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Structure and function of proteins involved in milk allergies

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Abstract

Allergy to milk proteins has been defined as any adverse reaction mediated by immunological mechanisms to one or several of proteins found in milk. The milk allergy has been classified according to the onset of symptoms as immediate or delayed type. The milk allergy seems to be manifested by three major proteins found in milk: α-lactalbumin, β-lactoglobulin and caseins. The structural comparison of allergenic sites in α-lactalbumin and β-lactoglobulin with the structure of lactoferrin has clearly shown that yet another major milk protein lactoferrin also possesses allergenic sites and thus may qualify to be an allergen. The heat treatment of milk proteins considerably reduces their allergenicity. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

Allergy is a widespread affliction affecting up to 15% of the population at any one time. Man can become allergic to a vast range of both natural and synthetic substances following their inhalation, ingestion and injection or by contacting them with his skin or mucous membranes. Hence, as milk and milk products are so commonly and widely ingested, the potential for allergic sensitization to milk proteins would seem to be great. Allergic reactions may be classified into two broad groups: immediate and delayed. The immediate reactions are mediated by immunoglobulin E (IgE) antibodies and usually occur within minutes of contacts with allergen. Examples of immediate reactions include hayfever provoked by grass pollens during spring and anaphylaxis following a bee-sting. These examples show that symptoms associated with immediate reactions can range from mild irritation of mucous membranes to life threatening bronchospasm and cardiovascular collapse.

Delayed reactions are cell-mediated. These include various contact sensitivities by irritants such as poison ivy or certain chemicals. Sometimes immediate and delayed reactions occur in the same individual following exposure to a particular sensitizing agent. In such cases, the delayed reaction usually occurs some hours after the immediate response. Both reactions may be manifested as impaired airway and lung functions or as swollen and inflamed skin reactions. While events underlying the immediate reactions are fairly well understood, we still have

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a poor understanding of mechanisms involved in late responses.

Allergens are those antigens that can induce an immediate hypersensitivity reaction in the airways, gastrointestinal tract, skin or mucous membranes of following interactions with cell bound, specific IgE molecules. Allergens are generally proteins [1,2]. So far only a small number of allergens have been characterized structurally thus providing insufficient data to determine common sites which confer allergenicity on a macromolecule. A majority of allergen sources contain more than one allergen molecule [3].

The real challenge is to determine as to how the proteins are allergenic as there is no generally accepted, established, definitive procedure to define or predict a protein's allergenicity. Comparing the structures of the proteins with known allergens and allergen epitopes could be one approach.

2. Suspected milk protein allergens

The allergy to milk proteins is considered to be mediated by immunological mechanisms to one or several of proteins found in milk. The milk allergy seems to be manifested by three major proteins found in milk: α-lactalbumin, β-lactoglobulin, and caseins but these are usually the only proteins tested. In highly allergic children, it has been found that every protein tested has reacted with IgE antibodies in the patient's serum. However, the role of milk proteins in the allergic reactions is not yet clearly understood. In some studies, β-lactoglobulin was found to be the fraction with the greatest sensitizing potential, while the α-lactalbumin fraction gave weaker positive reactions whereas casein was the least sensitizing of the fractions studied [4–6]. However, in a different investigation, casein molecule has been found to be the major allergenic and antigenic protein of milk [7]. Among caseins, α(s) 1-casein is a major allergen in the milk. The determinants of IgE, IgG4 and T cells specific for α(s) 1-casein from the same individual patients were investigated by using its synthetic peptides and cyanogen bromide-digested fragments [8]. By using ELISA for epitope mapping, a C-terminal region of α(s) 1-casein was identified as a common binding site for IgE whereas those for anti-α(s)-casein IgG4 were located in multiple regions of α (s) 1-casein.

α-Lactalbumin and β-lactoglobulin are major allergens involved in allergy to milk proteins. Hydrolyzing these proteins did not totally suppress their allergenicity; moreover their immuno-reactivity seems to have increased [9,10].

3. Structural studies of milk allergens

B-cell epitopes of casein, one of the major allergens of milk, have been identified by a screening method based on synthetic peptides [11]. In the case of β-lactoglobulin, three peptides were identified as major epitopes recognized by a large majority of human IgE antibodies. Numerous other epitopes are scattered all along the β-lactoglobulin sequence. A continuous stretch of four amino acids common to α-lactalbumin and β-lactoglobulin that might be responsible for this cross-reactivity has been identified.

Thus, the most known allergenic proteins in milk are α-lactalbumin, β-lactoglobulin and casein. In the allergen proteins, there should be some sites of interaction with IgE antibodies [12,13]. Since the three-dimensional structures of α-lactalbumin [14] (Fig. 1) and β-lactoglobulin [15] (Fig. 2) are known and the allergenic sites have already been predicted, the other proteins such as lactoferrin and lactoperoxidase which are present in large concentrations in milk [16] may be structurally compared to determine the possible allergenicity in these proteins. The most clearly understood allergenic sites in α-lactalbumin and β-lactoglobulin have been indicated in Figs. 1 and 2, respectively. As observed from Figs. 1 and 2, the conformations of the allergenic loops appear to be very similar in these proteins suggesting a characteristic conformation for the allergenic sites in the proteins. The loops are well defined as these are held tightly like a string at the two ends by antiparallel strands in both proteins. The least-squares fit for the allergenic segments of these proteins gives an r.m.s. difference of 1.0 Å (Fig. 3). Based on these structural data, we have searched for the similar sites in other milk proteins. As stated earlier, another well known protein in milk is lactoferrin whose structure is known from various species [17–22]. As a result

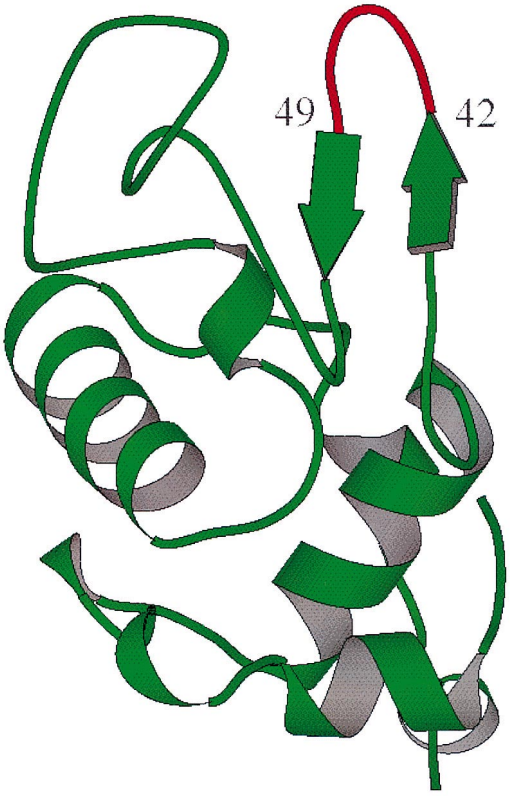

Fig. 1. Ribbon diagram of α-lactalbumin drawn using MOLSCRIPT [26]. The allergenic site (Val 42–Giu 49) is shown in red colour.

of a detailed comparison with allergenic segments from known allergens, two sites have been identified in lactoferrin, one each in N- and C-lobes. The superpositions consisting of eight residues these sites in lactoferrin with the corresponding residues in α-lactalbumin and β-lactoglobulin give the r.m.s differences of 0.7 Å (Fig. 4) and 0.8 Å (Fig. 5), respectively. The results of this analysis clearly suggest that the lactoferrin possesses at least two allergenic sites which are widely separated (Fig. 6).

4. Abolishment of allergy

There are many of allergenicity being reduced, but not eliminated by heating but heat-denatured proteins can also present new antigenic sites, uncovered by

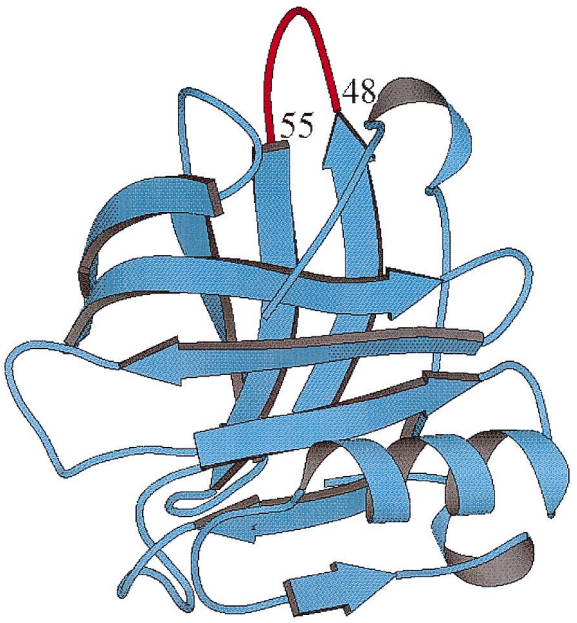

Fig. 2. Ribbon diagram of β-lactoglobulin drawn using MOLSCRIPT [26]. The allergenic loop (Pro 48–Glu 55) is indicated in red colour.

the unfolding process or created by new chemical reactions with other molecules present in the food (e.g. β-lactoglobulin associating with α-lactalbumin in milk) [23]. Heat-denatured β-lactoglobulin has been found to have at least one new epitope, not found in the native state [24]. Heating milk does not

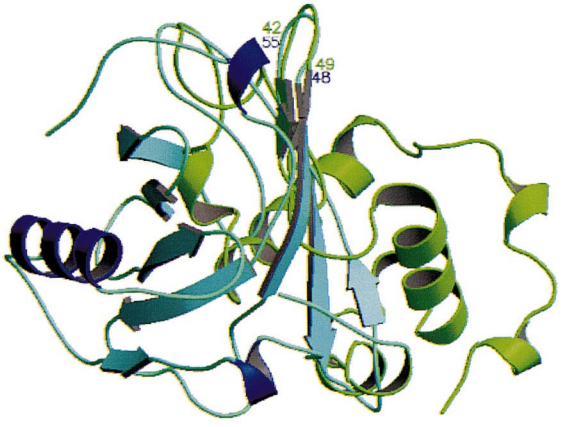

Fig. 3. The superposition of allergenic loop in α-lactalbumin (green) on the corresponding loop in β-lactoglobulin (grey). The figure was drawn using MOLSCRIPT [26].

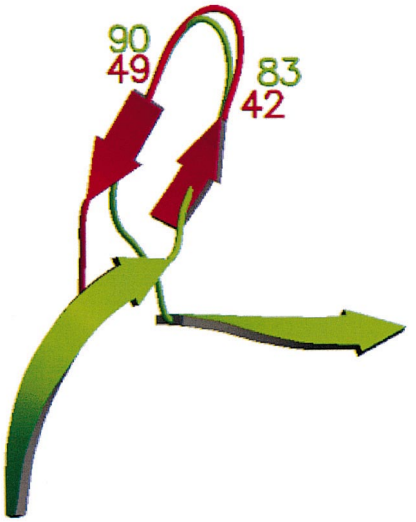

Fig. 4. Superposition of allergenic loop of α-lactalbumin (green) on the allergenic loop of lactoferrin N-lobe (red).

necessarily reduce allergenicity to its component proteins [6]. However, in another study, whey that had received severe heat treatment failed to sensitize and did not elicit anaphylaxis when injected into animals. These results suggest that heat denaturation of whey protein may be a logical and simple strategy of producing milk with reduced allergenicity.

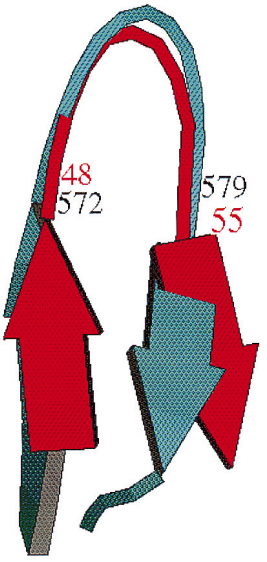

Fig. 5. Superposition of allergenic loop of β-lactoglobulin (grey) on the allergenic loop of lactoferrin in C-lobe (red).

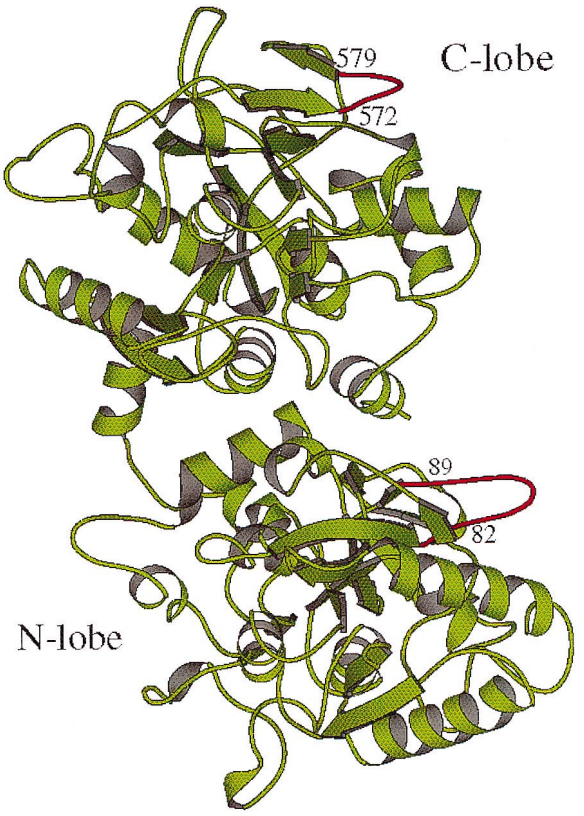

Fig. 6. Ribbon diagram of buffalo lactoferrin showing N- and C-lobes. The allergenic loops in N-lobe (Gly 83–Thr 90) and C-lobe (Leu 572–Lys 579) have been indicated in red colour.

5. Recombinant allergens

A possible solution to avoid milk allergy is the production of recombinant allergens [see chapter on Recombinant allergens in this volume]. These recombinant allergens and their respective natural counterparts possess comparable properties with respect to structure, function and interaction with the immune system. Recent studies documented that in vitro as well as in vivo diagnosis of IgE-mediated allergic diseases can be successfully improved by the application of recombinant allergens. Recombinant allergens, produced by side-directed mutagenesis with modified amino acid sequences show very low IgE binding capacity but strong T cell-stimulatory activity and represent a possible candidate for a safe and effective immunotherapy.

6. Milk hydrolysates

Milk hydrolysates, although frequently used as substituted in case of milk allergy, show a reduced but never a complete abolishment of antigenicity and allergenicity. In order to determine the lower molecular mass limit of peptides to elicit skin reactions and to bind IgE antibodies in vitro, Van Hoeyveld et al. in 1998 [25,26] fractionated an ultrafiltrated whey hydrolysate using FPLC, in different molecular mass fractions. Skin-prick tests were performed with the hydrolysates and its fractions in five milk allergic children, and RAST inhibition test were done using the serum of these children. On the basis of the lowest extinction values between two peaks of the chromatogram, seven fractions with molecular masses between 15 000 and 125 Da were obtained. Peptides of >2600 Da elicited a clearly positive skin reaction and inhibited IgE-binding, which peptides of >1400 Da did not give any positive skin reaction but were still able to inhibit to a small extent IgE-binding to the hydrolysate. These findings suggested that for skin reactivity, peptides of >1400 Da were needed. The minimal molecular mass for IgE binding in vitro appears to be situated between 1400 and 970 Da. Such peptides might be used to develop a safe formula for patients reaction to milk hydrolysates or even for tolerance induction.

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