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Lysine Residues in Bee Venom Phospholipase A₂ Are Important for Binding to Human Monoclonal or Polyclonal Antibodies of the IgG4 Isotype

Key Words

Bee venom phospholipase A₂
Discontinuous B cell epitope
Lysine residue

Abstract

Discontinuous antigenic sites on bee venom phospholipase A₂ (PLA) have been mapped using human monoclonal antibodies or human polyclonal serum antibodies (hpAbs) of the IgG4 isotype from beekeepers or of the IgE isotype from individuals allergic to PLA. Lysine residues of PLA have been specifically modified by acetylation or acylation by treatment with citraconic anhydride of their ε-amino groups to analyze their role in antigen-antibody binding. After the modifications, the binding of PLA to the human monoclonal antibodies is lost, whereas the binding to IgG4 hpAbs is significantly decreased. In contrast, the effect on the binding of PLA to IgE hpAbs appears to be more heterogeneous. The data indicate the importance of lysine residues as being part of B cell epitopes in PLA-specific antibodies of the IgG4 isotype, but less so for those of the IgE isotype.

Introduction

The most immunogenic component in bee (*Apis mellifera*) venom is phospholipase A₂ (PLA) [1–3]. The humoral immune response to PLA in individuals frequently exposed to bee venom such as beekeepers is dominated by the production of IgG4 antibodies against PLA [4]. Hence, this isotype has been associated with protection [5]. The epitopes on PLA recognized by IgG4 human polyclonal serum antibodies (hpAbs) and their potential biological function related to antigen clearance or inhibition of anaphylaxis [6–8] are not known.

For a precise analysis of antigenic sites on PLA recognized by IgG4 antibodies, we have developed two human monoclonal antibodies (hmAbs) of this isotype [9]. These

hmAbs have been found to represent part of the polyclonal IgG4 immune response. They are directed against conformational epitopes on PLA as they do not cross-react with synthetic peptides, and the epitopes recognized by these hmAbs do not contain the sugar residue [9]. Hence, the approach of using chemical protein modification described in this report was evaluated to gather information on the discontinuous epitopes recognized by the hmAbs and hpAbs specific for PLA.

Previous reports suggested that the initial stages in the association between antigen and antibody may involve charged residues [10–12]. The electrostatic interactions between antigen and antibody may be complemented by other binding forces displayed by the two molecules to stabilize the complex [13]. Information from the X-ray crystal struc-

ture of PLA indicates the presence of several surface-exposed charged residues, many of which are lysines [14]. This amino acid can be chemically modified by acetylation or citraconylation of its ϵ -amino group using sulfo-NHS-acetate (ester formed between acetic acid and N-hydroxy-succinimide-3-sulfonic acid) or citraconic anhydride [15], respectively. We have used enzyme-linked immunosorbent assay (ELISA) techniques with the chemically modified antigens together with hmAbs or hpAbs to determine the involvement of lysines in antigen-antibody interactions. Possible differences may exist in the binding of antibodies to natural and chemically modified antigens when comparing IgG4 hpAbs with IgE hpAbs because of the narrow spectrotype of the IgG4 but not IgE antibodies specific for PLA [16].

In this paper we show that chemical alteration of lysine residues on PLA significantly inhibits its binding to hmAbs or hpAbs of the IgG4 isotype. In contrast, lysine modification affects the binding of IgE hpAbs to a lesser extent.

Materials and Methods

PLA-Specific Antibodies

Two stable and independent hybridoma lines were established from peripheral blood mononuclear cells of a beekeeper [9]. Briefly, mononuclear cells were transformed with Epstein-Barr virus. Two independent and stable lymphoblastoid cell lines producing antibodies against PLA were then fused with human heteromyeloma F3B6 or LA55 by the plate fusion technique [17, 18]. The hybridomas were tested for secretion of anti-PLA antibodies cloned by limiting dilution. The two independent hybridomas secreting PLA-specific antibodies are designated BVA1 and BVA2. The sources of hpAbs were sera from 14 beekeepers with several years of professional experience and sera from 14 patients allergic to PLA treated at the University Hospital, Zürich, all having CAP classes 3–6 (Pharmacia).

Acetylation of PLA

For exhaustive acetylation of the ϵ -amino group of lysines, 1 mg of PLA (Boehringer Mannheim, Germany; concentration determined by the Bradford method using the Bio-Rad Protein Assay from Bio-Rad, Richmond, Calif., USA) was dissolved in 1 ml of 100 mM NaHCO₃ (pH 8.5), and 1 mg of sulfo-NHS-acetate (Pierce, Rockford Ill., USA), dissolved in 100 μ l dimethyl sulfoxide, was added. This corresponds to a 4.2-fold molar excess of acetylating reagent over lysine residues on PLA. All cysteines present in PLA are involved in disulfide bridges and thus cannot be acetylated. The reaction was kept for 1 h at room temperature, and the sample was extensively dialyzed against phosphate-buffered saline (PBS; pH 7.4).

Citraconylation of PLA

PLA (1 mg; Boehringer Mannheim) was dissolved in 200 μ l of 250 mM sodium pyrophosphate buffer (pH 9.0). For exhaustive citraconylation of the ϵ -amino group of lysines, 6 μ l of citraconic anhydride (Pierce), corresponding to a 70-fold molar excess of reagent over

lysines, was added to the protein in 2- μ l aliquots with thorough mixing. After addition of each aliquot, the mixture was readjusted to pH 9.0 with 3 M NaOH. After an additional incubation for 4 h at room temperature, the sample was dialyzed against PBS (pH 9.0) for 2 days at 4°C. To deacylate citraconylated PLA (PLA citraconylated at the ϵ -amino group of lysine residues with citraconic anhydride; Cit-PLA), the same samples were dialyzed against 10 mM formic acid (adjusted to pH 3.5 with aqueous ammonia) for 2 days at 4°C.

Determination of the Enzymatic Activity of Natural and Modified PLA

The phospholipase activity of natural and modified PLA was determined using a spectrophotometric assay with the short-chain phospholipid substrate racemic 1,2-bis(hexanoylthio)propyl-3-phosphomethanol [19].

Enzyme-Linked Immunosorbent Assay

Antigen-specific ELISA and inhibition ELISA were performed as described [9]. Briefly, polystyrene microtiter plates (Maxisorp; NUNC, Roskilde, Denmark) were coated with 5 μ g/ml of antigen in PBS (pH 8.0). The remaining free sites were then blocked with buffer consisting of 1% casein hydrolysate (Oxoid, Basingstoke, UK) and 5% Tween 20 in PBS (pH 7.4). For the antigen-specific ELISA, hmAbs or hpAbs diluted in blocking buffer were then added to the antigen-coated plates, and the antibodies bound were determined using an appropriate detection system. For the inhibition ELISA, hmAbs or hpAbs appropriately diluted in blocking buffer were preincubated with different amounts of antigen inhibitor. Thereafter the antigen-antibody mixture was transferred to the antigen-coated and blocked plates. The antibodies bound to the solid phase were then analyzed by the appropriate isotype-specific detection system. To study the pH dependence of the association of antibodies with PLA, hmAbs or hpAbs were diluted in PBS (pH 5.0–10.0) and added to antigen-coated and blocked microtiter plates. The reactions were kept for 2 h at 37°C. Subsequently, the plates were washed with PBS (pH 7.4) containing 0.05% Tween 20. The assay was then continued as described for the antigen-specific ELISA. To determine the pH dependence of the dissociation of the antigen-antibody complex, hmAbs and hpAbs were diluted in blocking buffer and incubated with the antigen-coated plates. After washing, PBS (pH 5.0–10.0) was added to the wells in an additional step and incubated for 2 h at 37°C. The plates were washed, and the amount of antibody bound was determined as described for the antigen-specific ELISA.

Determination of the Relative Avidity of hpAbs to Acylated PLA

The binding of IgG4 and IgE hpAbs to chemically modified PLA was analyzed by comparing their relative avidities in individual patients. The relative avidity to modified PLA compared to natural PLA, indicating a factor by which natural PLA is a more potent inhibitor than acetylated PLA (PLA acetylated at the ϵ -amino group of lysine residues with sulfo-NHS-acetate; Ac-PLA) and Cit-PLA, respectively, was defined as [Ac-PLA]/[native PLA] and [Cit-PLA]/[native PLA] where [native PLA] is the concentration of native PLA yielding the same inhibition in ELISA as the corresponding modified PLA at the concentration of 10 μ g/ml.

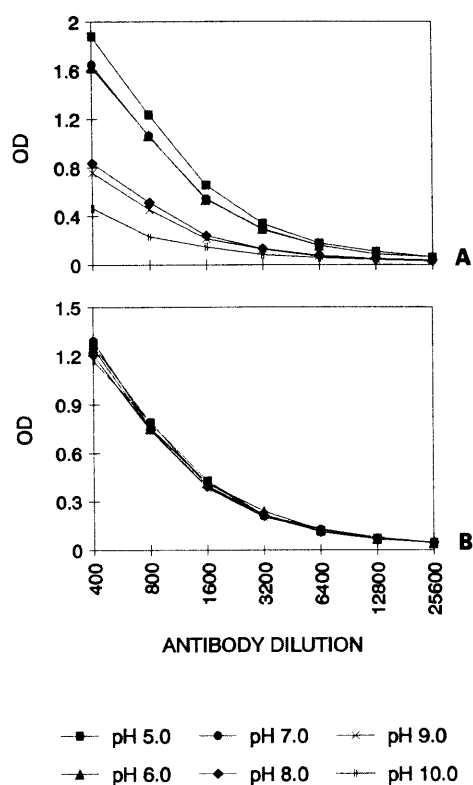


Fig. 1. Antigen-specific ELISA to determine the pH dependence of the rate of association and dissociation of the hmAb BVA2 with PLA. **A** Association study: BVA2 in serial twofold dilutions in PBS (pH 5.0–10.0) was added to the antigen-coated plates. **B** Dissociation study: BVA2 was diluted as in **A**, but using PBS at pH 7.4, and added to PLA-coated plates. Thereafter, the antigen-antibody complex was incubated with PBS (pH 5.0–10.0) for 2 h at 37°C. The antibody bound in **A** or **B** was analyzed using an appropriate detection system. The dilution of BVA2 is plotted against the OD_{405 nm} determined.

Results

The Rate of Association of PLA with Specific IgG4 Antibodies, but Not the Rate of Dissociation of the Antigen-Antibody Complex is Dependent on pH

The contact between antibody and antigen may be mediated by charged residues [10, 11, 13]. To assess the contribution of the charge to the interaction of PLA with antibodies, the influence of the pH on the association of PLA with hmAbs and hpAbs was analyzed. hmAbs were diluted in PBS (pH 5.0–10.0) and incubated with the antigen-coated plates. Under the experimental conditions, the association-dissociation of antigen and antibody did not reach an equilibrium [20]. As shown in figure 1A, maximal binding of

antibody occurred between pH 5.0 and 7.0. Incubation at pH 8.0 resulted in a more than 50% drop in the amount of antibody bound. At pH 10.0, there was only about 25% of the antibody binding seen at pH 5.0. In contrast, dissociation was not affected when the hmAbs were first bound to PLA-coated plates at pH 7.4 and subsequently treated with PBS at different pH values ranging from 5.0 to 10.0 (fig. 1B). Similar but less striking results were obtained using sera from beekeepers (data not shown). The data indicated that positively charged residues either on the antigen or the antibody or both are important for binding of PLA to hmAbs and hpAbs from beekeepers. Lysine residues on PLA can be selectively acylated and are, therefore, amenable to analysis of their involvement in antibody binding.

Acetylation and Citraconylation of the ϵ -Amino Group of Lysine Residues on PLA

To acylate the lysine residues, PLA was treated with either sulfo NHS-acetate or citraconic anhydride (fig. 2). Sulfo-NHS-acetate irreversibly modifies primary amino groups, whereas the chemical reaction of citraconic anhydride with amino groups is reversible [15]. At pH 8.0 or higher, Cit-PLA is stable, but at pH values below 4.0, Cit-PLA undergoes deacylation. Cit-PLA at pH 7.4 is stable for more than 24 h. Conditions for the exhaustive acylation of the ϵ -amino group of lysine residues on PLA were found empirically. Increasing amounts of sulfo-NHS-acetate or citraconic anhydride were added to PLA, and the modified PLA molecules were then tested by antigen-specific ELISA and inhibition ELISA using hmAbs or selected hpAbs from beekeepers. Increasing the degree of substitution of the ϵ -amino groups led to a decrease in the binding of hmAbs or hpAbs until a plateau was reached with a fourfold molar excess of sulfo-NHS-acetate or a 70-fold molar excess of citraconic anhydride over lysine residues of PLA. Under the experimental conditions used for the ELISA, the ϵ -amino groups of lysines are positively charged, the acetylated lysine residues are neutral, and the citraconylated groups are negatively charged. The van der Waals volumes of the acetyl group and the citraconyl group are 43.8 and 73.3 Å³, respectively, whereas the hydrogen atom of the unsubstituted lysine has a volume of 7 Å³. The enzymatic activity of the chemically modified PLA molecules was determined using a water-soluble short-chain phospholipid analogue. There was no significant difference in the enzymatic activity between the chemically modified PLA preparations and natural PLA (fig. 3). Although surface lysine residues are likely to be important for the binding of PLA to a membrane surface, the results of this study show that surface lysine residues on PLA are not important for the activity of the

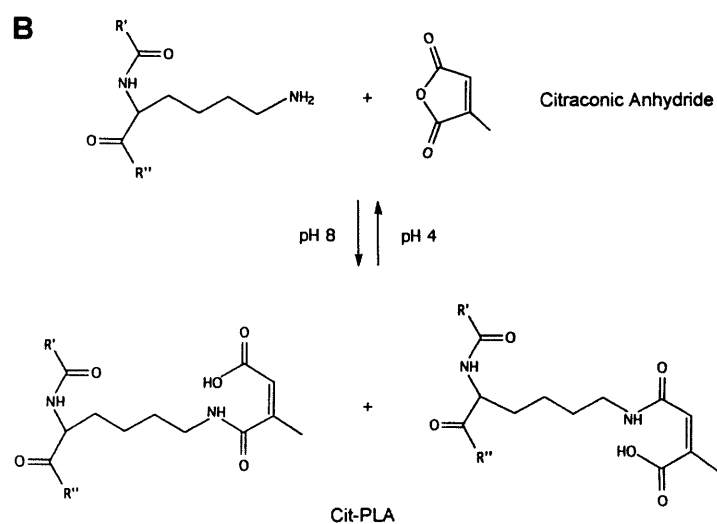
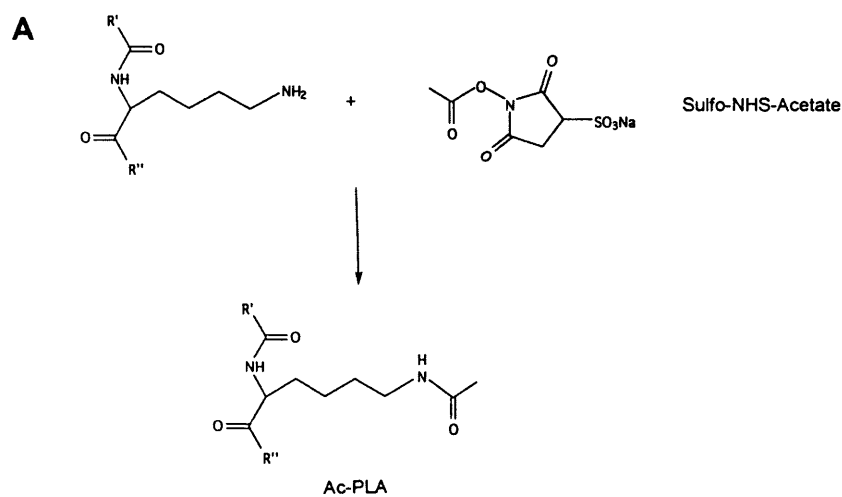


Fig. 2. Chemical modification of lysine with sulfo-NHS-acetate (**A**) or with citraconic anhydride (**B**).

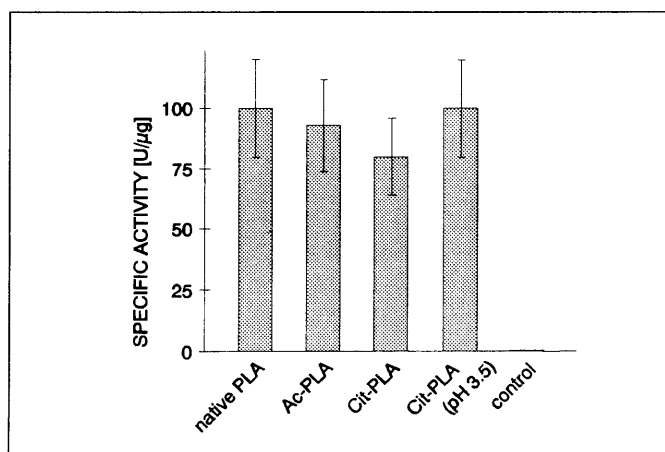


Fig. 3. Enzymatic activity of natural and modified PLA. The activity of native PLA was considered to be 100%. The control sample contained denatured PLA [21].

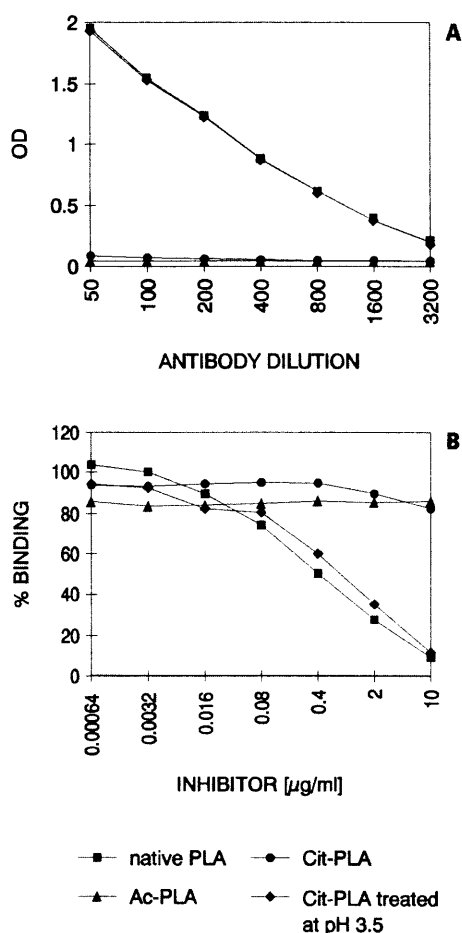


Fig. 4. A Antigen-specific ELISA with the hmAb BVA1 comparing natural and modified PLA. Serial twofold dilutions of BVA1 added to the antigen-coated microtiter plates are given along the abscissa, and the resulting OD_{405 nm} values measured after adding secondary antibody are given along the ordinate. **B** Inhibition ELISA with BVA1 using natural and modified PLA as inhibitors of solid-phase-bound PLA. The inhibitor concentration is plotted against the resulting OD_{405 nm}.

enzyme acting on a water-soluble substrate. The data indicate that the chemical modification of the ε-amino group of lysines does not cause gross alterations of the three-dimensional structure of PLA.

Lysine Residues Are Part of the B Cell Epitopes of hmAbs BVA1 and BVA2

The hmAbs BVA1 and BVA2 were analyzed for their binding to native or chemically modified PLA using an antigen-specific ELISA or inhibition ELISA. Solid-phase-bound Ac-PLA and Cit-PLA were not recognized by BVA1

and BVA2. The fact that treatment of Cit-PLA at pH 3.5 resulted in an antigen that displayed essentially the same antibody-binding properties as the unmodified PLA establishes that reversal of the lysine modification fully restores the antibody-binding capacity (fig. 4A). This indicates that the citraconylation of PLA does not cause an irreversible conformational change. The data obtained by antigen-specific ELISA were confirmed by inhibition ELISA. No inhibition was seen using either Ac-PLA or Cit-PLA as inhibitor. In contrast, natural PLA and Cit-PLA treated at pH 3.5 inhibited the binding to solid-phase-bound PLA equally well, resulting in complete inhibition at 10 μg/ml (fig. 4B). Recombinant PLA with a 15 amino acid fusion peptide at the N-terminus [21] was also used in inhibition ELISA, and the results are essentially identical to the results obtained with native PLA as inhibitor (data not shown). This indicates that acylation of the N-terminal amino acid isoleucine of native PLA is not the reason for the effect of chemical modification seen on antibody binding. The data suggest that the ε-amino group of at least one lysine residue is a decisive part of the antigenic structure recognized by the two hmAbs.

Lysine Residues Are Part of the Epitopes Recognized by IgG4 hpAbs of Beekeepers

The inhibition ELISA experiments were carried out with 14 sera from beekeepers using the same inhibitors as assayed with the hmAbs. In all 14 sera, the binding of hpAbs of the IgG4 isotype to Ac-PLA or Cit-PLA was lower than to native PLA. To compare the inhibitory efficacy of the different PLA competitors, the concentration of native PLA yielding the same degree of inhibition in ELISA as 10 μg/ml of the modified PLA preparations was evaluated by regression analysis, and relative avidities were calculated (table 1, left part). To obtain the same inhibition as with the natural PLA, 40- to 4,000-fold higher concentrations of the chemically modified PLA molecules were required in inhibition ELISA. Two main inhibition patterns were observed, as shown for representative examples (fig. 5). In the first group, comprising nine sera, similar inhibition patterns were seen in ELISA using either Ac-PLA or Cit-PLA (fig. 5A; table 1, left part). The relative avidities for Ac-PLA and Cit-PLA differed only by a factor 1–4. In the second group, comprising another five sera, Ac-PLA was 5–27 times more potent in inhibiting the binding of hpAbs to solid-phase-bound PLA than was Cit-PLA (fig. 5B; table 1, left part). On the average 800 times more of Ac-PLA and 2,000 times more of Cit-PLA were required for the inhibition ELISA response corresponding to the response obtained with native PLA (table 1, left part). The larger effect on antigen-antibody interactions seen with Cit-PLA as compared

with Ac-PLA is consistent with the fact that the citraconyl group is not only larger than the acetyl group, but also bears a charge opposite to that of lysine.

Binding of IgE hpAbs to Lysine-Modified PLA

Similar inhibition experiments were performed using IgE hpAbs from individuals allergic to PLA. The binding of IgE to solid-phase-bound PLA was inhibited to various degrees by Ac-PLA and Cit-PLA (table 1, right part). Relative avidities of IgE hpAbs of eleven sera ($n = 14$) were similar for Ac-PLA and Cit-PLA; both acetylation and citraconylation reduced the antigen-antibody interactions to similar extents (table 1, right part). IgE hpAbs from two of the sera (HO and LL) bound better to Ac-PLA than to Cit-PLA, whereas with one of the sera (SC), Cit-PLA was the better binder. The relative avidities of the IgE hpAbs of the 14 sera tested dropped by a factor of 1–800, the mean being 250 (table 1, right part). With two sera, CI and UE, there was no significant difference in the inhibition ELISA when the binding of IgE hpAbs to native PLA was compared with the binding to chemically modified PLA (table 1, right part). The epitopes recognized by polyclonal IgE hpAbs from serum CI have been previously shown to contain the carbohydrate residue of PLA [9]. In contrast, the epitopes recognized by IgE hpAbs in serum UE were found not to contain the sugar residue.

Discussion

Data from previous experiments [9] have shown that human monoclonal and polyclonal antibodies recognized discontinuous epitopes on PLA, the main bee venom allergen, as demonstrated by their inability to bind synthetic peptides. For the eventual high-resolution mapping of B cell epitopes on PLA by using site-directed mutagenesis of recombinant PLA, a rationale was sought for a targeted approach.

The pH dependence of the association rates of hmAbs or hpAbs to PLA suggests that charged residues were involved in antigen-antibody interaction (fig. 1A). It is likely that protonated lysine residues on PLA form favorable electrostatic interactions with glutamate or aspartate residues of the antibodies; the latter are expected to be ionized over the entire range of pH values used in the association experiments. Therefore, the rate of association of antibody with antigen will be proportional to the concentration of the protonated lysine-containing protein, and this depends on the pH during association. However, the rate of dissociation of the antigen-antibody complex is expected to be insensitive to pH in the range studied. This is because of the pKa values

of protonated lysines that are close in space to the carboxylates or acidic amino acids are expected to be well above 10, and thus such lysines will not lose their protons as the pH is increased to 10. In addition, other factors may play a role in the pH-independent dissociation of the antigen-antibody complex such as the fact that solid-phase-bound antigen-antibody complexes may have slower dissociation rates when compared to antigen-antibody complexes present in fluid phase [22].

To further dissect the positively charged amino acids involved in antigen-antibody interaction, lysine residues in the PLA molecule were chemically modified by acetylation or by citraconylation, yielding a neutral or an anionic product, respectively. The modification of lysines did not alter the gross structure of the PLA, as shown by the fact that the modified PLA molecules retain full catalytic activity. In addition, deacylation of Cit-PLA at low pH restored the full antigenicity of PLA. Finally, the chemical alteration did not significantly change the binding by IgE from two sera to the modified proteins (table 1, right part).

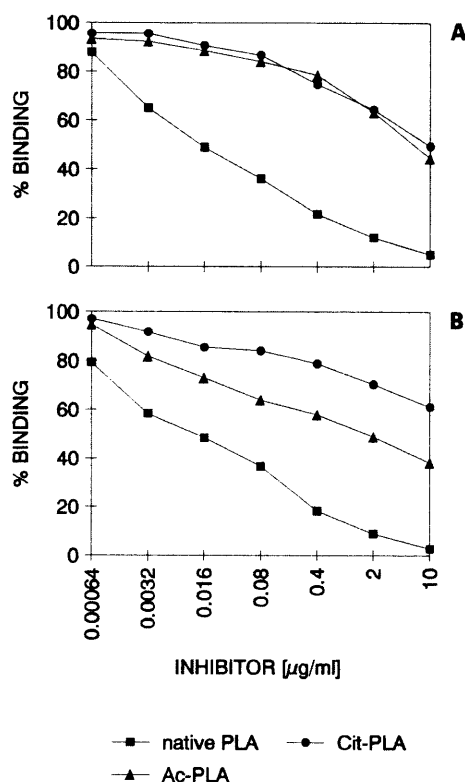


Fig. 5. Inhibition ELISA as described in figure 4 using two different sera from beekeepers. **A** Serum of beekeeper MR. **B** Serum of beekeeper RB.

Table 1. Relative avidities of IgG4 antibodies from beekeeper sera and IgE antibodies from sera of allergic individuals to modified PLA

Serum	[Ac-PLA] [native PLA]	[Cit-PLA] [native PLA]	Serum	[Ac-PLA] [native PLA]	[Cit-PLA] [native PLA]
<i>Relative avidities of IgG4 antibodies from beekeeper sera to modified PLA</i>			<i>Relative avidities of IgE antibodies from sera of allergic individuals to modified PLA</i>		
HM	229	180	CI	1	1
MR	266	643	UE	6	5
KH	300	844	NU	16	22
AG	357	1,256	HA	19	27
PZ	1,180	975	EE	102	110
HH	1,202	2,535	WE	160	177
BB	1,839	1,935	EL	266	298
CH	1,946	3,862	AO	278	418
WF	2,638	3,168	YS	498	392
BR	35	268	MY	682	755
GB	37	1,013	TI	796	762
RB	140	3,508	HO	8	35
HW	368	3,174	LL	10	213
AB	635	3,245	SC	651	8
Mean	798	1,900	Mean	250	230

Relative avidities of IgG4 and IgE hpAbs to Ac-PLA and Cit-PLA determined as described in text.

In antigen-specific ELISA and inhibition ELISA, the binding of both hmAbs to the acylated PLA preparations was totally abolished (fig. 4). The data, therefore, show that positively charged lysine residues on PLA are part of the epitopes recognized by BVA1 and BVA2.

The acylation of PLA significantly lowered the binding to IgG4 or IgE hpAbs which indicates that lysine residues are an essential part of the epitopes recognized by a considerable fraction of polyclonal IgG4 or IgE. However, the fact that lysine-modified PLA retained a low degree of binding to hpAbs suggests that some epitopes do not involve lysine residues at all.

Two different inhibition ELISA patterns were found by analyzing serum IgG4 hpAbs from beekeepers. In the first group there was no difference in inhibition using either Ac-PLA or Cit-PLA. In the second group Ac-PLA was a more potent inhibitor than Cit-PLA. With IgE hpAbs of bee venom allergic individuals we found different types of inhibition patterns. In one group of sera, Ac-PLA and Cit-PLA were equally potent as inhibitors. In the second and third types, either Ac-PLA or Cit-PLA, respectively, were more potent inhibitors.

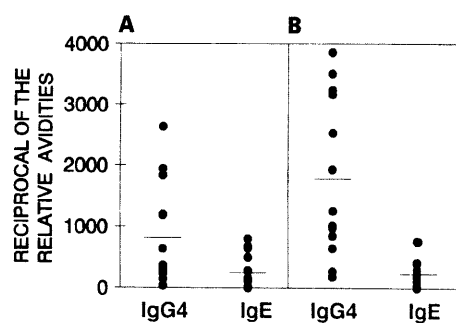
If only one major B cell epitope on PLA was recognized by these IgG4 and IgE hpAbs, the reaction pattern in the different groups could be explained by the differences in the

recognition of this one epitope. On the other hand, IgG4 and IgE hpAbs could recognize different epitopes on PLA, since this protein has twelve lysine residues potentially accessible for antibody binding [14]. The effects of acetylation and citraconylation of the lysine residues on the binding of IgE hpAbs, however, appeared to be more heterogeneous than the effect on the binding of IgG4 hpAbs.

The relative avidities of IgG4 hpAbs from 14 beekeepers to acylated PLA as compared with natural PLA drop by 40- to 4,000-fold. In contrast, the relative avidities of IgE hpAbs from individuals allergic to bee venom drop by 1- to 800-fold (fig. 6, table 1). The epitopes of IgG4 hpAbs were, therefore, more affected by acetylation and citraconylation of lysine residues on PLA than the epitopes of IgE hpAbs (fig. 6).

The evidence for lysine residues on PLA as being part of the B cell epitopes indicates that higher resolution epitope mapping studies employing site-specific mutagenesis of lysine residues are warranted. Indeed, data from such studies indicate the validity of this approach [23].

The approach presented in this study may benefit similar attempts to localize B cell epitopes on other antigens, especially when the epitopes are discontinuous as it is the case with more than 90% of the antigens studied [24].



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Fig. 6. Relative avidities of IgG4 hpAbs from beekeepers or of IgE hpAbs from individuals allergic to PLA to Ac-PLA (**A**) and to Cit-PLA (**B**). The determination was done as described in text. The horizontal lines represent the mean values.

References

- King TP, Sobotka AK, Kochoumian L, Lichtenstein LM: Allergens of honey bee venom. *Arch Biochem Biophys* 1976;172:661-671.
- Sobotka AK, Franklin RM, Adkinson NF Jr, Valentine M, Baer H, Lichtenstein LM: Allergy to insect sting. II. Phospholipase A₂: The major allergen in honey bee venom. *J Allergy Clin Immunol* 1976;57:29-40.
- Müller UR: Insect Sting Allergy: Clinical Picture, Diagnosis and Treatment. Stuttgart, Fischer, 1990.
- Aalberse RC, van der Gaag R, van Leeuwen J: Serologic aspects of IgG4 antibodies. I. Prolonged immunization results in an IgG4-restricted response. *J Immunol* 1983;130:722-726.
- Nakagawa T, Miyamoto T: The role of IgG4 as blocking antibodies in asthmatics and in beekeepers. *Int Arch Allergy Appl Immunol* 1985;77:204-205.
- Lessof MH, Sobotka AK, Lichtenstein LM: Effects of passive antibody in bee venom anaphylaxis. *Johns Hopkins Med J* 1978;142:1-7.
- Müller UR, Morris T, Bischof M, Friedli H, Skvaril F: Combined active and passive immunotherapy in honeybee sting allergy. *J Allergy Clin Immunol* 1986;78:115-122.
- Bousquet J, Fontez A, Aznar R, Robinet-Levy M, Michel F-B: Combination of passive and active immunization in honeybee venom immunotherapy. *J Allergy Clin Immunol* 1987;79:947-954.
- Schneider T, Lang AB, Carballido JM, Santamaria Babi LF, Dudler T, Kägi MK, Blaser K, Suter M: Human monoclonal or polyclonal antibodies recognize predominantly discontinuous epitopes on bee venom phospholipase A₂. *J Allergy Clin Immunol*, in press.
- Van Oss CJ: Antigen-antibody reactions; in Van Regenmortel MHV (ed): *Structure of Antigens*. Boca Raton, CRC Press, 1992, pp 99-126.
- Geysen HM, Tainer JA, Rodda SJ, Mason TJ, Alexander H, Getzoff ED, Lerner RA: Chemistry of antibody binding to a protein. *Science* 1987;235:1184-1190.
- Hopp TP: Protein surface analysis. Methods for identifying antigenic determinants and other interaction sites. *J Immunol Methods* 1986;88:1-18.
- Alexander H, Alexander S, Getzoff ED, Tainer JA, Geysen HM, Lerner RA: Altering the antigenicity of proteins. *Proc Natl Acad Sci USA* 1992;89:3352-3356.
- Scott DL, Otwinowski Z, Gelb MH, Sigler PB: Crystal structure of bee venom phospholipase A₂ in a complex with a transition-state analogue. *Science* 1990;250:1563-1566.
- Atassi MZ, Habeeb AFSA: Reaction of proteins with citraconic anhydride. *Methods Enzymol* 1972;25:546-553.
- Wetterwald A, Skvaril F, Müller U, Blaser K: Isotypic and idiotypic characterization of anti-bee venom phospholipase A₂ antibodies. *Int Arch Allergy Appl Immunol* 1985;77:195-197.
- Lang AB, Bruderer U, Senyk G, Pitt TL, Larrick JW, Cryz SJ Jr: Human monoclonal antibodies specific for capsular polysaccharides of *Klebsiella* recognize clusters of multiple serotypes. *J Immunol* 1991;146:3160-3164.
- Lang AB, Schürch U, Zimmermann F, Bruderer U: Selective generation of antigen-specific human hybridomas optimized for large scale growth in serum-free medium. *J Immunol Methods* 1992;154:21-26.
- Yuan W, Quinn DM, Sigler PB, Gelb MH: Kinetic and inhibition studies of phospholipase A₂ with short-chain substrates and inhibitors. *Biochemistry* 1990;29:6082-6094.
- Peterman JH: Factors which influence the Binding of Antibody to Solid-Phase Antigens: Theoretical and Experimental Investigations; PhD thesis, University of Iowa, 1986.
- Dudler T, Chen W-Q, Wang S, Schneider T, Annand RR, Dempcy RO, Crameri R, Gmachl M, Suter M, Gelb MH: High-level expression in *Escherichia coli* and rapid purification of enzymatically active honey bee venom phospholipase A₂. *Biochim Biophys Acta* 1992;1165:201-210.
- Suter M, Butler JE, Peterman JH: The immunochemistry of sandwich ELISAs. III. The stoichiometry and efficacy of the protein-avidin-biotin capture (PABC) system. *Mol Immunol* 1989;26:221-230.
- Dudler T, Schneider T, Annand RR, Gelb MH, Suter M: The antigenic surface of the bee venom allergen phospholipase A₂: Structural and functional analysis of human IgG4 antibodies reveals potential role in protection. *J Immunol*, in press.
- Horsfall AC, Hay FC, Soltys AJ, Jones MG: Epitope mapping. *Immunol Today* 1991;12:211-213.