRLDGRIVGGRPADIDYYPYQLS	47
Der p 9	-----MKFMILFALIAIGTSVAIGEQAIRLPLDAITEKFPWMINEPLNDE-RERIVGGSNASPGDAVYQIA	66
Der f 9	-----MDSPDLGTDDD-----DKAMADIGSEFIVGGENASPGDAIYQIA	40
Der f 3	LQSS-SHFCGGSIILDEYWI LTA AHCVNG---QSAKLSIRYNTLKHASGGEKIQVAEIQHENYDSMTIDNDVALIKLKTP	121
Eur m 3	LQSS-SHFCGGTILDEYWI LTA AHCVNG---QTASKLSIRYNSLKHASGGEKLSVAQIYQHEKYDSWTIDNDIALIKLOSP	123
Der p 3	LQSS-SHFCGGTILDEYWI LTA AHCVAG---QTASKLSIRYNSLKHSLGGEKI SVAKIFAHEKYDSYQIDNDIALIKLKSP	123
Blo t 3	LQRT-SHFCGGSIIDADNY LTA AHC IQG---LSASSLTTRYNTLRHNSGGITVKASRIIGHEKYDSNTIDNDIALIQTASK	129
Tyr p 3	LQHT-SHFCGGSIISERWI LTA AHCIDADDLSNPGGMSVRYNTLNLS-GTLVKVKSIVKHEQYSNVTSNDIALLETVAS	130
Per a 10	10FEYYGSHMCGASIIISPNV VTA AHCVDG---VSASSATFRAGGSIRESGSVHQATQLIANPNYDYITDFDVAVARVSPA	124
Der p 9	LFRKDSFTCGGSLISSRT VTA AHCVFG-DEATPSYFKIRYNTLDRTN-GPPIGVSKIYRHNLYSSPIDYDVATLILSQP	145
Der f 9	LLRKDSFTCGGSLISSRT VTA AHCVFG-DEASPSYFKIRYNTLDRTN-GPTIGVSKIYRHSLSSTTIDYDVATLILSEP	119
Der f 3	TLDQTNAPVPLPAQGSQSDVKVGDKIRVSGWGYLQEGSYSLPSELQVRDIDVVSREQCQQLYSKAGADVSENMICGGDVAN	201
Eur m 3	TLDQKNAKSVQLPSQGSQSDVKVGDKVRVSGWGYLKEGYSILPSDMYRVIDIVAREQCQNKLYEEAGATITDNMICGGNVAD	203
Der p 3	KLNQKNAKAVGLPAKGSQSDVKVGDQVRVSGWGYLEEGSYSLPSELRRVDIAVVSKECNELYSKANAETDNMICGGDVAN	203
Blo t 3	STGTNAQAIAKLPEQGSQSDPKASSEVLITGWTLSSGASSLPTKLQKVTVPIVDRKTCNANYGAVGAEITDNMFCAGILNV	209
Tyr p 3	MNLNQTNAAKLPKAGNDPQ-DGDLFLSGWGLTHSGDTTIPTNLQKVTVPLTNRSVCAEAYTGI-NITENMFCAGKMG	209
Per a 10	10FSYGTG--VQPIPLASSEPSAGQIATVSGWGTTEGGSTLPSQLQVSVPIVSRSECNOAYS DY-GITDDMICAAEQQ-	199
Der p 9	FTPSAN--ADIIPLTTSEPADGTLQITGWGRLLKSGG-TLPTILQIASVTKMSRTKCSSTWGSV-AITNRMLCAHNSN-	219
Der f 9	FTPSAN--ADII SLTTAEPADNTQLQITGWGRLLKSGG-TIPTLLQIATISKMSRTKCSNIWGSV-AITNRMLCAHSKK-	193
Der f 3	GGVDSQCGDSGGPVDVATKQIVG-IVSWGYG-CARKGYPGVYTRVGNFVDWIE--SKRSQ-----	258
Eur m 3	GGVDSQCGDSGGPVDVASNQIVG-IVSWGYG-CARKGYPGVYTRVGSFIDWID--SKRSQ-----	261
Der p 3	GGKDSQCGDSGGPVDVKNQVVG-IVSWGYG-CARKGYPGVYTRVGNFIDWIE--SKRSQ-----	261
Blo t 3	GGKDACQCGDSGGPVA--ANGVLVG-AVSWGYG-CAQAKYPGVYTRVGNYSWIK--GKGVPV-----	266
Tyr p 3	GGVDSQCGDSGGGAM--LNKELVG-VVSFVG-CGDPKYPGVYTRVSYLDWIELSAKSSATTLVAVNITLFLTLFIGAIW	285
Per a 10	10GGKDACQCGDSGGPLV--VNGQLAG-IVSWGVG-CAEQGYPGVYSNVASLKGFI TEQTGVN-----	256
Der p 9	--QASCNGDSGGPLVS--NGHLVG-VVSWGPGSTCLSTKYPTIYSNVANLRNWI ISNTV-----	273
Der f 9	--QSACNGDCGGPLVY--NGHLVG-VVSWGPGSTCLSNITYPTIYSNVATLRNWI ISNTV-----	246

FIG 2 Multiple sequence alignment of Per a 10 protein with different mite proteins. Per a 10 showed 27 to 38% homology with mite trypsins.

sensitive patients' sera (IgE) individually showed histamine release on challenge with cockroach extract, nPer a 10, and rPer a 10. Histamine release by individual patient's sera ranged from 60 to 85% and 47 to 64% upon challenge with nPer a 10 and rPer a 10,

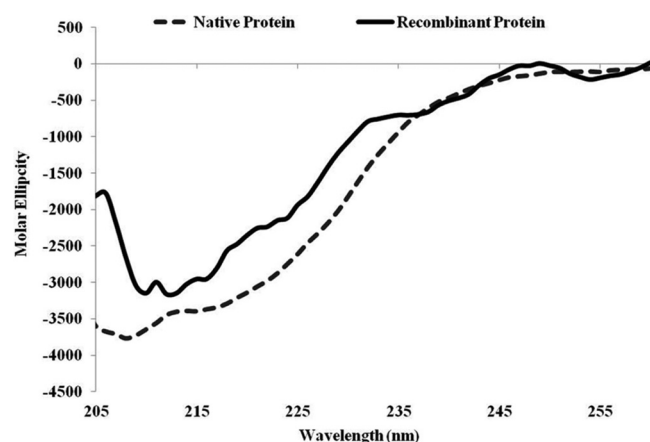


FIG 3 Circular dichroism spectra of rPer a 10 and nPer a 10. Protein was taken at a concentration of 0.1 mg/ml in 10 mM phosphate buffer, and spectra were recorded in the far-UV range (190 to 260 nm).

respectively, and showed a positive correlation ($R = 0.88$) (see Fig. S1a in the supplemental material), whereas the cockroach extract showed 38 to 52% histamine release (Fig. 7a).

The heat-inactivated nPer a 10 and rPer a 10 showed reduced histamine release. There were histamine releases of 30 to 39% and

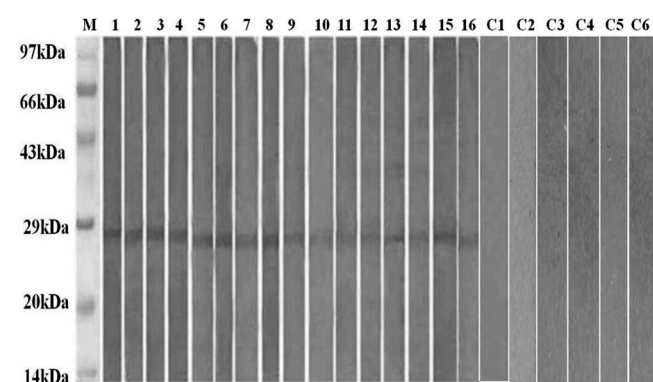


FIG 4 Immunoblot of IgE binding with individual patient's and control sera. Lane M, molecular mass marker; lanes 1 to 16, IgE binding of rPer a 10 protein with individual patient's sera; and lanes C1 to C6, rPer a 10 with sera from healthy subjects.

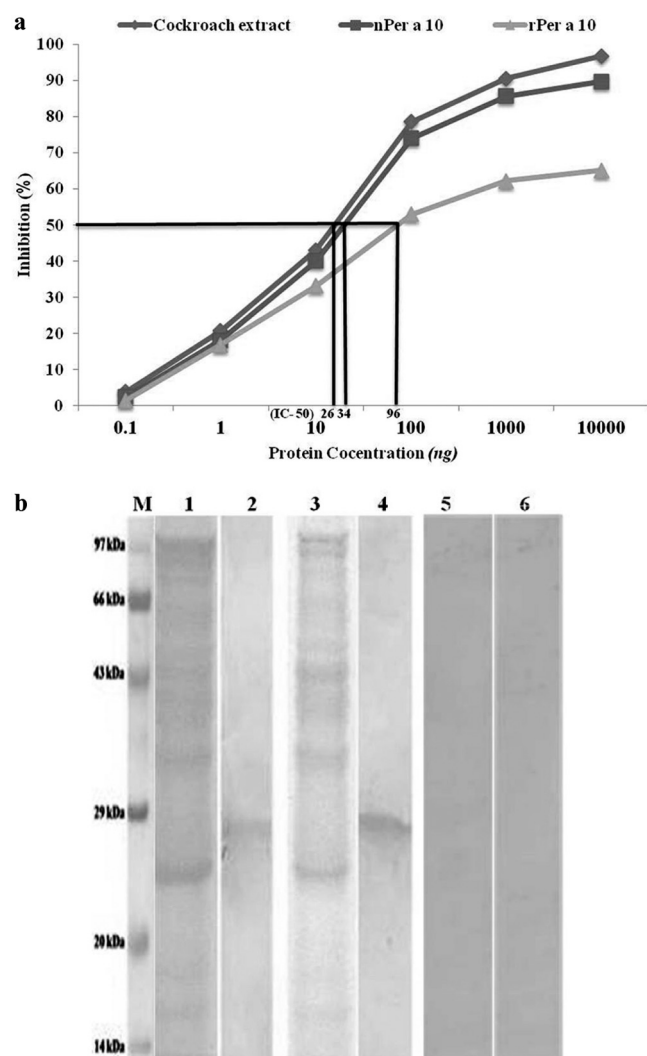


FIG 5 (a) ELISA inhibition was carried out using cockroach extract, nPer a 10, or rPer a 10 as an inhibitor. Cockroach extract, nPer a 10, or rPer a 10 (0.1 to 10,000 ng) was preincubated with pooled hypersensitive patients' sera, added to an ELISA plate coated with cockroach extract (solid phase), and detected using anti-human IgE peroxidase. (b) Immunoblot inhibition of cockroach extract and rPer a 10 with self and *C. lunata* extracts. Cockroach-hypersensitive patients' pooled sera were preincubated with 500 µg of extracts, as shown by lane: lane M, molecular mass marker; lane 1, *Curvalaria* extract; lane 2, purified serine protease from *Curvalaria*; lane 3, pooled cockroach-hypersensitive patients' sera used as a positive control on cockroach extract; lane 4, rPer a 10; lane 5, cockroach-hypersensitive patients' pooled sera with cockroach extract; lane 6, rPer a 10 inhibited with 500 µg of self-protein.

27 to 37% with heat-inactivated nPer a 10 and rPer a 10, respectively, which is positively correlated ($R = 0.97$) (see Fig. S1b in the supplemental material), whereas cockroach extract showed 20 to 29% histamine release. Basophils stimulated with control subjects' sera showed 6 to 9% histamine release in response to nPer a 10, rPer a 10, or cockroach extract (Fig. 7b).

DISCUSSION

Allergenic extracts prepared from natural sources for diagnosis and therapies are a complex mixture of proteins and other molecules which may differ qualitatively in various batches. Extracts of a major allergen may be helpful for obtaining a standardized prep-

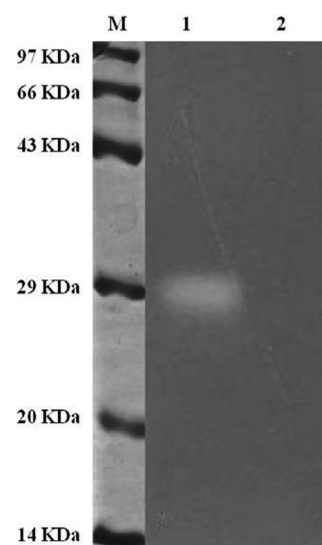


FIG 6 Proteolytic activity of Per a 10 in a zymogram. Shown is 12% gel copolymerized with 0.1% gelatin as the substrate and resolved with nPer a 10 and rPer a 10. The clear colorless region of depletion in the gel against the gray background shows proteolytic activity. Lane M, molecular mass marker; lane 1, nPer a 10; lane 2, rPer a 10.

aration. Furthermore, recombinant allergens are considered safer and more effective than whole-allergen extracts for component-resolved diagnosis and therapy of allergy-specific disorders (15). In the present study, Per a 10 allergen was subcloned, expressed, purified, and compared with nPer a 10 by immunobiochemical methods.

Homology studies of Per a 10 showed 71% similarity to *B. germanica* and 27 to 38% similarity to mite serine protease allergens like *B. tropicalis*, *D. pteronyssinus*, and *Dermatophagoides farinae*. Serine proteases from fungi and mites were recognized as major allergens by IgE binding studies. The purified nPer a 10 showed 82% IgE binding on immunoblotting and ELISA to the *P. americana*-hypersensitive patients' sera (17). In the present study, rPer a 10 showed 2-fold reduction (43%) in specific IgE binding to cockroach-hypersensitive patients' sera in ELISA and immunoblotting compared to >80% reduction with nPer a 10.

The study by Jeong et al. showed that the cockroach extract of *P. fuliginosa* and *B. germanica* showed 83% and 51% inhibition, respectively (20). In the present study, rPer a 10 showed 2.8 times less inhibition than nPer a 10 for reduction in 50% IgE binding on solid-phase-coated cockroach extract. Cockroach allergens are species specific, and group 1 allergens (tropomyosin) like Bla g 1 and Per a 1, are cross-reactive, which have been sequenced from both *P. americana* and *B. germanica* (21). Furthermore, IgE binding of rPer a 10 was completely inhibited when pooled patients' sera were preabsorbed with 500 µg of self-protein in immunoblot inhibition, and there was no inhibition in *C. lunata* serine protease, which showed clear bands. Serine proteases are reported from many sources, and most of them are cross-reactive allergens (22–24).

The biological activity of recombinant allergens was determined by basophil histamine release assay (14, 25). In this study, rPer a 10 and nPer a 10 induced significant histamine release by 47 to 64% and 60 to 85%, respectively, which was positively correlated. The heat-inactivated proteins of nPer a 10 and rPer a 10

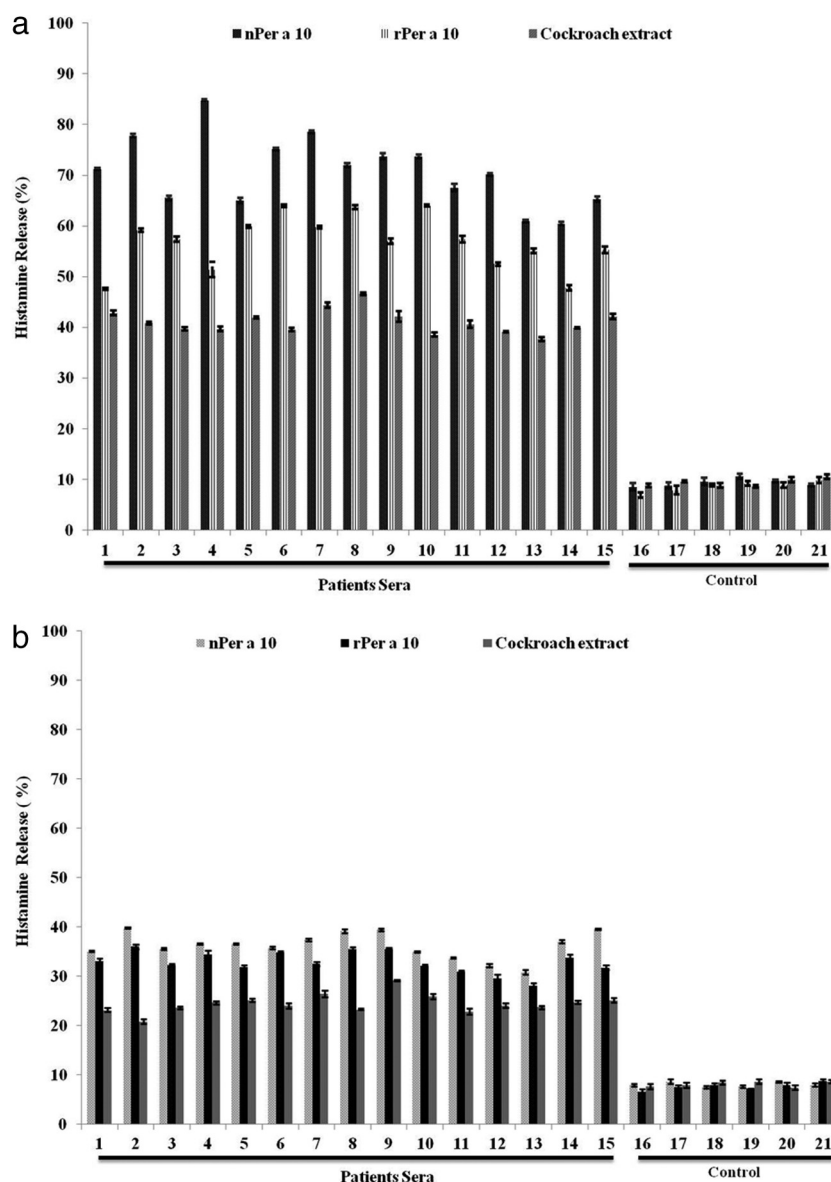


FIG 7 Histamine release assay with active (a) and heat-inactivated (b) protein. Basophils in leukocyte suspension were stripped off the bound IgE. Stripped basophils were sensitized with sera of cockroach-sensitive patients. The sensitized basophils were stimulated with rPer a 10 or nPer a 10 or cockroach extract. The histamine released in cell supernatant was quantified and expressed as a percentage of total histamine content. Lanes 1 to 15, histamine release with individual patient's sera; lanes 16 to 21, control sera from healthy subjects.

induced histamine release by 30 to 39% and 27 to 35%, respectively, which is correlated positively, and release was comparatively less toward the active proteins. The IgE binding and biopotency of rPer a 10 were low compared with that of nPer a 10. This may be due to the fact that the conformational B-cell epitopes in recombinant protein are in reduced form due to its improper folding (26, 27).

Serine proteases in cockroach extract may induce proinflammatory effects on human bronchial epithelial cells, leading to the progression of airway inflammatory diseases (3). Per a 10 has trypsin-like activity (17), and proteolytic activity enhances the entry of other allergens into the epithelium and plays a role in other allergen presentation (11). The absence of proteolytic activity of rPer a 10 may be due to improper folding of the protein. The proteolyti-

cally inactive Per a 10 with a reduced Th2 inflammatory response induced IL-10 in a mouse model (28). *E. coli*-expressed recombinant proteins get aggregated in insoluble form, whereas rPer a 10 is a soluble protein, and ease of purification may have an advantage for clinical use.

Conclusions. Per a 10 was subcloned, and the protein was purified to homogeneity. rPer a 10 showed reduced IgE binding and histamine release and showed no proteolytic activity. These data suggest that rPer a 10 has potential for immunotherapy.

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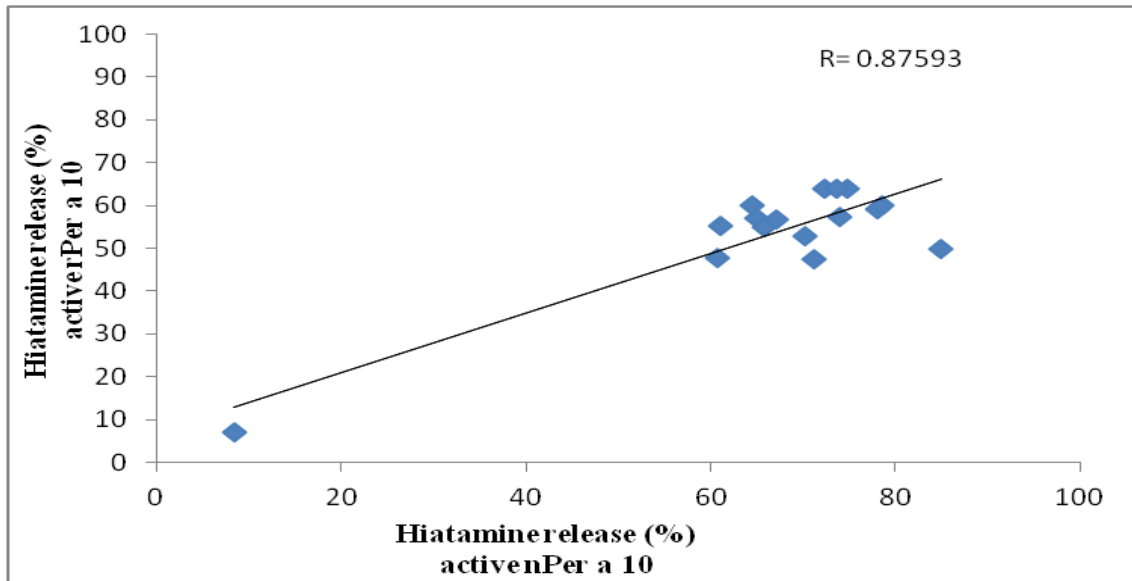
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Figure S1. Correlation between active (a) and heat inactivated (b) nPer a 10 and rPer a 10 for histamine release assay which are positively correlated.

(a)



(b)

