



SEQUENCING AND IMMUNOCHEMICAL CHARACTERIZATION OF THE AMERICAN COCKROACH Per a 3 (Cr-PI) ISOALLERGENIC VARIANTS

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Abstract—Two additional members of the American cockroach (*Periplaneta americana*) Per a 3 (Cr-PI) allergen, C13 and C28, were isolated and sequenced. They encoded proteins of 470 and 393 amino acids with two and no potential *N*-glycosylation sites, respectively. The molecular weights for C13 and C28 cloned proteins are 56 200 and 46 7000, with PI values of 7.06 and 6.54. C13 and C28 display 95.4% identity with several overlapping predicted central antigenic determinants. Both allergens were also found to have a 95% sequence homology with previously cloned C20 and share similar antigenic determinants, as defined by the structural prediction and ELISA analysis. However, the recombinant C13 and C28 allergens showed 26.3 and 94.7% skin reactivities on asthmatic patients while C20 elicited 47.4%. While no sequence similarity was found to other known allergens, these two aromatic amino acid-rich allergens were highly related to insect hemolymph proteins (28.7–36.5%), as with C20 cloned protein. Results suggest that these two are isoallergenic variants of C20. Sequence variations among isoforms, resulting a significant difference in skin reactivities, will be useful in elucidating the allergenic determinants. © 1997 Elsevier Science Ltd. All rights reserved.

Key words: cDNA cloning, American cockroach, Per a 3 allergen, isoallergenic variants.

INTRODUCTION

The most common and major domiciliary cockroaches are the American and the German cockroaches both in Taiwan (Lan *et al.*, 1988; Wu and Lan, 1988) and the United States (Arruda *et al.*, 1995a). Two important allergens, Bla g 2 and Bla g 4 of the German cockroach (*Blattella germanica*) have recently been cloned and sequenced (Arruda *et al.*, 1995a,b). Bla g 2 is a protein of M_r 36 000, sharing sequence homology to the aspartic proteases, and Bla g 4, an allergen of M_r 21 000, is a ligand binding protein or calycin. Previous studies have isolated and identified Per a 3 and the Cr-P11 allergenic fractions from the CRA-A (Wu and Lan, 1988). The major allergens of Per a 3, of M_r 78 000 and 72 000, cause T cell proliferation in cockroach allergic patients (Jeng *et al.*, 1996), and the M_r 45 000, 32 000 and 28 000 proteins

in the Cr-P11 have also been identified as additional major allergens of *Periplaneta americana* (Wu *et al.*, 1996a).

Previously, the construction of a λ gt22A cDNA library (Wu *et al.*, 1993, 1995) and the subsequent identification of several clones recognized by anti-Per a 3 mAb has been reported (Wu *et al.*, 1990). Among them, clones C12, C20, C13 and C28 expressed fusion proteins of molecular weight 78 000, 72 000, 54 000 and 46 000, respectively, in transformed *Escherichia coli* BL21 (DE3). These proteins were recognized by human specific IgE antibodies as determined by immunoblotting (Wu *et al.*, 1995). Recently, two cDNA clones were sequenced (Wu *et al.*, 1996b) and the predicted molecular weights for C12 and C20 cloned proteins were 79 300 and 75 500, with PI values of 6.26 and 6.63, which are compatible with the determined size (78 000 and 72 000) and PI (6.2) of Per a 3 (Wu and Lan, 1988; Wu *et al.*, 1996a). A high degree of identity (69.1%) including several overlapping predicted central antigenic determinant residues and non-conserved 3' noncoding region was found between these two allergens. While no sequence similarity was found to other known allergens, including Bla g 2 and Bla g 4, these two aromatic amino acid-rich allergens were found to have striking sequence identities to insect hemolymph proteins (20.1–36.4%). Results suggested that two prominent allergens of *Periplaneta americana* are ancestrally related to these insect hemolymph proteins and represent

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Abbreviations: PI, isoelectric point; CRA-A, crude American cockroach extract; mAb, monoclonal antibody(ies); PBS, phosphate-buffered saline; SDS, sodium dodecylsulfate; ELISA, enzyme-linked immunosorbent assay; ABTS, 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid); FAST, fluoroallergosorbent test; IU, international unit; bp, base pair(s); SD, standard deviation.

a new group of proteins in the hemocyanin superfamily. On the basis of sequence homology and similar immunochemical properties, the M_r 79 300 (C12) and 75 500 (C20) cloned allergens, according to WHO/IUIS nomenclature, (King *et al.*, 1995) are isoallergens and hereby designated Per a 3.01 and Per a 3.0201, respectively.

Several allergens have been shown to be composed of multiple isoforms either by immunochemical properties, direct sequence analysis, or by comparison of deduced protein sequence of isolated cDNA clones (Mole *et al.*, 1975; Roebber *et al.*, 1983; Griffith *et al.*, 1991; Matthiesen and Lowenstein, 1991; Nilsen *et al.*, 1991; Rafnar *et al.*, 1991; Silvanovich *et al.*, 1991; Singh *et al.*, 1991; Larsen *et al.*, 1992; Thomas *et al.*, 1992; Breiteneder *et al.*, 1993; Chua *et al.*, 1993; Kuo *et al.*, 1993; Ong *et al.*, 1993; Batanero *et al.*, 1994; Bufe *et al.*, 1994; Nishiyama *et al.*, 1994; Petersen *et al.*, 1995; Suphioglu and Singh, 1995; Smith and Thomas, 1996; Smith *et al.*, 1996). The existence of these isoallergens indicates the importance of determining the polymorphisms in allergens that may determine and define which protein isoforms play a role in allergic properties. This paper reports the nucleotide and deduced amino acid sequences of two additional partial cDNA clones, C13 and C28, encoding variants of Per a 3.0201 with significant difference in skin reactivities among asthmatic patients.

MATERIALS AND METHODS

Sequencing of cDNAs

cDNA clones, C13 and C28, were isolated from a λ gt22A cDNA library (Wu *et al.*, 1995) derived from American cockroach mRNA (Wu *et al.*, 1993). The insert DNA released by Not I and EcoRI digestion was subcloned into the plasmid pSPORT 1 (Gibco BRL/Life Technologies, Grand Island, NY, U.S.A.). DNAs were prepared from transformed *Escherichia coli* DH5 α colonies, and dideoxynucleotide sequencing (Sanger *et al.*, 1977) was performed using the Multi-Pol DNA Sequencing System (Clontech Laboratories Inc., Palo Alto, CA, U.S.A.), according to the manufacturer's directions. A set of nested deletions was created using the "Erase-a-base" Exo III/S1 nuclease system (Promega, Madison, WI, U.S.A.). [^{35} S]-labeled products were analysed on 6% polyacrylamide gels and autoradiographed.

Computer analysis

Sequence data were analysed using the PC/GENE software (IntelliGenetics Inc., Mountain View, CA, U.S.A.) and the FASTA program (Pearson and Lipman, 1988) was used for the sequencing homology search. The antigenic index was calculated according to the algorithm of Jameson and Wolf (Jameson and Wolf, 1988).

Expression and purification of fusion protein

Purified insert DNA was ligated into the multiple cloning sites of pET System (Novagen Inc., Madison, WI, U.S.A.) and used to transform *E. coli* BL21(DE3) as

described (Wu *et al.*, 1995). Recombinant proteins were recovered under denaturing conditions from inclusion bodies of the host cells, and purification of fusion proteins under denaturing conditions was performed using the rapid affinity column chromatography with pET His-Tag System as described by Novagen. Refolding was performed using dialysis with a gradual removal of the guanidine in 0.1 M Tris-HCl buffer (pH 8.0) and lyophilized. The purified fusion protein in a range of 1.0–2.0 μ g/ml was finally dialysed in 0.1 M Tris-HCl buffer, pH 9.8.

Preparation of antisera to fusion protein

The fusion protein was used as an immunogen, and polyclonal antibodies were produced from immunized mice by the method described (Mahana and Paraf, 1993; Wu *et al.*, 1996b). Ascitic fluids were harvested and purified by Protein A-agarose affinity chromatography (Wu *et al.*, 1990) using Affi-Gel Protein A MAPS II kit, according to the manufacturer's instructions (BioRad, Hercules, CA, U.S.A.).

Cockroach extracts and preparation of CRa-A and Per a 3

CRa-A was prepared by extracting the whole body of insects with Coca's solution in the laboratory, and the Per a 3 was purified from the CRa-A by Sephadex G-150 SF (Pharmacia, Piscataway, NJ, U.S.A.) gel filtration (Wu and Lan, 1988). Commercial cockroach skin test extracts were obtained from Allergy Laboratories Inc. (American cockroach, 1:20 w/v, Lot No. A5366; Oklahoma City, OK, U.S.A.), Berkeley Biologicals (American cockroach, 1:10 w/v, Lot No. 624072; Berkeley, CA, U.S.A.), Center Laboratories (American cockroach, 10000 PNU, Lot No. 3M00041; Port Washington, NY, U.S.A.), Greer Laboratories (American cockroach, 1:20 w/v, Lot No. GB26-30B-1A5 and German cockroach, 1:20 w/v, Lot No. GB46-3B-1A3; Lenoir, NC, U.S.A.) and Hollister-Stier Laboratories (American and German cockroach mix, 1:10 w/v, Lot. No. F63J7104; Spokane, WA, U.S.A.).

Detection of Per a 3 in cockroach extracts by anti-fusion protein antibody based ELISA

Microtiter plates (Immunolon II, Dynatech, Alexandria, VA, U.S.A.) were coated with either 100 μ g/ml of CRa-A (10 μ g/ml), Per a 3 (5 μ g/ml), Cr-P11 (5 μ g/ml) fusion protein (5 μ g/ml) or 1:20 dilution of commercial cockroach extracts in 0.1 M sodium carbonate buffer (pH 9.6) for 2 hr at 37°C. After incubation, coated wells were washed with PBS containing 0.05% Tween (pH 7.4, PBST) and then blocked with 2% bovine serum albumin overnight at 4°C. Wells were washed and incubated with 100 μ l of 1:10 dilution of anti-fusion protein antibodies (10 μ g/ml) for 2 hr at room temperature in triplicate. Following washing, peroxidase-labeled anti-mouse immunoglobulin (100 μ l of 1:4000, Sigma, St. Louis, MO, U.S.A.) was reacted with the wells for 2 hr at room temperature. Wells were then washed with PBST, and the

reactions were developed using 100 μ l of ABTS (ABTS, 55 μ g/ml in 0.1 M sodium citrate buffer, pH 4.2, containing 0.03% of H₂O₂) for 30 min, terminated with 50 μ l of 5% SDS. The results were determined at 415 nm on an EAR 400 AT Easy Reader (SLT-Labinstruments, Salzburg, Austria).

Skin testing

Commercial American cockroach extracts (1:20 w/v, Greer Laboratories), *Per a 3* and recombinant proteins at concentrations of 100 μ g/ml in PBS, containing 50% glycerol, were used for skin testing. PBS containing 50% (v/v) glycerol and 1.0 μ g/ml histamine were included as negative and positive control, respectively. Allergens and controls were applied to the testheads with a Dip 'N Touch dispenser (Center Laboratories, Port Washington, NY, U.S.A.) and epicutaneous skin test was performed with a sterile disposable Multi-Test applicator of eight testheads (Lincoln Diagnostics, Decatur, IL, U.S.A.). The reaction was read after 15 min and graded against the controls. Reaction comparable to negative control was considered as negative. Response with a wheal 2.0 mm larger, or an area of erythema 5.0 mm greater than negative control and a wheal 4.0 mm larger, or an area of erythema 10 mm greater than negative control was graded as 1+ and 2+, respectively. Reaction compatible to the positive control that usually had a wheal of 7–10 mm and an area of erythema greater than 20 mm was graded as 3+. Reaction greater than the positive control that was sometimes associated with pseudopodia and itching was graded as 4+.

IgE antibodies to CRa-A and Per a 3

Serum IgE antibodies to CRa-A and *Per a 3* were determined by FAST using an enzyme immunoassay kit (IgE FAST-Plus Test; BioWhittaker Inc., Walkersville, MD, U.S.A.), according to the manufacturer's instruction (Wu *et al.*, 1996b). IgE concentration (IU/ml) was determined by a BioWhittaker fluorometer using calibrators containing 0.35, 0.8, 4.0 and 20.0 IU/ml (1.0 IU/ml = 2.4 ng/ml) of perennial ryegrass-specific IgE as standards. A class-scoring system was assigned according to the manufacturer's recommendation as follows: class 0 < 0.34 IU/ml; class I, 0.35–0.99 IU/ml; class II, 1.0–2.99 IU/ml; class III, 3.0–6.99 IU/ml; class IV, 7.0–16.9 IU/ml; class V, 17.0–43.99 IU/ml; and class VI > 44.0 IU/ml.

RESULTS

Sequence analysis

The cDNA sequences from C13 and C28 inserts are presented in Fig. 1. The C13 insert contained a 1410 bp open reading frame that begin with a leucine codon (CTC). The C28 insert included a 1 bp 5' proximal end sequence and 1179 bp open reading frame that begins with a glutamic acid codon (GAA). The eukaryotic polyadenylation signal AATAAA was not found. Instead,

the variant AATATA signal (Hanson *et al.*, 1989) was located in both untranslated regions. Alignment of complete nucleotide sequences revealed 94.1% identity between the C13 and C28 clones (Fig. 1). The comparison of deduced amino acid sequences of C13 with C28 reveals 95.4% (375/393) identity between these two proteins (Fig. 2). The complete C13 and C28 inserts correspond to 470 and 393 amino acid polypeptides, of calculated molecular weights 56 200 and 46 700, and the predicted PI values for C13 and C28 are 7.06 and 6.54. The deduced amino acid sequences of C13 and C28 both contain three cysteines. The alignment of these sequences reveals that these cysteines are very well conserved. Clone C13 contains two potential *N*-glycosylation sites, while no potential *N*-glycosylation site was found for clone C28. It is striking that these allergens, as with *Per a 3.0201*, are rich in aromatic amino acids containing a combined 17.0% of phenylalanine and tyrosine residues.

Structure predictions

A computer-assisted analysis of antigenic sites with the deduced primary amino acid sequences was performed. The peaks reaching an antigenic index in the 1.3 to 1.7 area represent potential central epitopes, as recommended previously (Jameson and Wolf, 1988). Eleven and nine predicted central antigenic determinants for clones C13 and C28 are presented in Fig. 2, while eight overlapped regions are found between them. The predictions of membrane associated alpha helices were performed using Eisenberg's (Eisenberg *et al.*, 1984) and Rao's (Rao and Argos, 1986) methods and no region could be found to satisfy the criteria recommended by the authors.

Amino acid sequence homologies

The computer-assisted sequence homology search (SWISS-PROT release 31) revealed that C13 and C28 sequences, as with C20 cloned protein, have a significant high degree of identity with several insect storage proteins (28.7–36.5%), insect juvenile hormone-suppressible proteins (28.8–35.2%) and arthropod hemocyanins (30.9–35.7%) (data not shown). C13 and C28 deduced amino acid sequences were found having about 95% identities to C20 (Fig. 2), and no similarity was found to other known allergens.

Detection of Per a 3 by anti-fusion protein antibodies

A polyclonal antibody-based ELISA using anti-C12, anti-C13, anti-C20 and anti-C28 was performed to detect *Per a 3* allergens in the cockroach extracts and recombinant proteins. The response of *Per a 3* to all anti-fusion protein antibodies in the ELISA was dose dependent, and no *Per a 3* allergens were detected when pre-immunized mouse serum was substituted for anti-fusion protein antibodies, as a coating antibody for the ELISA (data not shown). Each of the polyclonal antibodies to the different recombinant fusion proteins was able to detect the CRa-A, *Per a 3* and other recombinant proteins with optical

C20	DIGDHYDIEANIGHYKYPHVVKNFISYKKGLLPRGEPFVYKHKHREQAIKLELFFAANDYDTFYKTACWARDRVNMGFMVYALTVAAFHRED	95
C13	-----	
C28	-----	
C20	TKDLVLPPEVNPYLFVEDDVIQQAQKYWTKESTGTDKHVEHVIPVNFARSQEDLVAFREDVDLNAFNMYFYIYPSWFNTLYGKSFDI ~~Q~~	190
C13	-----	30
C28	-----	
C20	GFYTYHQIYARYFLERLSNLPDVKPFQYKSKPLKTGYNEPLRYHNGEEMPAR ~~psn~~ MYPTNFDLFYVSDIKNYERRVEKAIDFGYAFDEHRTFYS	285
C13	GL..... ~~psn~~ Q..... ~~psn~~ D.....	124
C28	-----	47
C20	LYHDQHGMDYLGOMIE ~~qt~~ INSPHQYFYGSVFHFYRLLVGHVVD ~~pyhkn~~ GLAPSALEHPQOTALRDPAPFNQWLKRI ~~DHI~~ VQKYKRLPRYTYDELSF	380
C13	 ~~tsn~~ Y.....I..... ~~pyhkn~~ H.....Y..... ~~kykn~~	219
C28	 ~~tsn~~ Y.....I..... ~~pyhkn~~ H.....Y..... ~~kykn~~	142
C20	PGVKIENVGVKLYTFE ~~FEH~~ FEHSLGNAMYLGLKLE ~~DYMKASIRARHYRLNHNKPF~~ TYNIEVSSDKAQDVYVRI ~~FLG~~ PKYQSLGHECELDERRHYVE	475
C13	 ~~VL~~ ..N..... ~~pkvd~~	314
C28	 ~~IL~~ ..N..S..... ~~pkvd~~	237
C20	MDRFVHKVEAGKT ~~VIER~~ KSHDSSIIISD ~~shdsy~~ FLNLFKKVSDALQEKDQYVIDKSHKYGYPENLLLPK ~~akkg~~ QGTFTFVIVTPYVKQDEHDEP	570
C13	 ~~shdsy~~ ~~shdsy~~ ~~Eckd~~ ~~dnsh~~ ~~kakkg~~ L.S	409
C28	 ~~shdsy~~ ~~shdsy~~ Y...A...E.....N. ~~pe~~ ~~kakkg~~ EQ.....	332
C20	YHYKAFSYCGVGH ~~grkyp~~ ddkpl ~~GF~~ FFDRKIHVDYFTPNMYFKDVVIFHKKYDEVHDVTH	631
C13	T..... ~~grkyp~~ ddkpl..... ~~NETN~~	470
C28	 ~~grkyp~~ ddkpl.....E...	393

Fig. 2. Alignment of the deduced amino acid sequences. The single-letter amino acid code is used, and spaces (-) are introduced to optimize the alignment. The potential N-glycosylation sites, NFT, NTT and NET are underlined. () represents homology with clone C20 and the potential central antigenic determinant residues as described in the text are underlined and in lowercase characters.

Table 1. Detection of recombinant proteins by anti-fusion protein antibody based ELISA

Cockroach allergen	Optical density at 415 nm (average $\pm$ SD)			
	Anti-C12	Anti-C13	Anti-C20	Anti-C28
CRa-A	0.722 $\pm$ 0.006	1.034 $\pm$ 0.031	0.967 $\pm$ 0.034	0.889 $\pm$ 0.018
Per a 3	0.963 $\pm$ 0.012	1.284 $\pm$ 0.034	1.132 $\pm$ 0.022	1.218 $\pm$ 0.013
C12	1.593 $\pm$ 0.014	1.491 $\pm$ 0.052	1.379 $\pm$ 0.017	1.136 $\pm$ 0.039
C13	1.425 $\pm$ 0.013	2.474 $\pm$ 0.042	2.320 $\pm$ 0.032	2.094 $\pm$ 0.032
C20	1.138 $\pm$ 0.023	1.458 $\pm$ 0.049	1.587 $\pm$ 0.035	1.513 $\pm$ 0.015
C28	0.423 $\pm$ 0.014	1.364 $\pm$ 0.025	0.856 $\pm$ 0.016	1.373 $\pm$ 0.019

density ranging from 0.722 to 2.474 (Table 1). Moreover, all anti-fusion protein antibodies were able to detect all five commercial cockroach extracts, although there were differences between the quantity of allergens in the extracts (optical density ranging from 0.088 to 0.840). No detectable allergens were found in the German cockroach extract and the American cockroach Cr-P11 allergens using anti-fusion protein antibodies.

Allergy skin test and IgE antibodies

Thirty-nine allergic patients (16 to 57 years old) with asthma, and 10 non-allergic adults were skin tested with the commercial CRa-A. One out of 10 control subjects had positive skin reaction. Of 39 patients, 60% (23/39) gave a CRa-A positive immediate skin reaction. Among these 23 CRa-A-positive patients, 19 (82.6%) were sensitive to Per a 3. These 19 patients and non-allergic adults were tested for specific IgE to CRa-A and Per a 3 using FAST. All patients tested were found to carry CRa-A and

Per a 3 specific IgE, and controls showed no cockroach-specific IgE. C13 and C28 recombinant proteins showed 26.3% and 94.7% positive skin reaction on these 19 CRa-A- and Per a 3-positive patients, respectively. Results are summarized in Table 2

DISCUSSION

In this study, we presented the nucleotide sequences and preliminary immunochemical properties of two American cockroach Per a 3 allergens, clones C13 and C28. Coding regions of nucleotide sequence revealed that C13 and C28 are 96% identical to Per a 3.0201. Besides the high degree of identity and similarity to insect hemolymph proteins, several predicted antigenic sites were found overlapping among Per a 3.0201, C13 and C28. Recombinant proteins of clones C13, C20 and C28 can be recognized by both anti-Per a 3 mAb and human IgE. The anti-fusion protein antibodies-based ELISAs were

Table 2. Skin tests to recombinant allergens and serum IgE antibodies to CRa-A and Per a 3 on asthmatic patients

Patient	Skin test					FAST Class	
	CRa-A	Per a 3	C13	C28	C20	CRa-A	Per a 3
1	4+	2+	—	1+	1+	I	III
2	3+	1+	—	1+	—	I	II
3	3+	2+	—	2+	—	II	V
4	3+	2+	—	2+	1+	II	V
5	2+	2+	—	1+	—	II	III
6	2+	2+	—	1+	—	II	II
7	1+	1+	—	—	1+	I	I
8	3+	2+	1+	2+	1+	I	IV
9	2+	1+	—	1+	—	I	II
10	1+	1+	1+	1+	—	I	I
11	1+	1+	—	1+	—	I	I
12	2+	1+	—	1+	1+	I	III
13	3+	2+	—	2+	2+	I	II
14	1+	2+	2+	2+	2+	II	II
15	2+	2+	—	2+	—	I	II
16	2+	1+	—	1+	—	II	II
17	4+	3+	2+	1+	1+	II	V
18	2+	1+	—	1+	—	I	I
19	2+	2+	1+	2+	2+	I	II

able to detect CRa-A, Per a 3, recombinant proteins of C12, C13, C20 and C28 (Table 1) and five commercial cockroach extracts (Wu *et al.*, 1996b). Taken together, these recombinant allergens possess immunobiologically closely related properties, suggesting that they are Per a 3.0201 isoallergenic variants.

The occurrence of natural polymorphism in allergens within interspecies and subspecies in the population have been reported. Four Amb a 1 isoforms have been identified from short ragweed pollen, while one particular plant was found to express seven unique Amb a 1 mRNA transcripts (Kuo *et al.*, 1993). Moreover, no specific distribution pattern of isoforms can be found when examining of Amb a 1 isoform distribution in plants collected from different geographical locations. Four isoforms of canary grass allergen, Pha a 5, were isolated from cDNA library constructed from total RNA of different plants collected from various locations and the genes have been sequenced (Batanero *et al.*, 1994). In addition, it has been reported that the major house dust mite allergen Der p 1 also consists of five polymorphic forms, differing in only one to three amino acids among each cloned protein (Chua *et al.*, 1993). The CSL colony of mites isolated from Melbourne, Australia, used in the studies has been maintained for 20 years. In the present study, batches of live adult insects trapped at the same location in Taichung were identified and used to extract total RNA for constructing a cDNA library (Wu *et al.*, 1993). However, whether these Per a 3 variants, as with Pha a 5 isoforms, are expressed in the same insect or in the different insects is not yet known. Taken together, these results suggest that isoallergens may be expressed either in the same plant, cultured colony, or in the wider environment. More studies are required to firmly establish the occurrence of natural polymorphisms within interspecies and subspecies in the population.

Variable biological effects of the variants showing significant (Breiteneder *et al.*, 1993) and no differences (Nishiyama *et al.*, 1994) on IgE binding of allergens have been reported. The Per a 3 variants described here showed high amino acid sequence identities. They differ significantly in skin reactivities, whereas all patients tested reacted with native Per a 3 (Table 2). C13 and C28 cloned proteins showed 26.3% and 94.7% skin reactivities among asthmatic patients. This dramatic change in skin reactivity might be due to an amino acid changed from proline (position 332) in C28 to serine (position 409) in C13 (Fig. 2). It has been known that proline can strongly influence the three-dimensional structure of a protein (Brandts *et al.*, 1975). Thus, changing a proline to serine might affect the antibody affinity to possible T- and/or B-cell epitopes. Moreover, these cDNA clones have several differences at the level of amino acid sequences that would largely affect the T cell epitopes. More studies, such as epitope mapping, are needed to address this issue and the immunobiological properties of Per a 3 family members are currently being pursued.

Our studies indicated that Per a 3 elicits 73% (Wu and Lan, 1988) and 82.6% skin reactivities in CRa-A-sensitive atopsics. These preliminary data also indicated

that the Per a 3 allergens cause T cell proliferation in cockroach allergic patients (Jeng *et al.*, 1996). Per a 3.01, C13, Per a 3.0201 and C28, sharing high sequence identity (69.1–96%), elicit 47.4%, 26.3%, 47.4% and 94.7% skin reactivities in CRa-A-sensitive asthmatic patients, respectively. This reconfirmed that Per a 3 is the prominent allergen of the American cockroach with sequence polymorphisms sharing common antigen determinant(s). Many other isoallergens with same or different molecular weights containing few to many conserved and variable regions have been reported. Therefore, the variations of Per a 3 isoallergen sequences presented here are not unusual. However, the comparisons among the deduced amino acid sequences of these isoallergenic variants will be helpful in elucidating B cell and T cell epitopes of the Per a 3 for devising an effective therapeutic course. The C13 and C28 cloned allergens are Per a 3.0201 variants and hereby designated as Per a 3.0202 and Per a 3.0203, respectively, according to WHO/IUIS nomenclature (King *et al.*, 1995).

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