

Chemical modification studies on *Cocos nucifera* pollen allergens

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MS received 3 February 1992; revised 21 September 1992

Abstract. Two highly active allergens C_n II (*M_r* 158,000) and C_n VII (*M_r* 2900) isolated from *Cocos nucifera* pollen extract were treated with various protein modifying reagents in order to ascertain the amino acid residues responsible for their allergenicity. In C_n II modification of carboxy group and tryptophan residue led to 30 and $\geq 75\%$ loss in allergenicity and those of lysine and tyrosine reduced 62 and 38% activity. Lysine, tyrosine, tryptophan and carboxy group of C_n VII were also modified causing 81, 17, ≥ 70 and 26% loss of allergenicity respectively. Allergenicity of both was highly affected by pronase and moderately affected by heat. Periodate destroyed about 50% of their allergenicity and other chemical reagents except urea had no remarkable effect.

Keywords. Allergen; chemical modification; allergenicity; *Cocos nucifera*.

1. Introduction

The atopic allergens are usually proteins (King 1976, 1979) or glycoproteins (Geraci *et al* 1978; Howlett and Clarke 1981; Lowenstein 1978; Chakraborty *et al* 1981; Haavik *et al* 1985; Mitra and Chatterjee 1990) producing Type I hypersensitivity reaction. Although allergenicity of various pollens has been denoted by immunological reactions little information is received about the causative agent of the allergenic molecule. Periodate oxidation and proteolytic enzyme treatment of allergen may be considered useful methods to know the identity of the active allergenic component such as whether the carbohydrate or protein or both are responsible for allergenicity. Coconut (*Cocos nucifera*) tree grows all over the world specially in tropical belt. The allergenicity of coconut pollen has been established earlier by immunochemical studies (Jaggi *et al* 1989). Roy Karmakar *et al* (1992) have isolated two active allergenic fractions C_n II (*M_r* 158,000) and C_n VII (*M_r* 2,900) from coconut pollen extract (CPE) by DEAE-Sephadex ion-exchange chromatography to follow the changes in human serum IgE and IgG levels to CPE immunotherapy which demonstrates that IgE and IgG reactivities against those two allergenic fractions are nearly identical and comparable to those against CPE.

The present paper describes a detailed study on the chemical modification of various amino acid residues of C_n II and C_n VII and the effects of chemical reagents including periodate and proteolytic enzymes on their allergenicity to understand the structure function relationship within the molecule.

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2. Materials and methods

2.1 Materials

Citraconic anhydride, O-methyl isourea, cyclohexane-1, 2-dione, 5,5'-dithio-bis-(2-nitrobenzoic acid) (DTNB), α -aminobutyric acid methyl ester, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC), N-bromo-succinimide (NBS), 2-hydroxy-5-nitrobenzyl bromide (HNB), ethoxy formic anhydride (EFA), glutaraldehyde, bovine serum albumin (BSA), Tween 20, goat antihuman IgE (batch No 67F-8814), *o*-phenylenediamine, pronase and pepsin were the product of Sigma Chemical Co., St. Louis, Mo, USA. Bovine pancreas trypsin was purchased from E Merck, Germany and papain was obtained from Boehringer, Mannheim GmbH, Germany. All other reagents used were of highest analytical grade.

2.2 Allergen

Two highly active allergens were isolated from coconut (*C. nucifera*) as reported (Roy Karmakar *et al* 1992). Briefly, coconut whole pollen extract (CPE) was prepared by extracting the defatted pollen with 0.15 M PBS (1:50, w/v), pH 7.8. CPE, following ammonium sulphate precipitation (0–90%), was fractionated by ion-exchange chromatography on DEAE-Sephadex A-50 column into seven fractions (C_n I, C_n II, ..., C_n VII) of which C_n II and C_n VII gave high response to skin prick test. C_n II and C_n VII when passed through Sephadex G-150 and G-50 columns respectively, appeared as a single peak and were found to be homogeneous. The purity of both fractions was further judged by SDS-PAGE (12%) according to Laemmli (1970). Isoelectric focussing (IEF) was performed by the method of Malik and Berrie (1972) using 5% Polyacrylamide gel containing 2% carrier ampholytes (pH 3–10).

2.3 Analytical method

Protein content of the allergen fractions was estimated by the method of Lowry *et al* (1951) using BSA as the standard. Total neutral carbohydrate was estimated according to Dubois *et al* (1956) using D-galactose as the standard. For identification and estimation of individual sugars, each allergen fraction (2 mg) was hydrolyzed separately with 0.1 M HCl for 48 h at 100° C with inositol as the standard and the hydrolyzates were analysed by GLC (Hewlett-Packard model 5730A) as their alditol acetates using 3% of ECNSS-M on Gas Chrom Q (100–120 mesh) at 170° C (Sarkar and Chatterjee 1983). For determination of amino sugars, each allergen (2 mg) was hydrolyzed separately with 4 M HCl for 6 h at 100° C using inositol as the standard and the hydrolyzates after being neutralized were analysed by GLC as their alditol acetates using 3% of Poly A-103 column on Gas-Chrom Q (100–120 mesh) at 190° C (Ahmed and Chatterjee 1988). Amino acid analysis was performed by separately hydrolyzing C_n II (4 mg) and C_n VII (4.2 mg) with 6 M HCl for 24 h and analysing the hydrolyzates by HPLC on a PICO TAG C 18 reverse phase column (Heinrikson and Meredith 1984) as their isothiocyanate derivatives. Tryptophan was determined separately on unhydrolyzed allergens by spectrophotometry method (Goodwin and Morton 1946).

2.4 ELISA-inhibition

The inhibitory capacity of the modified C_n II and C_n VII was determined in duplicate by ELISA-inhibition in PVC plates according to Feo *et al* (1984). Briefly, C_n II and C_n VII (10 µg in 100 µl each) were coated separately at 4°C overnight, washed 4 times with PBS-T (10 mM, PBS, pH 7.4 containing 0.05% Tween 20) and incubated with 1% BSA for 1 h at 37° C. Sera (100 µl 1:5 dilution) from *C. nucifera* sensitive patients were incubated with the modified allergens (10 µg in 100 µl) for 24 h at 4°C with unmodified allergens served as controls. The inhibited serum pool was then incubated separately with C_n II and C_n VII allergens solid phase for 1 h at 37° C and then overnight at 4° C. Each well was then washed with PBS-T as above and 100 µl of goat-antihuman IgE-horseradish peroxidase (1:100 dilution with PBS containing 0.05% BSA) prepared according to Lind (1956), was then added to each well. Following incubation at 37° C for 1 h and usual washing, *o*-phenylenediamine (100 µl, 1 mg/ml in 0.05 M citrate phosphate buffer, pH 5.0, containing 0.01% H₂O₂) was added. The absorbance of each well was measured at 492 nm in a Titertek Multiskan Plus MK II ELISA plate reader (Flow Lab, UK) after incubation at 25° C for 30 min in the dark.

2.5 Cross reactivity between C_n II and C_n VII

Allergenic cross reactivity between C_n II and C_n VII was established by ELISA-inhibition. C_n VII coated plates were used as the immobilized allergens and varying concentrations of C_n II (0.1 to 50 µg/ml) were taken as inhibitors.

2.6 Effect of pH

ELISA was performed with separate solutions of C_n II and C_n VII (100 µg/ml) to check their allergenicity at different pH (0.2 M citrate-phosphate buffer, pH 3.5-7.0; 0.2 M Tris-HCl buffer, pH 7.5-9.0 and glycine-NaOH buffer pH 9.5-10.5).

2.7 Chemical modification of allergens

C_n II and C_n VII were treated with amino acid modifying reagents (thrice in duplicate). Allergens undergone the same steps of procedure but without modifying reagents served as controls. After treatment the excess reagents were removed by dialysis against water followed by 10 mM PBS, pH 7.4 at 4°C. Finally the activity of the modified allergens was tested by ELISA-inhibition. After modification if the allergenicity reduced ($\geq 20\%$), the effect of modification was considered significant.

2.7a Modification of lysine and tyrosine residues: To C_n II and C_n VII (5 mg each) in 2 ml of 50% saturated sodium acetate, pH 8.3 at 0° C, four-1 µl portions of acetic anhydride were added for 1h with stirring. The pH was maintained by 0.1M NaOH (Rice and Etzler 1975) and the samples were dialyzed. To acetylated allergens (1 mg/ml) an equal volume of 2 M NH₂ OH·HCl was added and after adjusting the pH to 7.5 the solutions were left for 10 min at 20° C and dialyzed.

C_n II and C_n VII (2 mg each) in 1 ml of 0.2 M sodium borate buffer, pH 9.0 at 4°C

were reduced with 0.5 mg of NaBH_4 followed by six 5 μl portions of 3.5% formaldehyde at 10 min intervals (Means and Feeney 1968) and extensively dialyzed.

To each allergen, 9 mg in 3 ml of 0.1 M Tris-HCl buffer (pH 8.0), at 20° C, nine 3 μl portions of citraconic anhydride were added with constant stirring for 30 min. The pH was maintained to 8–9 by 0.1 M NaOH (Dixon and Perham 1968) and the samples were dialyzed. Each citraconylated allergen (3 mg) was acetylated as before, decitraconylated by dialysis against 0.2 M sodium acetate buffer, pH 3.9 at 4° C and finally dialyzed as described above.

C_n II and C_n VII (5 mg each) in 3 ml of 0.2 M glycine/NaOH buffer, pH 10.5 was mixed with 3 ml of 0.6 M O-methylisourea. The pH was maintained by 0.1 M NaOH and the solution was kept at 4° C for 24 h and exhaustively dialyzed as before.

2.7b Modification of arginine and histidine residues: Each allergen (2 ml, 2 mg/ml) was diluted to 4 ml by 0.4 M sodium borate buffer, pH 9.0, containing 0.05 M cyclohexane-1, 2-dione, flushed with nitrogen for 4 h in dark and treated with 1 ml of 5% acetic acid (Patthy and Smith 1975). The content was dialyzed against 1% acetic acid, then water and finally PBS.

C_n II and C_n VII (1 mg/ml each) in 0.02 M PBS was treated with 26 μl of EFA (Melchior and Fahrney 1970) for 1 h at 25° C and then dialyzed.

2.7c Modification of thiol groups: Thiol-group was modified by DTNB and quantified spectrophotometrically (Ellman 1959). To each allergen solution (2 mg in 1 ml PBS) 1 mM DTNB (20 μl) was added, mixed and absorbance was recorded at 412 nm after 1 h.

2.7d Modification of carboxy group: C_n II and C_n VII (4 mg each) were dissolved in 1 ml of 1 M α -aminobutyric acid methyl ester, pH 4.75. Two 50 μl portions of 1.1 M EDC were added to each solution at 30 min interval and stirred for 5 h. The pH was maintained at 4.75 by HCl (Carraway and Koshland 1972).

2.7e Modification of tryptophan residues: To 2 mg of each C_n II and C_n VII in 2 ml of 0.1 M sodium acetate buffer, pH 6.0, five 10 μl portions of 10 mM aqueous NBS were added with rapid mixing. After 10 min of each addition the absorbance at 280 nm was recorded. The number of tryptophan residues oxidized was then calculated (Spande and Witkop 1967). Finally, the reaction mixtures were dialyzed as usual. Modification of tryptophan residues with HNB was also done by the procedure of Barmann and Koshland (1967). To 4 mg of each C_n II and C_n VII in 2 ml of 0.1 M sodium acetate buffer (pH 3.5), were added ten 20 μl portions of HNB (50 mg/ml) in dry acetone at 25° C. The excess reagent was removed by dialysis as above.

2.8 Enzyme treatment

Each allergen (2 mg/ml) in 10 mM PBS, pH 7.4 was incubated separately with 500 μg of papain (*Papaya carica*), pronase (*Streptomyces griseus*), trypsin (bovine

pancreas) and pepsin (porcine stomach mucosa) for 24 h at 25° C. The reaction mixture was heated at 50° C for 30 min, centrifuged and the supernatant was dialyzed against PBS. The residual allergenicity was then tested by ELISA-inhibition.

2.9 Chemical effect and thermostability

Each allergen (2 mg/ml) in 0.05 M sodium acetate buffer (pH 5.1) was mixed with an equal volume of 0.25 M sodium metaperiodate solution in the same buffer and the mixture was kept at 4° C in the dark. Aliquots were removed at intervals of 2, 8, 24 and 48 h respectively, treated with ethylene glycol and dialyzed against 10 mM PBS. Ethylene glycol was set up as control. The digestion of allergens with 0.05 M H₂ SO₄ and 0.1 M NaOH was carried out for 6 h at 4° C. The effects of 6 M urea, 1% SDS and 1% β -mercaptoethanol on the allergens were studied at 37° C for 30 min. Finally, the reaction mixtures were dialyzed against PBS. The allergenicity of the oxidized and chemically treated allergens was measured by ELISA-inhibition.

The effects of temperatures on the allergens were studied by incubating separately in water-bath at 60° C and 100° C for 1 h. The allergenicity was measured by ELISA-inhibition study.

3. Results and discussion

Both C_n II and C_n VII resulted in a single band by SDS-PAGE and were found to be homogeneous (figure 1a). IEF produced two bands of pI values 3.1 and 3.5 in C_n II and one band of pI value 3.8 in C_n VII (figure 1b).

The carbohydrate analysis of C_n II revealed the presence of L-rhamnose, D-xylose, D-galactose, D-glucose and N-acetyl-D-galactosamine whereas C_n VII contained D-mannose, D-glucose, N-acetyl-D-galactosamine and trace amount of L-rhamnose (table 1). C_n II and C_n VII contains 11% and 16% neutral carbohydrate, a result, in close agreement with that obtained by GLC (10.84% in C_n II and 18.96% in C_n VII). Like many other glycoprotein allergens (Howlett and Clarke 1981; Feo *et al* 1984; Paulsen *et al* 1985; Barnett and Howden 1986; Nilsen and Paulsen 1990) amino acid analysis of C_n II was characterized by a high content of acidic amino acids, glycine, alanine, and a little amount of histidine, methionine, cystine and isoleucine (table 1). C_n VII contains high amount of glycine, relatively moderate amount of acidic amino acids, serine, alanine, valine, cystine and a low amount of lysine, arginine, proline, tyrosine, methionine, phenylalanine and tryptophan.

From figure 2 it is apparent that C_n II and C_n VII show 39% allergenic cross-reactivity which indicates that C_n II and C_n VII despite of being different proteins have 39% common allergenic determinants or epitopes. Figure 3 shows that both C_n II and C_n VII have the highest IgE-specific reactivity at pH 7.5.

In the present study, in each experiment, a control which had undergone the same modification steps without modifying agents was set up. The relative ELISA-inhibition of the control was taken as 100% so that the error due to reagent or pH change could be nullified. The effect of amino acid modifying agents on the allergenicity of C_n II and C_n VII are shown in table 2 as their relative ELISA-inhibition. Acetic anhydride and reductive methylation modified lysine including

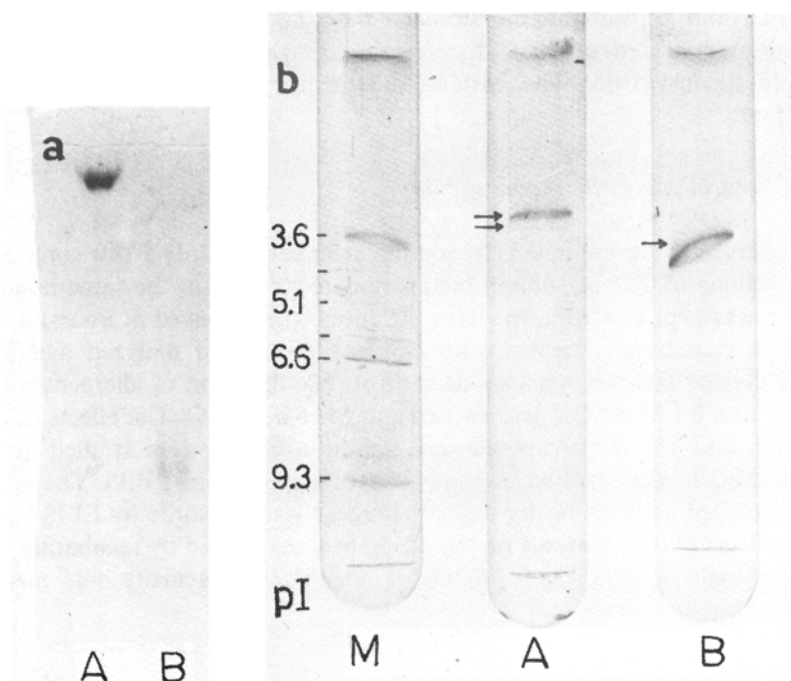


Figure 1. (a) SDS-PAGE of C_n II (A) and C_n VII (B). (b) IEF (pI gradient 3–10) of C_n II (A) and C_n VII (B); Marker (M): amyloglucosidase (pI 3.6), trypsin inhibitor (4.6), β -lactoglobulin (5.1), carbonic anhydrase II (5.9), carbonic anhydrase I (6.6) and trypsinogen (9.3).

terminal α -amino group and phenolic hydroxyl group such as tyrosine and led to 8.5 % decrease in allergenicity of both C_n II and C_n VII. De-O-acetylation removed O-acyl of tyrosine but not N-acyl of lysine. The activity of C_n VII allergen remained almost unchanged. Whereas restoration of 25% activity was achieved in C_n II after regeneration of phenolic -OH group of tyrosine. Citraconic anhydride which modified lysine residue including N-terminal-amino group decreased 64% allergenic activity in C_n II and 89% in C_n VII. Treatment with O-methylisourea retained 38% allergenicity in C_n II but only 19% in C_n VII. After citraconylation, treatment with acetic anhydride modified tyrosine residues and on subsequent decitraconylation N-terminal NH₂ groups of lysine were regenerated whereas hydroxy groups of tyrosine were not. This resulted in 26% restoration of allergenicity in C_n II and 72% in C_n VII. Loss of allergenicity of C_n II by acetylation and partially compensated by de-O-acetylation suggests that the amino group of lysine and the hydroxyl group of tyrosine were important for allergenicity of C_n II which was further evidenced by selective acetylation of hydroxy group of tyrosine and citraconylation of free amino group of lysine. C_n VII after such treatment responded to its allergenicity and no significant loss of activity was observed by modification of tyrosine (table 2) suggesting that the allergenicity of C_n VII was highly dependent on its lysine residues and not on tyrosine. The role of lysine

Table 1. Carbohydrate and amino acid compositions of C_n II and C_n VII allergens. allergens

	Allergens					
	C _n II			C _n VII		
	g/100 g	Mol/ Mol	Nearest mol integer/mol	g/100 g	Mol/ Mol	Nearest mol integer/mol
Sugar						
L-Rha	0.52	4.99	5	1.86	0.35	—
D-Xyl	1.06	11.12	11	—	—	—
D-Man	—	—	—	3.92	0.67	1
D-Gal	5.70	50.05	50	—	—	—
D-Glc	2.64	23.19	23	9.18	1.57	2
D-GalNAc	0.92	6.54	7	4.02	0.56	1
Amino acid						
Asp	8.49	100.8	101	6.79	1.53	2
Glu	10.34	111.15	111	7.13	1.47	2
Ser	5.35	80.51	81	6.42	1.87	2
Gly	13.53	285.04	285	11.32	4.53	5
Lys	5.52	59.68	60	4.41	0.93	1
Arg	4.12	37.38	37	2.74	0.5	1
His	1.52	15.54	16	ND	—	—
Thr	3.73	49.45	50	0.42	0.11	—
Ala	6.56	116.42	116	4.28	1.48	2
Pro	5.32	73.11	73	4.22	1.1	1
Tyr	2.52	2.01	22	3.53	0.59	1
Val	2.79	37.77	38	5.78	1.48	2
Met	1.61	17.12	17	5.97	1.2	1
Cys	0.86	11.21	11	6.92	1.72	2
Leu	2.86	34.5	35	0.65	0.15	—
Ile	1.40	16.92	17	0.54	0.12	—
Phe	4.61	44.17	44	2.63	0.48	1
Trp*	7.32	56.67	57	6.84	1.41	1

ND, Not determined.

*Trp was determined separately on unhydrolyzed allergens by the method of Goodwin and Morton (1946).

residues for allergenicity in C_nII and C_nVII was further confirmed by treatment with O-methylisourea.

When C_nII and C_nVII were treated with EDC followed by α -aminobutyric acid methyl ester a considerable decrease in allergenic activity was observed in both C_nII (30%) and C_nVII (26%) indicating that allergenicity of both were partially dependent on glutamic acid and aspartic acid. King *et al* (1974) also demonstrated the important role of lysine, glutamic acid and aspartic acid in maintaining the allergenic activity of the allergen molecule.

Modification of thiol groups resulted in 16% and 13% loss in allergenicity of C_n II and C_n VII respectively. After modification of arginine allergenic activity of C_nII (89%) and C_nVII (86%) was not found to be changed significantly. Modification of histidine residues of C_n II and C_nVII showed no appreciable change in their allergenic activity. After modification of thiol groups with DTNB,

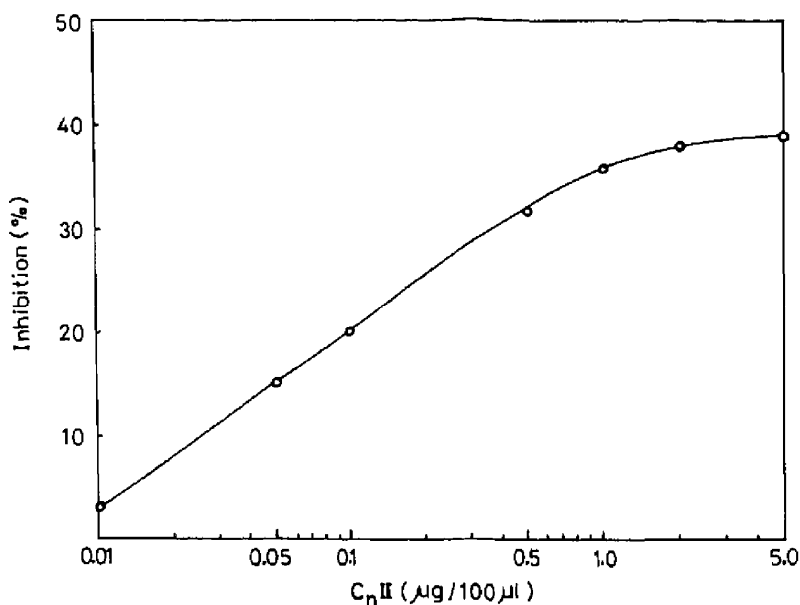


Figure 2. Allergenic cross-reactivity between C_n II and C_n VII: ELISA-inhibition was performed with C_n II as inhibitor and wells coated with C_n VII as immobilized allergen. Each point is the mean of four determinations.

guanidino groups with cyclohexane 1,2-dione and imidazole groups with EFA allergenicity was not reduced significantly and this suggested that allergenicity of both C_n II and C_n VII is independent of the above groups.

Modification of tryptophan residues by HNB led to a loss of 76% and 72% allergenic activity of C_n II and C_n VII, respectively, the results corroborated to that obtained by NBS. Controlled addition of NBS resulted in the sequential modification of tryptophan residues in C_n II. Modification of maximum 20 residues of C_n II incurred 75 % loss of allergenic activity (figure 4). After modification of tryptophan residues, the significant loss ($\geq 70\%$) of allergenic activity in C_n II and C_n VII indicates that indole group of tryptophan is important for allergenicity of both. The oxidation of allergens with NBS is rapid and quantitative at acidic pH, with a large reduction of protein absorption due to conversion of the indole to oxindole chromophore. NBS is known to attack both tyrosine and tryptophan residues in the pH range 3.4–4.0 but under experimental conditions adopted herein, the reagent modified only the tryptophan residues (Sikdar and Chatterjee 1990).

All enzymatic, chemical and thermal effects on C_n II and C_n VII were measured by ELISA-inhibition and calculated as mentioned under table 2. In each experiment the SD has been found $\leq \pm 6$. The proteolytic activity of pronase, pepsin, papain and trypsin reduced the allergenic activity of C_n II by 75, 60, 69 and 70% respectively and of C_n VII by 73, 55, 64 and 71% respectively. The results suggested that allergens on digestion with proteolytic enzymes reduced the allergenic activity. Such decrease in activity had also been reported in *D. farinae* mite allergen (Ishii *et al* 1973) and house dust allergens (Mitra *et al* 1992).

Periodate oxidation of C_n II for 2, 8, 24 and 48 h resulted in loss of allergenicity

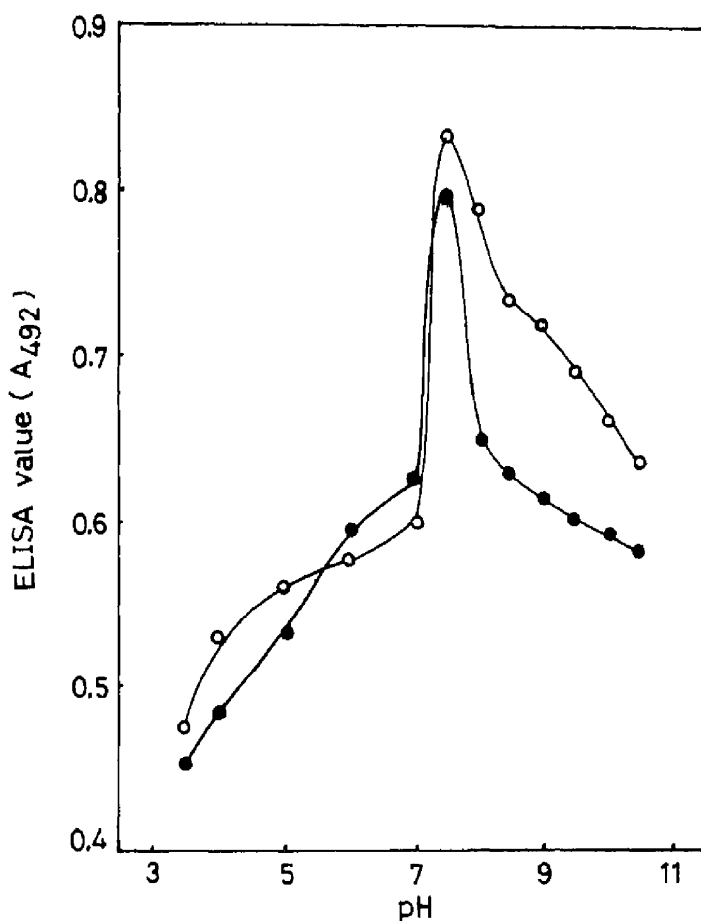


Figure 3. Effect of pH on C_n II (●) and C_n VII (○).

by 6, 14, 33 and 55% respectively and of C_n VII by 10, 19, 30 and 49% respectively which suggests that carbohydrate moiety is involved in IgE-binding activity of both the allergens. The responsiveness of carbohydrate moiety in allergenicity was also reported in the allergens of *D. farinae* mite (Ishii *et al* 1973), *Xanthium strumarium* pollen (Jaggi and Gangal 1987) and house dust (Mitra *et al* 1992). Exposure to 6 M urea, 1% SDS and 1% β -mercaptoethanol resulted in 31, 19 and 20% loss of allergenicity respectively in C_nII while C_n VII lost 35, 16 and 18% allergenicity respectively. Conversely, *X. strumarium* allergens were almost unaffected by denaturing agents except 6 M urea (Jaggi and Gangal 1987). C_n II and C_n VII after acid treatment resulted in 6 and 26% loss of allergenicity respectively whereas the same was 20 and 10% respectively after alkali treatment. Few other allergens (Fredricks and Tebo 1977; Chakraborty *et al* 1981; Barnett and Howden 1986; Jaggi and Gangal 1987) also exhibited similar effect with acid and alkali.

The allergenic activity of C_n II was decreased by 11, 27, 33 and 41% when incubated at 60° C for 15, 30, 45 and 60 min respectively whereas upon same treatment the decrease in activity in C_n VII was 13, 19, 25 and 30% respectively. Heating at

Table 2. Effects of amino acid modifying agents on C_n II and C_n VII allergens.

Reagent	Type of groups modified	Relative ELISA-inhibition ^a (%)±SD ^b	
		C _n II	C _n VII
Acetic anhydride	Lys ε-amino, N-terminal α-amino, Tyr hydroxy	15 ± 5	11 ± 3
Reductive methylation	Lys ε-amino, N-terminal α-amino, Tyr hydroxy	16 ± 4	15 ± 3
Acetic anhydride followed by hydroxylamine, pH 7.5	Lys ε-amino, N-terminal α-amino	41 ± 3	21 ± 3
Citraconic anhydride	Lys ε-amino, N-terminal α-amino	36 ± 2	11 ± 3
Citraconic anhydride followed by acetic anhydride, then decitraconylation	Tyr hydroxy	62 ± 6	83 ± 6
O-Methylisourea	Lys ε-amino	38 ± 1	19 ± 2
Cyclohexane-1,2-dione	Arg guanidino	89 ± 7	86 ± 5
5,5'-Dithiobis-(2- nitrobenzoic acid)	Thiol	84 ± 6	87 ± 5
Carbodiimide, then α-aminobutyric acid methyl ester	Glu γ-carboxy and Asp β-carboxy	70 ± 2	74 ± 3
2-hydroxy-5-nitro- benzyl bromide	Trp indole ring	24 ± 3	28 ± 4
N-bromosuccinimide	Trp indole ring	25 ± 4	30 ± 3
Ethoxy formic anhydride	His imidazole	87 ± 7	91 ± 6

^aRelative ELISA-inhibition of the modified allergens was calculated as percentage of inhibitory capacity of C_n II and C_n VII taken as control and was considered as 100%.

^bEach modification was repeated thrice in duplicate and ELISA in duplicate. SD was determined from these twelve determinations.

100° C for 15, 30, 45 and 60 min reduced the allergenicity of C_n II by 23, 35, 42 and 47 % respectively and of C_n VII by 24, 28, 34 and 43 % respectively.

In conclusion chemical modification of C_n II and C_n VII indicates the involvement of lysine, glutamic acid, aspartic acid, tryptophan and tyrosine for allergenicity (IgE-binding property) of C_n II whereas participation of lysine, glutamic acid, aspartic acid and tryptophan residues is noted for that of C_n VII, which demonstrates that the protein modifying agents affects the reactivity of both fractions suggesting peptides form a major part of the allergenic region. It is further supported by the fact that proteolytic enzymes were found to have reduced the allergenic activity of both the fractions. C_n II and C_n VII retained their allergenicity after mild treatment with different chemicals and were moderately heat sensitive. The loss of their activities by periodate oxidation indicates that carbohydrate moiety is also involved in binding with IgE expressing allergenicity.

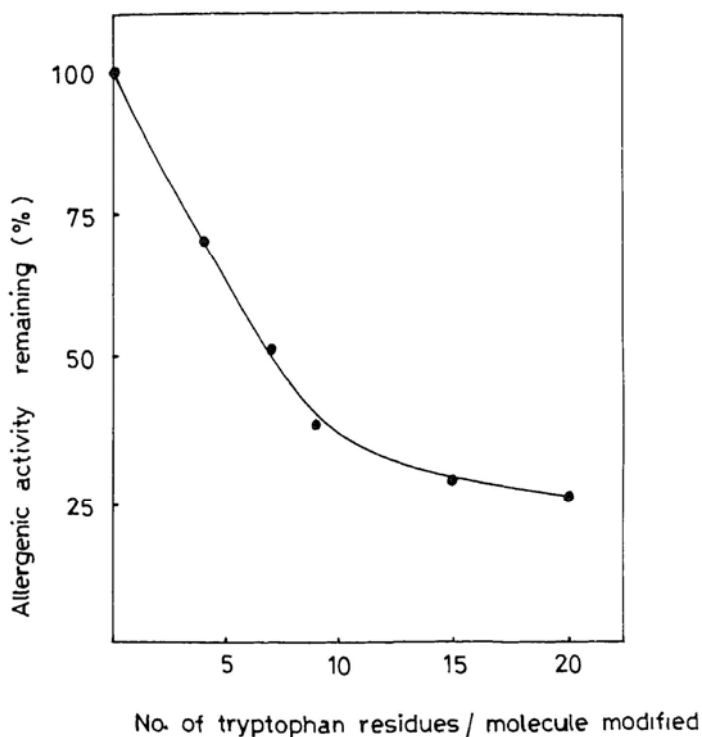


Figure 4. Effect of modification of tryptophan residues on the allergenic activity of $C_n II$. Percentage activity remaining after modification compared to that of native $C_n II$ is plotted against the number of tryptophan residues/molecule oxidized. The number of tryptophan residues modified was calculated according to Spande and Witkop (1967).

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