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3-D Model of the bee venom acid phosphatase: Insights into allergenicity

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ABSTRACT

The acid phosphatase Api m 3 is the major allergen of the honeybee venom. Except for the amino acid sequence, no other structural information for the enzyme is available. We applied homology modeling to assign the three-dimensional structure of Api m 3. The structure of the homodimeric human prostatic acid phosphatase was used to model the Api m 3 tertiary structure. IgE epitopes and antigenic sites were predicted using programs based on the structure of known epitopes and analysis of the 3-D model. The model of Api m 3 revealed an active site similar to those of the histidine—type acid phosphatases with conservation of the catalytically important residues. The observed substitutions in the phosphate ion binding site suggest differences in the substrate specificity in comparison to other acid phosphatases. The analysis of the Api m 3 three-dimensional model revealed a very likely mechanism of enzyme action.

Honeybee (*Apis mellifera*) venom has opposite effects on the human body. On one side it creates strong allergic reactions on hypersensitive humans, often leading to potentially deadly anaphylactic shock. On the other, the venom is used in pharmaceutical products such as tablets, creams, ointments, injection forms etc. for medical purposes (http://en.wikipedia.org/wiki/Bee_venom_therapy). Bee venom therapy was named "Apitherapy". The application in rheumatology, for arthritis and chronic pain has been known for a long time [1–3]. The major allergen in the honeybee venom is an acidic phosphatase, Api m 3 (orthophosphoric monoester phosphohydrolase, EC 3.1.3.2). It belongs to a group of enzymes hydrolysing phosphomonoesters at acidic pHs. While the structure, function, and substrate specificities of animal, plant and bacterial acid phosphatases have been intensively studied [4–10], relatively little is known about Api m 3. The molecular mass of the enzyme is 43.9 kDa and the isoelectric point is 5.64 [11]. The complete amino acid sequence of the polypeptide chain consists of 373 residues with four potential N-linked carbohydrate sites [11,12]. The natural substrate, biological role and structure-function relationships of Api m 3 have yet to be established. The purified honeybee venom phosphatase exists in two molecular weight forms (45 000 Da for the monomer and 96 000 Da for the homodimer) [13]. It is a potent allergen which is capable of releasing histamine from sensitized human basophils [13]. Api m 3 is a glycoprotein like the hyaluronidase and the phospholipase A_2 from the same venom [14]. Recently, Api m 3 was cloned from venom gland cDNA and expressed in eukaryotic insect cells [15].

An experimental three-dimensional structure of Api m 3 has not yet been determined. We exploited the catalytic and sequence similarity between Api m 3 and the human acid phosphatase to build a model of the honeybee venom enzyme. The model and sequence analysis were used to understand the structure and function of the enzyme and to predict epitopes for IgE binding as well as antigenic binding sites. Predicting methods have gained much interest and, at present, modeling by homology is a reliable method [16]. The results of the present work can be used for site-directed mutagenesis. Allergen engineering is a suitable tool for immunotherapy of allergy.

Materials and methods

Construction of a homology model of Api m 3. The sequence alignment technique BLAST [17] was applied to search for primary structure similarities between Api m 3 and other proteins. A homology homodimeric model, based on the cDNA deduced sequence of the honeybee venom enzyme, was constructed using the crystallographic C_α coordinates of the human prostatic acid phosphatase [6] and applying the SWISS-MODEL server [18]. The stereochemistry of the model was checked by the program PROCHECK [19]. The surface accessibility was calculated by the program AREAIMOL [20,21]. The root mean square (rms) distance between the C_α positions of the resulting model and the human acid phosphatase was calculated by the program LSQKAB [22].

Prediction of allergenicity, IgE binding epitopes, and antigenic determinants. Prediction of allergenic proteins and mapping of IgE epitopes was performed by the method described in [23] and using the respective programs. The data-set applied for testing consists

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of 578 allergens and 700 non-allergens. Saha and Raghava [23] used protein amino acid and dipeptide compositions, a database of known IgE epitopes and BLAST search against allergen representative peptides. A sequence is predicted to be allergenic by these programs when the score is less than the threshold. Antigenic determinants of Api m 3 were predicted by the method given in [24].

Results

Comparison of the amino acid sequences of honeybee venom, human and rat acid phosphatases

The cDNA derived amino acid sequence of Api m 3 [15] was used in a BLAST search [17] and two acid phosphatases were identified showing significant sequence similarity towards the target protein: human (HPAP) and rat (RPAP) prostatic acid phosphatases with 40% homology to the honeybee venom enzyme taking into account conservative substitutions. Fig. 1 shows the alignment of the primary structures and the similarity can be followed throughout the entire sequences. There is a variation in the lengths of the polypeptide chains of Api m 3, on one side, and HPAP or RPAP, on the other. The sequences were aligned using a "truncated" form of the last enzyme with the same chain length as those of the other two phosphatases.

Building of a homology model of Api m 3 based on the amino acid sequence and using the crystal structure of the human acid phosphatase as a template: analysis of the model

Finding a template protein, related to the target protein, is the first step in homology modeling. The model of the homodimeric molecule, generated from the amino acid sequence of Api m 3 and using the dimeric structure of HPAP [6] as a template, is shown in Fig. 2. The two enzymes share common active sites and some other conserved regions. Superposition of the modeled Api m 3 on the crystallographic structure of HPAP gave an average distance between the C_α positions of 0.6 Å. Comparison of the topology of the two acid phosphatases showed considerable similarities. It is known that the 3-D protein structure is more conserved than the amino acid sequence [25]. The secondary structure elements, designated in Fig. 1, include 12 α-helices and 7 β-strands. The monomer of the modeled protein contains two domains. The larger one is α/β—type domain composed of a central 7-stranded β—sheet and helices from both sides. Four of the strands are in parallel and three in antiparallel conformation. The smaller highly helical domain contains 6 α-helices (Fig. 2). In analogy with the human phosphatase, the active site of Api m 3 was supposed to be located in an open cleft between both domains, which represents the phosphate binding site. The centre of the HPAP active site consists of Arg11, His12, Arg15, Arg79, His257, and Asp258 [6]. The same type functional groups (imidazole, carboxylic and guanidine) are present in the active site of the honeybee venom phosphatase modelled structure (Fig. 3). Asp258 of HPAP is substituted by Glu259 in Api m 3 and Arg79 by Arg83. Moreover, the septapeptide active site sequence RHGXRXP of the N-terminal region of the polypeptide chain, which is characteristic for acid phosphatases, is also conserved in Api m 3. It was supposed that during the phosphoester hydrolysis His12 interacts with the substrate phosphate group with the formation of a phosphohistidine intermediate [26 and references therein]. However, some residues in HPAP, forming one part of the active site, Ile18, Tyr215, Phe171, Trp174, Tyr182, Leu275 as well as residues from the opposite side of the cleft (Tyr123 and Arg127) are not conserved in Api m 3, some of the substitutions being radical (Fig. 1).

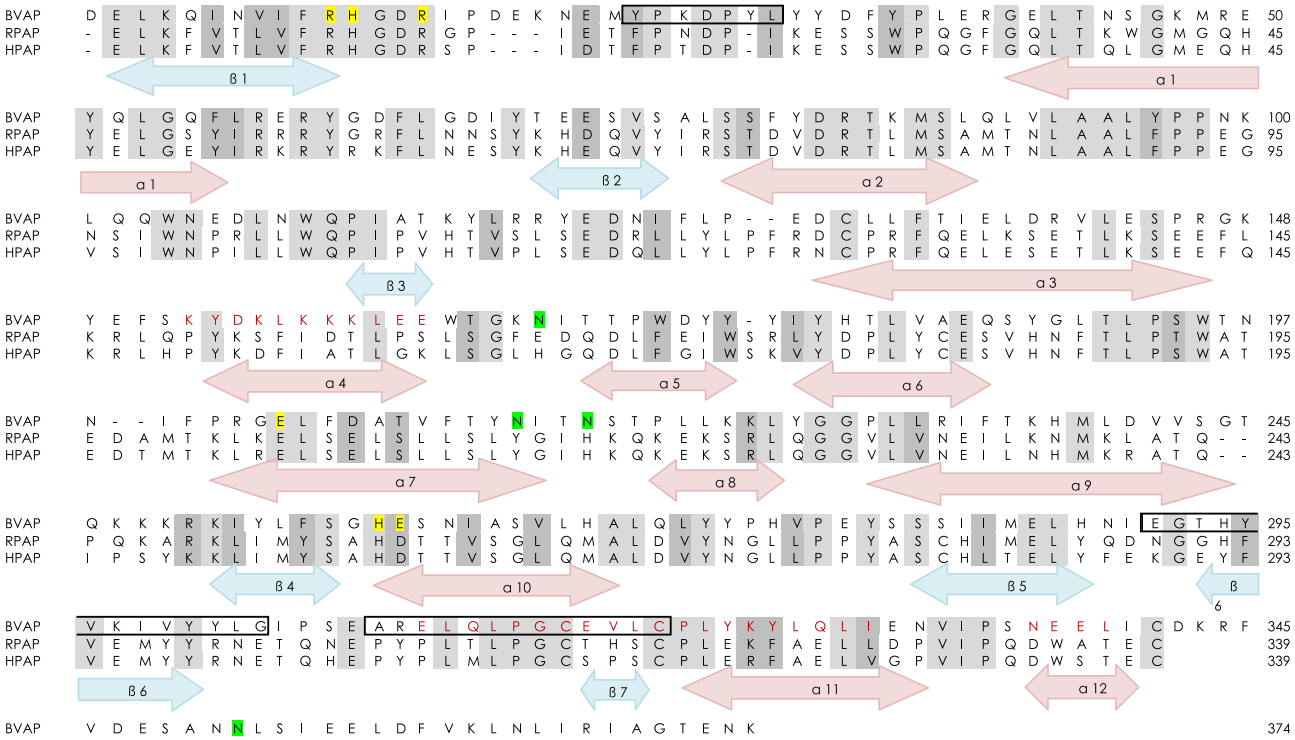

Fig. 1. Sequence comparison of the honeybee venom acid phosphatase (BVAP), rat prostatic acid phosphatase (RPAP) and human prostatic acid phosphatase (HPAP). α = α-helix; β = β-strand. Identical residues are highlighted in gray and the conservative substitutions in dark gray. The predicted allergenic determinants are labelled in red, the glycosylation sites—in green and the active site residues—in yellow. The predicted antigenic sites in BVAP are framed. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)