



Reaction with, and fine structural recognition of polyamines by human IgE antibodies

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Abstract

Human IgE antibodies from nine allergic subjects were found to react with poly-L-lysine (PLL) and other polyamines. Radioimmunoassay inhibition studies indicated that the two amino groups, but not the carboxyl, in lysine contributed to the antibody binding and 4-aminomethyl-1,8-octanediamine, a compound containing three primary amino groups, was a better inhibitor than compounds containing only two primary amino groups. Ethylamine showed weak but clear inhibition indicating that even a single amino group could bind to the antibody combining site. Substituted ethanolamine and quaternary ammonium compounds were well recognized by some sera but with others, substitution hampered recognition. Inhibition studies with compounds containing an amino and a carboxyl group at different distances revealed that an adjacent carboxyl group interfered with recognition of the amino group by some IgE antibodies. IgE binding to PLL was examined at different pHs and ionic strengths. Binding was greatest at pH 5–6 to 8 and decreased markedly outside this range. Ionic strengths higher than 0.3 M significantly diminished the binding. These results indicated that binding of specific antibody to polyamine was due to electrostatic interactions of positively charged amino groups in the polyamine with the antibody combining site. These results may be relevant to mechanisms underlying recognition of some allergens in some atopic conditions. © 2000 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Polyamines are found in all living organisms and are necessary for optimal growth and replication of all cell types. They have been shown to stabilize membranes, alter intracellular calcium levels, influence the transcriptional and translational phases of protein synthesis and may have important messenger and neurophysiological functions (Wallace, 1996; Morgan, 1998).

In routine diagnostic assays for the detection of IgE antibodies to drugs using sera from subjects with suspected drug allergies, we have found a number of sera which showed clear and apparently specific interaction with poly-L-lysine (PLL). Antibodies, mainly of the IgG class, have been shown to react with PLL in what appears to be a specific manner (Kroubouzos et al., 1992) but apart from the recent investigations of Delcros et al. (1996) and Bouille et al. (1999), who studied the structural basis of binding of a monoclonal antibody to the polyamine spermine, no work appears to have been directed at antibody recognition of small polycationic haptens and defining the complementary antigenic residues.

In this study, we describe experiment designed to

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examine the fine structural binding features of the antibodies from the atopic subjects with the view of elucidating the molecular basis of IgE antibody recognition of PLL and other polyamines.

2. Materials and methods

2.1. Sera

Sera from nine patients were included in this study. All the patients had suspected drug allergies (Table 1) and their sera tested positive in immunoassays designed to detect PLL-reactive IgE antibodies. Second samples of sera 235 and 9126 were collected 20 days and 4 months respectively after the first samples. Reactivity with PLL remained the same for serum 235 but diminished for serum 9126, which was designated 9126-1 and -2 to distinguish the first and the second samples.

Control sera were obtained from normal, non-allergic subjects and cord serum, used as an IgE-free control, was provided by the Department of Obstetrics, Royal North Shore Hospital, Sydney.

2.2. Drugs and chemicals

Poly-L-lysine hydrobromide (PLL₄₉) (DP(vis)=49, MW(vis)=10200), poly-L-lysine hydrobromide (PLL₁₈) (DP(vis)=18, MW(vis)=3700), poly-L-lysine hydrobromide (PLL₆) (DP(capillary electrophoresis)=6, MW=1000), L-lysine HCl, D-lysine HCl, L-lysine methyl ester 2HCl, choline Cl, *N*-methyl-L-lysine HCl, ethanolamine and 1,3-diaminopropane 2HCl were obtained from Sigma, St Louis, MO, USA. 1,5-Diaminopentane, 6-aminocaproic acid, 4-aminobutyric acid, 5-amino-*n*-valeric acid, 2,6-diaminopimelic acid, 4-aminomethyl-1,8-octanediamine, *N*-methylethanolamine and spermine 4HCl were from Fluka Chemie, Buchs, Switzerland. Glycine and *N,N,N',N'*-tetramethylethyle-

nediamine were products of Ajax Chemicals, Auburn, NSW, Australia and Eastman Kodak, Rochester, NY, respectively. Ethylamine HCl and *N,N*-dimethylethanolamine were purchased from Aldrich, Milw., WI.

2.3. CNBr activated paper discs

Whatman 542 filter paper discs (3.75 g) of 6 mm diameter were soaked in 3 M NaOH (40 ml) for 15 min at room temperature. After three washes with distilled water and suspension in 2 M Na₂CO₃ (225 ml) at 0°C, the discs were activated by adding 15 g CNBr dissolved in 7.5 ml acetonitrile and stirred for 10 min at 0°C. After successive washes with 3.75 l ice-cold distilled water, 1.5 l ice-cold 0.1 M NaHCO₃, 450 ml ice-cold acetone:H₂O (1:2), 450 ml ice-cold acetone:H₂O (1:1) and 450 ml ice-cold acetone, the discs were dried in a foil covered container overnight and stored at -70°C.

2.4. PLL₄₉ paper disc complex

CNBr-activated paper discs were soaked with agitation in a solution of PLL₄₉ in freshly made 0.1 M NaHCO₃ at 4°C for 20 h at a concentration of 50 µg of PLL per disc. Discs were then washed three times with 0.1 M NaHCO₃ and the remaining reactive sites were blocked by the addition of 0.1 M ethanolamine in 0.1 M NaHCO₃ (pH 9.0) at room temperature for 3 h. During this period the solution was changed twice at 30 and 120 min respectively. After three successive washes with 0.1 M sodium acetate buffer (pH 4.0) and phosphate-buffered saline (PBS), discs were stored in PBS at 4°C.

2.5. Detection of PLL-reactive IgE antibodies by radioimmunoassay and inhibition assay

2.5.1. Radioimmunoassay

Antibodies were detected as previously described

Table 1
Clinical details of subjects included in this study^a

Subject	Sex	Age	Clinical condition	Tests requested	Test positive to
235	F	19	Rash	IgE to Amox	BPA, Ce
272	F	25	Allergy to antibiotics	IgE to inhalants and antibiotics	Ce, Cx
8667	M	?	Urticaria	IgE to grass and dust mite	PG, PV, Amp, Amox, Ce, Cx, Cf
9126	F	?	Urticaria	IgE to keflex and trimethoprim	PG, PV, Ce, Cx, Cf
9005	F	26	Allergy to antibiotics	IgE to antibiotics	PG, PV, Amp, Ce, Cx, Cf
9661	F	70	Multiple antibiotic allergies	IgE to antibiotics	BPA, AXO
9856	F	68	Dermatitis	IgE to ciprofloxacin	BPA, AXO
9857	F	16	Serum sickness	IgE to penicillin	BPA, AXO, Ce, Cx, Cf
9901	M	27	?	IgE to cephalosporins	BPA, AXO, Ce, Cx, Cf

^a Amox: Amoxicillin; Amp: Ampicillin; AXO: Amoxilloyl determinant; BPA: Penicillanyl determinant; Ce: Cephalothin; Cf: Cefaclor; Cx: Cephalixin; PG: Penicillin G; PV: Penicillin V.

(Harle and Baldo, 1990). Briefly, serum (20 μ l) was incubated for 3 h at room temperature with a disc that had previously been coupled to PLL₄₉. Discs were then washed three times with PBS containing 0.05% Tween 20 and 0.05% sodium azide before incubation overnight with about 30000 cpm of ¹²⁵I-anti-human IgE (Bioclone, NSW, Australia). Discs were washed again as above and counted in a Packard Auto-Gamma Spectrometer. The presence of specific IgE antibodies was determined by the percent radioactive uptake of ¹²⁵I-anti-human IgE, i.e. percent of counts added.

To assess the influence of pH on antibody reactivity, the sera were diluted (1–4) in buffers containing 100 mM of phosphate (pH 6, 7.2 or 8), carbonate (pH 9.2) or acetate (pH 4 or 5) together with 0.14 M NaCl. To check the effect of ionic strength on antibody binding, the sera were diluted (1–4 for serum 235 and 1–8 for serum 9005) in 10 mM Tris buffer (pH 7.2) containing different concentrations of NaCl (0.14–1.5 M). The assay was then continued as described above.

2.5.2. Inhibition test

For inhibition studies, the inhibitors were dissolved in PBS pH 7.2 and the pH of the solutions were adjusted to 6.5–7.5 with HCl or NaOH. Serum (20 μ l), appropriately diluted, was incubated for 2 h at room temperature with 20 μ l of inhibitor solution before the addition of a PLL₄₉ paper disc. The assay was then continued as described above.

3. Results

3.1. Binding of serum IgE antibodies to PLL and preliminary inhibition findings

All nine sera showed significant IgE binding to

PLL₄₉ conjugated to paper discs. Percentage uptakes of ¹²⁵I-anti-IgE ranged from 2.3 to 26.4% (Table 2). Uptakes with sera from cord blood and normal, non-allergic subjects were less than 1% (data not shown). With each of the patients, antibody binding was significantly inhibited by PLL (PLL₁₈ or PLL₄₉) and/or the diamine, diaminopentane. L-Lysine and D-lysine showed similar inhibitory potencies with most of the sera and were significantly weaker than the PLLs and diaminopentane. On the basis of the inhibitory potencies of L- or D-lysine, sera were separated into two groups — those where IgE binding to the PLL-solid phase was clearly inhibited (more than 30%) and those where L-lysine caused weak inhibition (less than 20%). Detailed inhibition findings with PLLs, lysines and *N*-methyl-L-lysine are shown for representative sera from each of these two groups — serum 9126 for the former group (Fig. 1a) and sera 235 and 9005 for the latter (Fig. 1b and c; Table 3). With serum 9126, inhibition with *N*-methyl-L-lysine was approximately equal to that observed with the lysines (Fig. 1a) but with sera 235 and 9005, the *N*-methylated derivative was less active (Fig. 1b and c).

3.2. Recognition of (poly)amines

Compounds containing one or more amino groups were compared side-by-side in inhibition studies with all three sera. In each case, 4-aminomethyl-1,8-octane-diamine which contains three primary amino groups, was the most potent inhibitor followed by 1,5-diaminopentane, 1,3-diaminopropane (each with two primary amino groups) and spermine (two primary amino and two secondary amino groups) which showed similar inhibitory potencies with each of the sera (Table 3). Ethylamine, with one primary amino group, was a much weaker inhibitor with all three sera (Fig. 2a).

Table 2
Inhibition (%) of serum IgE binding to PLL paper discs by polylysines^a, lysines^b and diaminopentane^b

Inhibitor	Serum								
	235	272	8667	9005	9126-2	9661	9856	9857	9901
PLL	94	—	67	88	50 ^c	93	85	84	84
DAPE ^d	54	—	55	76	56	91	47	73	—
L-lysine	0	36	36	11	66	35	0	6	50
D-lysine	0	—	25	16	76	37	14	20	—
Direct binding ^e	26.4	5.7	2.3	23.2	7.7	17.5	5.0	4.8	3.7

^a PLL (DP = 18). Used at an inhibitory concentration of 5.6 mM.

^b Used at inhibitory concentrations of 40 mM.

^c PLL (DP = 6) used at a concentration of 20 mM.

^d DAPE: 1,5-diaminopentane.

^e Percentage of ¹²⁵I-anti-IgE uptake when using undiluted sera with PLL paper discs without adding any inhibitor.

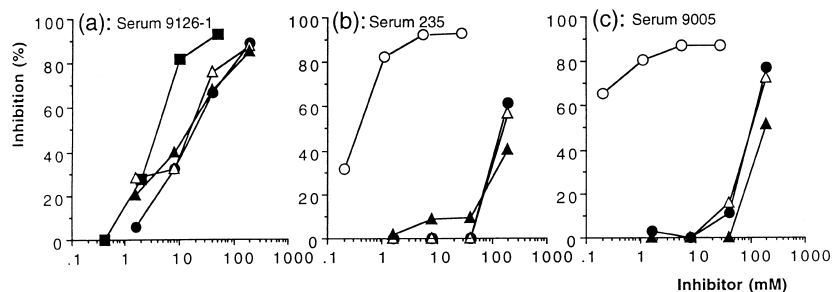


Fig. 1. Inhibition of IgE antibody binding to PLL₄₉-paper discs by PLL₄₉(■), PLL₁₈ (○), L-lysine (●), D-lysine (△), and *N*-methyl-L-lysine (▲).

3.3. Recognition of substituted amino groups

N-Methyl substituted amines with a hydroxyl group to increase their solubilities were compared side-by-side in inhibition studies. The results showed that the addition of a hydroxyl group did not affect their activities, i.e. ethanolamine and ethylamine were approximately equally inhibitory with all three sera. With serum 9126, the tertiary diamine, *N,N,N',N'*-tetramethylethylenediamine, clearly inhibited binding to the

solid phase and the other substituted monoamines showed similar inhibitory activities which were weaker than the substituted diamine (Fig. 2b; Table 3). With sera 235 and 9005, substitution hampered recognition, that is, inhibition became weaker with the higher substitution (Fig. 2b). This effect was clearer with serum 9005 where tertiary and quaternary amines showed weak or no activity and the order of inhibitory potency was primary amine > secondary > tertiary and quaternary (Fig. 2b; Table 3).

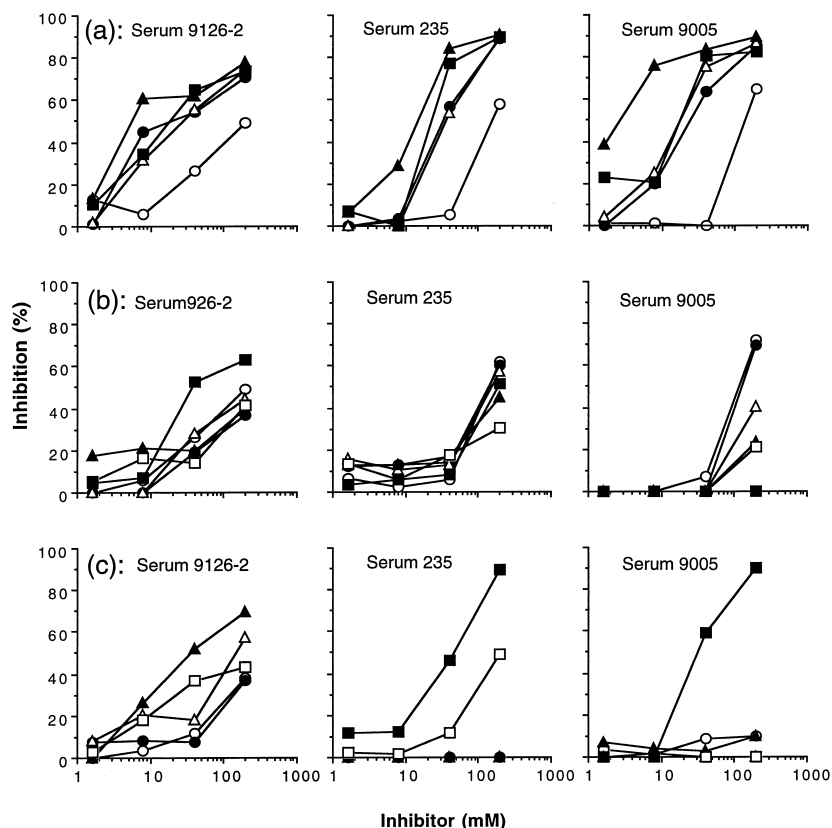


Fig. 2. Inhibition of IgE antibody binding in three different sera to PLL₄₉-paper discs by: (a) (poly)amines (ethylamine (○), 1,3-diaminopropane (●), 1,5-diaminopentane (△), 4-aminomethyl-1,8-octanediamine (▲), and spermine (■)), (b) substituted amines (ethylamine (○), ethanolamine (●), *N*-methylethanolamine (△), *N,N*-dimethylethanolamine (▲), choline (□) and *N,N,N',N'*-tetramethylethylenediamine (■)) and (c) amino acids (glycine (○), 4-aminobutyric acid (●), 5-amino-*n*-valeric acid (△), 6-aminocaproic acid (▲), 2,6-diaminopimelic acid (□) and L-lysine methyl ester (■)).

Table 3
Inhibition of the binding of IgE antibodies to polylysine by primary and substituted amino compounds

Compounds	Structure	IC ₃₀ (mM)				IC ₅₀ (mM)				Inhibition (%) at 40 mM			
		9126	235	9005	9126	9005	9126	235	9005	9126	235	9005	9005
Poly-L-lysine ₄₉	(L-lysine) ₄₉	2			3.2					93			
Poly-L-lysine ₁₈	(L-lysine) ₁₈		0.18	< 0.1		< 0.1					94	88	
L-Lysine	NH ₂ (CH ₂) ₄ CH(NH ₂)COOH	6.1	90	65	19	65	19	130	105	66	0	11	
D-lysine	NH ₂ (CH ₂) ₄ CH(NH ₂)COOH	3.1	90	60	13	60	13	140	105	76	0	16	
N-methyl-L-lysine	CH ₃ NH(CH ₂) ₄ CH(NH ₂)COOH	3.1	105	100	12	100	12	> 200	200	68	9	0	
Ethylamine	CH ₃ CH ₂ NH ₂	50	81	81	> 200	81	> 200	130	120	26	5	0	
1,3-Diaminopropane	NH ₂ (CH ₂) ₃ NH ₂	4.2	18	11	19	11	19	30	22	54	57	64	
1,5-Diaminopentane	NH ₂ (CH ₂) ₅ NH ₂	7.1	20	9	29	9	29	36	18	56	54	76	
4-Aminomethyl-1,8-octanediamine	NH ₂ (CH ₂) ₆ CH(CH ₂ NH ₂)(CH ₂) ₃ NH ₂	2.6	8.1	< 1.6	5.2	< 1.6	5.2	13	2.6	62	84	84	
Spermine	NH ₂ (CH ₂) ₃ NH(CH ₂) ₄ NH(CH ₂) ₃ NH ₂	6	16	10	18	10	18	21	18	65	77	81	
Ethanolamine	HO(CH ₂) ₂ NH ₂	100	75	80	> 200	80	> 200	130	120	19	14	0	
N-Methylethanolamine	HO(CH ₂) ₂ NHCH ₃	45	75	120	> 200	120	> 200	135	> 200	28	13	0	
N,N-Dimethylethanolamine	HO(CH ₂) ₂ N(CH ₃) ₂	90	80	> 200	> 200	> 200	> 200	> 200	> 200	20	17	0	
N,N,N',N'-Tetramethylethylenediamine	(CH ₃) ₂ NCH ₂ CH ₂ N(CH ₃) ₂	19	90	> 200	39	> 200	39	190	> 200	53	8	0	
Choline	(CH ₃) ₃ N(OH)(CH ₂) ₂ OH	100	190	> 200	> 200	> 200	> 200	> 200	> 200	15	17	0	
Glycine	NH ₂ CH ₂ COOH	110	> 200	> 200	> 200	> 200	> 200	> 200	> 200	12	0	9	
4-Aminobutyric acid	NH ₂ (CH ₂) ₃ COOH	115	> 200	> 200	> 200	> 200	> 200	> 200	> 200	8	0	0	
5-Amino- <i>n</i> -valeric acid	NH ₂ (CH ₂) ₄ COOH	61	> 200	> 200	120	> 200	120	> 200	> 200	18	0	0	
6-Aminocaproic acid	NH ₂ (CH ₂) ₅ COOH	10	> 200	> 200	35	> 200	35	> 200	> 200	52	0	3	
2,6-Diaminopimelic acid	HOOCCH(NH ₂)(CH ₂) ₃ CH(NH ₂)COOH	21	85	> 200	> 200	> 200	> 200	> 200	> 200	37	12	0	
L-Lysine methyl ester	NH ₂ (CH ₂) ₄ CH(NH ₂)COOCH ₃		18	18				45	31	46	59		

3.4. Recognition of amino acids. Effect of neighbouring carboxyl groups

Since the inhibitory activities of D- and L-lysine were generally less than the diamines, amines containing one or more carboxyl groups were examined to see whether such neighbouring groups had any effect on IgE antibody recognition. Four amino acids with increasing C chain length between the amino and carboxyl groups viz. glycine (1C), 4-aminobutyric acid (3Cs), 5-aminovaleric acid (4Cs) and 6-aminocaproic acid (5Cs) were selected and examined side-by-side with the dicarboxylic acid, 2,6-diaminopimelic acid. Like 1,5-diaminopentane, the latter compound contains two primary amino groups separated by a five C chain. With serum 9126, which showed clear recognition of D- and L-lysine, all the compounds showed some inhibition with the order of inhibitory potency being 6-aminocaproic acid > 5-aminovaleric acid > 4-aminobutyric acid = glycine. 2,6-Diaminopimelic acid was intermediate in inhibitory potency between the best and worst inhibitors (Fig. 2c; Table 3).

With sera 235 and 9005, which were found to be weakly inhibited, by lysines, all four monocarboxylic acids were without activity. 2,6-Diaminopimelic acid weakly inhibited IgE antibodies in serum 235 but was inactive with serum 9005. However, the methyl ester of L-lysine was a good inhibitor with sera 235 and 9005, demonstrating potency close to the diamines (Fig. 2c; Table 3).

3.5. Influence of pH and ionic strength on the binding of IgE to PLL

The binding of IgE antibodies in sera 235 and 9005 to PLL₄₉ discs was tested at different pHs and ionic strengths. The highest bindings were obtained in the pH range 6–8 for serum 235 and 5–8 for serum 9005. Outside these ranges the bindings decreased progress-

ively (Fig. 3a). Ionic strength higher than 0.3 M significantly diminished the binding (Fig. 3b). At approximately 0.8–1 M, binding reached its lowest point.

4. Discussion

Although polyamines are known to be involved in a number of different biological processes (Pegg, 1988; Seiler, 1994; Wallace, 1996; Morgan, 1998), their precise roles and molecular bases of their actions are not well understood. As pointed out by Bouille et al. (1999), this is at least partly due to a lack of knowledge of their cellular localisation and, with this in mind, immunocytochemical and quantitative immunoassay studies utilizing antibodies to polyamines such as spermine have been pursued (Garthwaite et al., 1993; Hougaard et al., 1987; Fujiwara et al., 1994a, 1994b; Valentino et al., 1996). The existence of “natural” antibodies reacting with polyamines in human sera was reported by Roch et al. (1979). In this study, antibody titres were found to be less in the sera of tumour patients than in normal sera.

Detailed specificity findings presented here with sera from allergic subjects reflect a human IgE antibody recognition spectrum ranging from preferential reactivity with primary amino groups (marked with serum 9005, less so with serum 235) to a broader recognition of substituted (di- and tri-), as well as primary, amino groups (serum 9126). Other specificity differences found amongst the polyamine-reactive serum panel were demonstrated in inhibition studies with amino acids where compounds with a chain length of 4 or 5 Cs between the amino and carboxyl groups were more strongly recognized than compounds with three or less methylene groups. This was the case with serum 9126, a representative example from the sera which showed clear recognition of D- and L-lysines as well as PLLs.

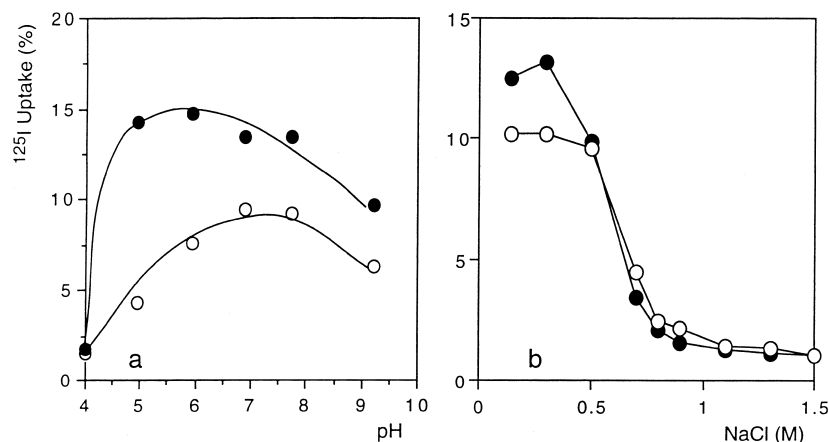


Fig. 3. Influence of pH and ionic strength on the binding of IgE antibodies in sera 235 (○) and 9005 (●) to PLL₄₉-paper discs.

However, with PLL-reactive sera that reacted only weakly with the lysines, for example, 235 and 9005, each of the amino acids tested from glycine (1 methylene) to 6-aminocaproic acid (5 methylenes) were without inhibitory effect. In contrast, conversion of the carboxyl group of L-lysine to the methyl ester restored inhibitory activity. Interestingly, 2,6-diaminopimelic acid which contains two amino groups separated by a 5 C chain and two carboxyl groups 1 C distant from each amino, showed significant reactivity with IgE in both sera 9126 and 235.

The inhibition findings with the amino acids, the diamino acid 2,6-diaminopimelic acid and L-methyllysine ester suggest that charge distribution underlies the differences in fine structure recognized by the different PLL-reactive IgE antibodies. Amino acids are amphotheric and exist as dipolar or zwitterions (hybrid ions) in which the carboxyl is the acidic group or proton donor and the amino is the basic group or proton acceptor. In addition, reactivity with the primary amino groups appears to be influenced by near neighbour effects of carboxyl groups — the greater the distance between the amino and carboxyl groups (Fig. 4), the stronger the antibody recognition.

Binding studies by Bouille et al. (1999) on an anti-spermine monoclonal antibody demonstrated marked dependence on pH and ionic strength indicating the importance of the electrostatic environment for interaction with spermine. Paratope modelling revealed that aspartic acid and tyrosine were important contact residues for spermine within the antibody combining site.

Specificity studies on the monoclonal antibody (Delcros et al., 1996) showed the importance of terminal primary amino groups, the number of such groups and that a four carbon chain was the optimum spacer between the amino groups. Affinity of the antibody for free polyamines was in the order pentamines > tetramines > triamines > diamines, emphasizing the importance of charge in the binding.

A comparison of heavy and light chain amino acid sequences of four anti-polyamine antibodies showed no conserved heavy chain sequences but three light chains of marked homology with only five non-conservative amino acid substitutions (Johnston et al., 1998). This may reflect a functional role for the light chain in antibody binding to polyamines.

The specificity of the polyamine-reactive mouse monoclonal antibody (Delcros et al., 1996; Bouille et al., 1999) and the human antibodies appears to be similar. The finding of naturally induced polyamine-reactive antibodies of the IgE class in the sera of some allergic subjects is intriguing since it is not obvious what biological role such antibodies may have or, indeed, if the antibodies are even relevant to the allergic state in the subjects in which they occur. It may be that antibodies that react with primary and substituted amino groups underlie some allergic or other adverse reactions to drugs and chemicals that contain the groups or to amino acid components of some peptide allergens. This is an hypothesis that may be tested and it should be remembered that drug-reactive IgE antibodies complementary to tertiary and quaternary am-

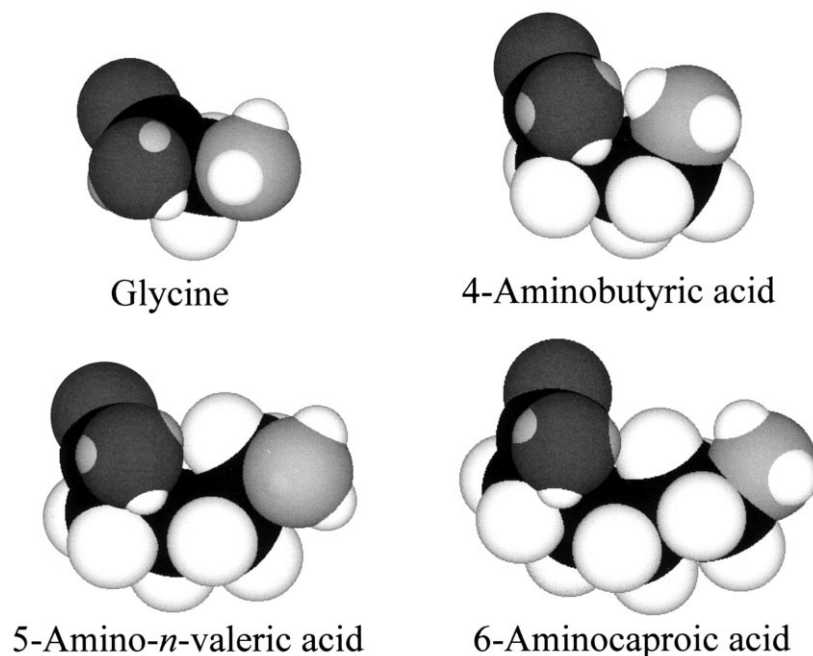


Fig. 4. Space-filling models of amino acid of increasing C chain length showing proximity of the carboxyl and amino groups. The hydrogen, nitrogen, oxygen and carbon atoms are shown as white, grey, dark grey and black respectively.

monium groups have already been identified, studied and employed routinely in diagnostic tests for some drug allergies (Baldo and Fisher, 1983; Baldo and Harle, 1990; Baldo and Pham, 1994).

The existence of “false positive” human IgE antibodies that react with PLLs should be kept in mind when PLL antigen conjugates are employed in immunoassay studies. With regard to the frequency of occurrence of polyamine-reactive IgE antibodies detected by reaction with PLL, it seems that such antibodies occur only infrequently. So far, positive reactions to PLL have been detected in a small number of sera from subjects with suspected drug allergies. Further studies designed to reveal the incidence of such antibodies in normal, non-allergic subjects and in groups of patients allergic to drug, inhalant and food allergens are planned.

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