

Studies on the specificity of human IgE-antibodies to the plant proteases papain and bromelain

X. BAUR

Pulmonary Section, Medical Clinic I, Klinikum Großhadern, Ludwig-Maximilians-Universität of Munich, Germany

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Summary

The sera of seven patients clinically hypersensitive to papain—in one case also to bromelain—and the sera of sixty asthmatic patients with allergies to other inhalant and food allergens were investigated for IgE antibody activity to the plant proteases papain and bromelain and to common allergens by RAST, confirmed in some sera by RAST inhibition.

There seems to be a relation between the antibody reactions to papain and bromelain, in several cases also between the reactions to these proteases and to grass pollen and flour. Studies by RAST inhibition showed that papain, bromelain, wheat flour, rye flour, grass pollen and birch pollen mutually inhibit IgE antibody to each antigen; but the degree of inhibition varies among the different sera and allergens.

Our results suggest that these allergens from various plants, besides having specific antigenic determinants, also possess similar or even identical antigenically active regions, leading to immunological cross-reactivity.

Introduction

Proteases are a constituent part of numerous plants, especially of their fruits and seeds; well known examples are carica papaya fruit, pineapple stem and fruit, fig lattices, several beans, wheat seed, white gourd, melon fruit, maya fruit, pollen grains (Paton, 1919; Mounfield, 1938; Greenberg & Winnick, 1945; Lennox & Ellis, 1945; Knox & Heslop-Harrison, 1970; Kato & Sasaki, 1974; Kaneda & Tominaga, 1975; Iordan & Belozerskii, 1976; Blanchard & Gardner, 1976; Murachi, 1976; Bousquet *et al.*, 1978).

Human exposure to some of these enzymes is common, especially as ingredients in foods and drugs; furthermore there is occupational exposure while processing the raw materials or dealing with purified proteases, e.g. in biochemistry and medicine. Several reports of hypersensitive reactions indicate that at least some of these substances are highly potent allergens in man if they are repeatedly inhaled over long periods of time

Correspondence: Dr X. Baur, Pulmonologische Abteilung, Klinikum Großhadern, D 8000 München 70, Germany.

(Milne & Brand, 1975; Tarlo *et al.*, 1978; Galleguillos & Rodriguez, 1978; Baur & Fruhmman, 1979a). As opposed to this, hypersensitive reactions following ingestion of one of these substances has been proven in only one case (Baur & Fruhmman, 1979b).

Studies in our pulmonary section demonstrated that patients exposed only to airborne papain also showed IgE reactions to bromelain. Furthermore, in many of these patients, the presence of IgE antibodies to grass pollen and in some cases of antibodies to flour could be established. On the other hand, several patients with hypersensitivity to grass pollen showed IgE reactions to papain and/or bromelain, despite their never having been exposed to these purified proteases.

The object of this investigation was to find out if IgE antibodies specific to bromelain or papain are responsible for the positive RAST results to these enzymes or if there exists an immunological cross-reaction between the two proteases and possibly also between these and other common allergens.

Materials and methods

RAST inhibition

To study cross-reactivity of IgE antibodies the patients' sera were incubated at the same time with two allergens, one of them coupled to cyanogen bromide-activated paper discs, the other—as a neutralising inhibitor—in soluble form in different concentrations. All the other steps were similar to those of the usual RAST method (Pharmacia, Uppsala, Sweden).

In this study reproducibility of the RAST results was $\pm 5.2\%$ for papain discs and $\pm 4.9\%$ for bromelain discs.

Coupling of paper discs was performed in our laboratory using commercially available extracts: mixed grass pollen 6% (Bencard, Brentford, England), papain 500 u/g (Sigma Chemical Co; St. Louis, U.S.A., lot 46C-0297) and bromelain 5 000 u/g (Merck, Darmstadt; West Germany, lot 8531 400).

Further pre-prepared allergen discs were purchased from Pharmacia, Uppsala, Sweden.

Commercially available allergen extracts were used for inhibition of RAST: wheat flour 10% and rye flour 10% (both from Hollister Stier, Spokane, Washington, U.S.A.; Distributor Köln, West Germany); mixed grass pollen 6%, birch pollen 6% and house dust mite *Dermatophagoides pteronyssinus* 1–2% (all three from Bencard, Brentford, England).

Results

Table 1 illustrates the RAST reactions to papain, bromelain and common allergens of asthmatic patients with or without previous contact with purified proteases. As expected, in nearly all cases there were strong reactions to such substances to which a clinically relevant sensitization existed. Of special interest is the finding that the sera of most of the patients exposed and sensitized to purified proteases showed high RAST values in tests with these enzymes. On the other hand, there were often weak to moderate positive RAST results without clinical relevance; e.g. five of six patients hypersensitive to papain who had not been exposed to purified bromelain showed reactions to bromelain and some patients of this group also to pollen and flour, despite the fact that they had not been suffering from hypersensitivity to these allergens.

Table 1. RAST results of sixty-seven patients with or without previous exposure to purified bromelain and/or papain. According to the Phadebas RAST method, values below 0.35 PRU/ml are regarded as negative (=0); values between 0.35 and 0.7 PRU/ml are regarded as doubtful

Patient	Clinically sensitized to*	RAST results (PRU/ml)							Other allergens to which there were positive RAST results
		Bromelain	Papain	Mixed grass pollen	Medow grass pollen	Birch pollen	Wheat flour	Rye flour	
F.M.	Bromelain, papain, penicillin V	76.7	4.8	0.5	0.6	2.8	0	0	Alder pollen
M.R.	Papain, wheat flour, rye flour	25.5	183.0	0	0	0	2.0	1.1	
S.H.	Papain	6.6	222.5	10.3	11.0	0	3.2	3.4	Milk
L.B.	Papain	4.5	60.0	0.4	0	0	0	0	
N.K.	Papain, spice	2.4	18.1	0	1.0	0	0	0	
G.A.	Papain, wheat flour	1.5	7.6	0.4	1.0	0	0.4	0.4	House dust mite, house dust
P.J.	Papain	0	0.8	0	0	0	0	0	House dust mite
K.L.	Wheat flour, rye flour	12.1	0.4	11.1	9.2	2.0	23.0	35.0	
H.L.	Grass pollen, house dust mite	25.0	3.4	10.0	16.0	1.1	13.4	9.2	House dust mite, house dust, cat epithelium
R.C.	Grass pollen, house dust mite	10.6	1.1	241.0	272.0	1.5	6.2	5.1	House dust mite, house dust, dog epithelium
U.K.	Grass pollen	5.8	0.4	5.8	4.8	0	1.6	1.4	
M.J.	Grass pollen, birch pollen, house dust mite	4.8	1.5	24.2	18.5	64.0	0.6	0.4	Several flower pollen, alder pollen, house dust mite
D.E.	Grass pollen, mould spores	2.5	0.4	2.1	2.7	0	9.6	9.0	Plantain pollen, <i>Alternaria tenuis</i>
S.J.	Grass pollen, cat fur, dog hair, mould spores	2.1	0	144.0	203.0	0	1.5	1.2	Cat and dog epithelium, <i>Alternaria tenuis</i>
M.U.	Grass pollen, tree pollen, mould spores	1.6	0.5	16.3	25.0	9.6	1.6	1.4	Dog epithelium, <i>Aspergillus fumigatus</i> , <i>Penicillium n.</i>
Eighteen further asthmatics with pollinosis	Grass pollen, various inhalent allergens	<0.7	<0.7	0.7-8.1	0.7-8.8	0-64	0-9.6	0-9.0	Various inhalent allergens
Thirty-four further asthmatics without pollinosis	Various inhalent and food allergens	<0.7	<0.7	0-5.1	0-5.0	0-1.5	0-19.5	0-18.5	Various inhalent and food allergens

*Clinical sensitization was proven by case history, skin test, RAST and in doubtful cases also by bronchial provocation test.

To prove IgE specificity of the results, serum samples of patient F.M. were incubated at 56°C for 4 hr prior to measurement by the RAST method; this procedure led to a loss of reactivity to papain of 92%, to bromelain of 94.5%, and to birch pollen of 99%.

As the positive RAST results without clinical relevance might be due to cross-reactivity of the tested substances of plant origin, we investigated four bakers highly sensitive to wheat flour and rye flour as well as twenty-five asthmatics with pollinosis. Seven patients of the latter group and one of the bakers (patient K.L.) showed RAST values above 0.7 PRU/ml when bromelain was tested and three out of the pollinosis group also when papain was tested (Table 1).

In studies with RAST inhibition it was shown that addition to serum samples of the same protease as that coupled to the incubated discs resulted in an increasing drop in IgE binding on the discs, reaching inhibition rates of 82 to 100% (Fig. 1a, 1b).

Similar results were obtained when the inhibitory effect of bromelain on RAST reactions to papain (Fig. 1c) and vice versa (Fig. 1d) was investigated. But in these

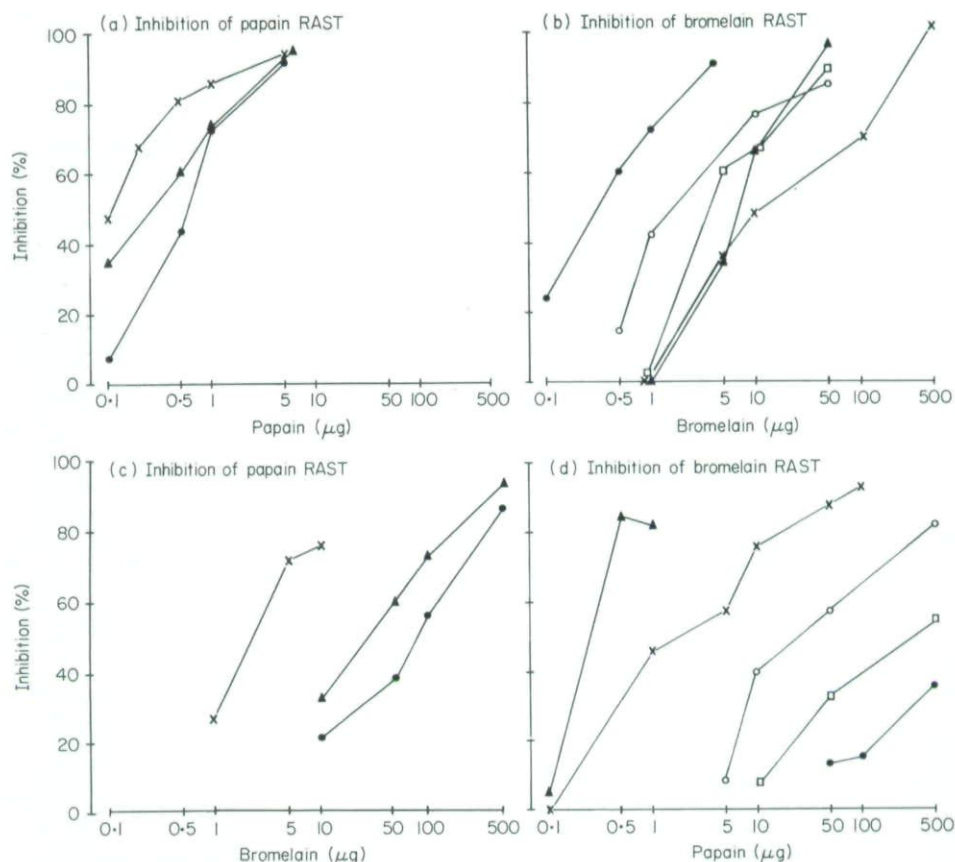


Fig. 1 a, b, c, d. Studies on cross-reactivity of papain and bromelain by RAST inhibition. Different amounts of papain or bromelain were added to serum samples incubated with papain discs (papain RAST) or bromelain discs (bromelain RAST); the results are expressed as percent reduction of IgE binding on the discs. Patients: (●) F.M.; (×) S.H.; (▲) L.B.; (□) H.L.; (○) R.C.

experiments the amounts of inhibitors showing a noticeable effect varied widely. Of special interest is the finding that IgE binding of serum F.M. (the only patient previously exposed to airborne bromelain) on bromelain discs could be inhibited only partly by papain, even when very large amounts were used; but the bromelain reaction of this serum was inhibited by bromelain more easily than were the bromelain reactions of other sera.

Reactions to bromelain of sera of papain-exposed patients were inhibited by smaller amounts of papain than of bromelain, whereas for sera of patient F.M. and of two patients not exposed to any airborne proteases, smaller amounts of bromelain were sufficient (Fig. 1b, 1d).

As illustrated in Table 2, IgE bindings on papain and bromelain of sera F.M. and S.H. were slightly to moderately inhibited by extracts of grass pollen, birch pollen, wheat flour and partly also by extracts of rye flour; but the same allergen extracts had a strong inhibitory effect when sera of patients not exposed to purified proteases (H.L.; R.C.) were investigated. In no case did the house dust mite allergen cause a comparable drop of IgE binding.

Table 2. RAST inhibition. Percent inhibition of IgE binding on papain discs and on bromelain discs by common allergens. Commercially available allergen extracts (20 μ l) were used as inhibitors

Patient	Papain discs					Bromelain discs				
	Inhibitor					Inhibitor				
	Mixed grass pollen	Birch pollen	Wheat flour	Rye flour	House dust mite	Mixed grass pollen	Birch pollen	Wheat flour	Rye flour	House dust mite
F.M.	9	3	12	0	-2	20	9	13	10	6
S.H.	12	4	9	6	4	15	17	28	6	-7
H.L.	63	63	74	67	11	81	69	89	90	-10
R.C.						86	51	83	88	7

Inhibitory rates were same when the enzymatic activity of the proteases coupled to the paper discs was blocked by tosyl-L-phenylalanine or phenylmethylsulfonyl fluoride according to the method of Whitaker & Villaseñor (1968).

In a different experiment, inhibition of IgE binding on discs coupled with common allergens by papain or bromelain was investigated (Table 3). Only in some cases were the reactions to grass pollen and wheat flour influenced noticeably by 20 μ g of papain; whereas the same amounts of bromelain inhibited in nearly all cases to a high degree the IgE binding on grass pollen and wheat flour and in one case also on birch pollen. Neither of the proteases caused a significant effect on reactions to cat epithelium, horse epithelium or to the house dust mite allergen.

Table 3. RAST inhibition

Patient	Percentage inhibition of IgE binding on different allergen discs by papain (20 μ g)					Percentage inhibition of IgE binding on different allergen discs by bromelain (20 μ g)				
	Mixed grass pollen	Birch pollen	Wheat flour	House dust mite	Cat/horse epithelium	Mixed grass pollen	Birch pollen	Wheat flour	House dust mite	Cat/horse epithelium
F.M.		-4					-5			
S.H.	0		36			3		68		
H.L.	13	-4	14	-4	6	74	8	61	9	10
R.C.	31	-5	3	0		35	35	65	1	
K.L.	6	0	5			65	4	27		
G.K.	9	-5				21	-5			
P.K.	0				-5	0				7
T.N.				-5	1				0	6
S.A.			0					18		
D.E.	6					39				
B.J.		-1								

Discussion

In the present study it is shown by RAST and RAST inhibition that human antibodies to papain cross-reacts with bromelain and vice versa. This explains why patients exposed and clinically sensitized only to papain also show positive skin test results and immediate asthmatic reactions to bromelain. Furthermore, small to large degrees of inhibition by grass pollen, birch pollen, wheat flour and rye flour on IgE reactions to papain and bromelain, as well as remarkable inhibitory effects of the two proteases on IgE reactions to these common allergens, suggests that there is an immunological cross-reactivity of human IgE antibodies to these substances.

As shown by Husain & Lowe (1970), Drenth *et al.* (1971) and Goto, Murachi & Takahashi (1976), there is evidence for structural homology in terms of the amino acid sequence of bromelain, papain and ficin. Immunological cross-reactions between these enzymes as well as between papain and chymopapain and between stem and fruit bromelain, are reported by Arnon & Shapiro (1968), Eder & Arnon (1973), Sasaki, Kato & Iida (1973) and Kato & Sasaki (1974); in these studies antibodies from the rabbit were used. In other investigations, Hoffman (1975), using RAST inhibition, was able to show cross-reactivity between antigens of cereal grains, rice and grass pollen.

The above studies as well as our results lead us to suggest that the cross-reactivity of papain, bromelain, pollen and flour is due to common antigenic determinants. It would be very interesting to examine if such common antigenic determinants in pollen grains and cereals could be related to the proteolytic enzymes contained in them. But as the reactions of sera to the tested allergens varied greatly and as the inhibitory activity of a certain allergen on these antigen-antibody reactions was different from serum to serum—obviously depending on the allergens to which the patients were predominantly exposed and clinically sensitized, there must also exist specific antigenic determinants.

In the studies with RAST and RAST inhibition it must be considered that the proteolytic activity of papain and bromelain could have an influence on the RAST results, e.g. through digestion of antibodies or antigens. This may occur especially when high concentrations are used. It is unlikely that such effects play an important role in our experiments because blocking the enzymatic activity of the proteases did not result in a significant change of the RAST values nor prevent the inhibitory reactions; moreover the inhibitory rate of a certain amount of papain or bromelain was not constant but differed from allergen to allergen and from serum to serum.

The well documented fact that some widely used proteases of plant origin are potent allergens capable of provoking hypersensitive respiratory and gastrointestinal reactions, leads us to believe that similar proteolytic active molecules are the substances proper actually responsible for the antigenic properties of other foodstuffs and airborne organic particles. Furthermore, it seems possible that immunological relationships between the antigenic determinants of the tested allergens as well as between antigenic determinants of other inhalent and food materials might underlie the step-wise sensitization to a broad spectrum of allergens so often seen in atopic patients.

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