



Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc



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

Dessislava Georgieva^a, Kerstin Greunke^b, Nicolay Genov^c, Christian Betzel^{a,*}

^a Institute of Biochemistry and Molecular Biology, c/o DESY, Building 22a, Notkestrasse 85, 22603 Hamburg, Germany

^b Protein Ligand Structural (PLS)-Design GmbH, Eichenstrasse 42, 20255 Hamburg, Germany

^c Institute of Organic Chemistry, Bulgarian Academy of Sciences, "Akad. G. Bonchev"-str. Bl. 9, 1113 Sofia, Bulgaria

ARTICLE INFO

Article history:

Received 13 November 2008

Available online 4 December 2008

Keywords:

Honeybee venom

Allergen

Acid phosphatase

Protein modeling

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.

© 2008 Elsevier Inc. All rights reserved.

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 A₂ 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

* Corresponding author. Fax: +49 40 8998 4747.

E-mail address: Betzel@unisgi1.desy.de (C. Betzel).

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 RHGXRP 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).

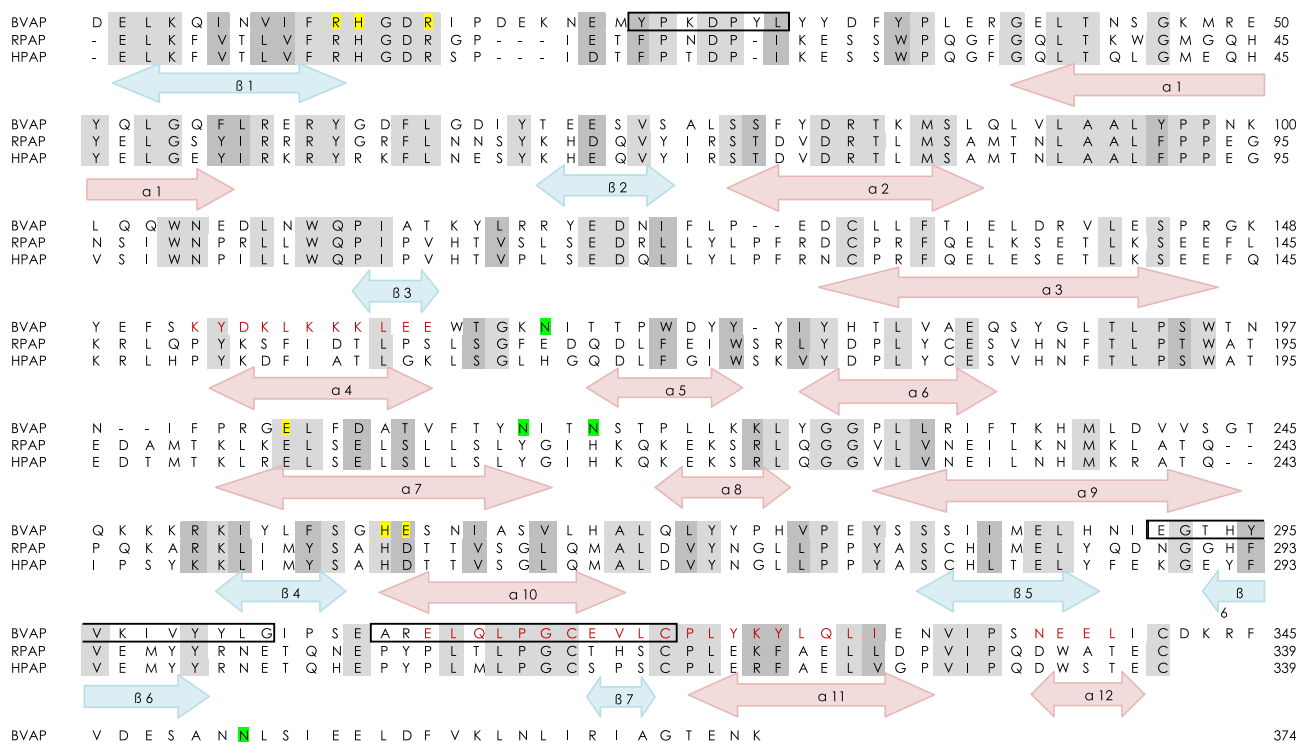


Fig. 1. Sequence comparison of the honeybee venom acid phosphatase (BVAP), rat prostatic acid phosphatase (RPAP) and human prostatic acid phosphatase (HPAP). α = α -helix; β = β -strand. Identical residues are highlighted in gray and the conservative substitutions in dark gray. The predicted allergenic 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.)

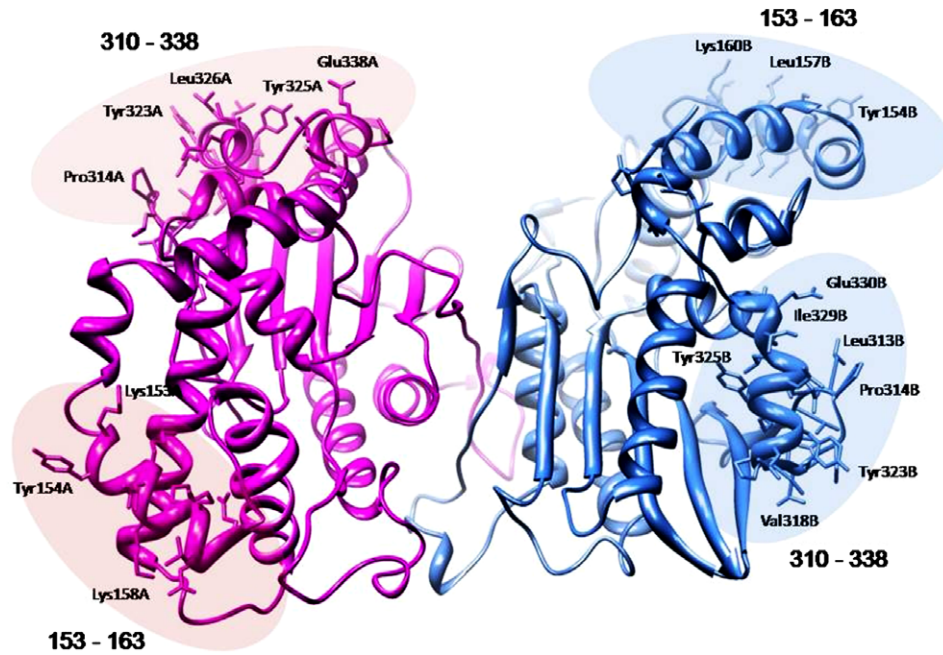


Fig. 2. Ribbon representation of the honeybee venom acid phosphatase. The predicted IgE binding sites, residues 153–163 and 310–338 are shown.



Fig. 3. View of the honeybee venom acid phosphatase active site.

Honeybee venom phosphatase exists in two molecular weight forms of 45 and 96 kDa; i.e. as a monomer and a homodimer [13]. A homodimer of Api m 3 was constructed using the dimeric molecule of HPAP as a template [6]. The accessible surface area (ASA) of the monomeric subunit is 18091 Å² and that of the dimer is 33089 Å². The complex formation decreases the ASA of the two subunits by approx. 3100 Å². The homodimer is stabilized mainly through hydrophobic interactions. The main interactions are between the β -strands Pro112–Thr115 from each monomer which are located antiparallel to each other at the interface (Fig. 2).

Prediction of allergenic determinants in the molecule of Api m 3

Regions of Api m 3 were assessed as possible allergenic determinants using the 3-D model of the phosphatase and the methods and programs of Saha and Raghava [23], integrating various approaches for predicting allergenic proteins and mapping of IgE epitopes. The same methods were used to investigate the related acid phosphatases HPAP and RPAP. The procedures based on the amino acid compositions and dipeptide analyses characterized the honeybee venom enzyme as “allergen” while the human and rat acid

phosphatases were classified as “non allergens”. Api m 3 does not contain a sequence identical to known IgE epitopes as assessed by the program. The same results were obtained with the truncated form containing residues 1–341. Examination of different parts of the polypeptide chain by the methods described in [23], showed the presence of sequences, which are potential IgE binding sites. The C-terminal extension was also classified as an allergen.

It was shown [27] that lysyl residues are essential for the binding of the immunoglobuline E to the major grass pollen allergen Phl p 5b C terminus and participate often in allergenic determinants. Inspection of the model revealed two surface regions (residues 153–163 and 246–250, respectively) containing clusters of exposed lysyl residues. The residues of the region were assessed as possible allergenic determinants by substituting with alanine. The program for dipeptide analysis [23] classified the phosphatase containing virtual mutations at residues 153 – 163 as “non allergen”. According to the same program the non-substituted enzyme is an “allergen”. The segment Lys153–Glu163 contains the sequence XEEY which is characteristic for 7 human IgE binding epitopes of the major latex allergen Hev b 5 [28]. Two or more glutamic acid residues participate in all 11 immunoglobuline E-binding regions of the allergen mentioned above [28] which indicates the important role of this amino acid residue as a constituent of allergen determinants. The programs predicting allergenicity did not confirm a participation of the sequence Gln246–Arg250 in IgE binding epitopes. The Api m 3 molecule with “substituted” by alanines residues at the C-terminal part of the polypeptide chain (res. 310–329) was classified by the program for dipeptide analysis [23] as “non-allergen”. Combination of the results from the application of the programs mentioned above and inspection of the 3-D model predicted three IgE binding regions: res. 153–163, 310–329 and 336–339.

Prediction of antigenic determinants on the Api m 3 structure

The method of Kolaskar and Tongaonkar [24] for the prediction of linear antigenic determinants, based on the structure of experimentally determined peptides, predicted 14 epitopes in the honeybee venom enzyme. The most likely antigenic epitopes were chosen following the rules of MIF Bioinformatics, Molecular Immunology Foundation (<http://immunax.dfci.harvard.edu/Tools/antigenic.html>), namely: the antigenic peptides to be accessible to the solvent, to contain both hydrophobic and hydrophilic residues, and preferably to be in loops, avoiding helical regions. We investigated the 3-D model of the honeybee venom acid phosphatase and having in mind the rules mentioned above selected the peptides Tyr24–Leu30 (exposed on the protein surface loop), Ile290–Gly303 (exposed β -strand) and Ala308–Cys320 (containing the exposed loop Gln312–Glu317) (Fig. 1) as possible antigenic determinants of Api m 3. These regions have a high antigenic propensity.

Discussion

Examination of the homology model and the cDNA deduced amino acid sequence of Api m 3 classified the enzyme as an orthophosphoric-monoester phosphohydrolase (EC 3.1.3.2) [29]. The presence of a histidyl residue at the active site of the modeled protein, His 12, which is probably phosphorylated during the catalysis, characterizes the enzyme as a member of the sub-class of “histidine acid phosphatases” [30] with a probable difference in the specificity in comparison to the human and rat acid phosphatases. The natural substrate of Api m 3 and its biological function are not known and have to be established. The N-terminal region of Api m 3 contains the sequence RHGDRIP which is conserved in the other

“histidine acid phosphatases” with variations in positions 13 and 15 (Asp 13 and Ile 15 are substituted by other amino acid residues) [31]. This sequence is involved in the binding of the substrate phosphate group [5 and references therein]. Primary structure analysis showed also other conserved sequence motifs among the honeybee venom enzyme, HPAP and RPAP (Fig. 1).

If no 3-D experimental structure of a protein is available and only its amino acid sequence is known, structural and functional insights can be obtained by homology modeling based on the known crystallographic structures of related proteins. The theoretical 3-D model provides enough information about the spatial arrangement of functionally important residues to allow a prediction with high probability of the mechanism of biological function and other properties, including prediction of IgE epitopes, antigenic determinants on protein antigens etc. The model of homodimeric Api m 3 revealed important information about the mechanism of enzymatic action and putative functional sites. The active site cleft of the modelled Api m 3 is likely to be located between the two domains. Important for the catalysis functional groups are conserved. The imidazole of His 12 and the carboxylic group of Asp 258 serve as a phosphate acceptor and a proton donor during the hydrolysis of phosphomonoester substrates by histidine phosphatases [32]. The high similarity of the catalytic sites leads to the conclusion for a common hydrolytic mechanism of the three phosphatases. However, the observed amino acid substitutions in the putative phosphate ion binding pocket, some of them radical like Arg127/Glu, suggest differences in the substrate specificity. The highly accessible and relatively large substrate-binding cleft can be occupied by various phosphomonoesters suggesting a broad specificity.

Api m 3 is the major allergen in honeybee venom [15] which is able to induce a wheal and flare reaction in humans [13]. Allergy due to the bee venom is a type I hypersensitivity of the human body. Knowledge of the structure, function and allergy determinants of potent allergens like the honeybee venom acid phosphatase is important to understand the allergen-IgE interactions which signal the basophils to produce histamine causing allergic symptoms. Usually, the allergenic proteins contain a number of epitopes and the honeybee venom phosphatase is not an exception. At least several IgE binding regions were predicted in Api m 3 using the programs of Saha and Raghava [23]. Two of them (res. 153–163 and 336–339) contain the sequence XEEY which is present also in six human IgE binding regions of the allergen Hev b 5 [28]. Moreover, such sequences are present in the rabbit IgG binding epitopes of the same allergen [33]. The other sequence XEEY in the polypeptide chain of the honeybee venom phosphatase (residues 70–73) is identical to that of the human IgE binding regions 5.2 and 5.6 of the major latex allergen [28]. However, this sequence can not serve as a 3-D binding region of IgE antibody due to the limited accessibility to the solvent. The segment Asn336–Leu339 is exposed on the protein surface of the homodimer and can be predicted as a part of epitope. The 3-D model of the homodimer showed also that the other large C-terminal region (residues 310–329) predicted as IgE binding site, involves the loop Gln312–Glu317 and the α -helix Pro321–Asn331 which are exposed on the surface of the two subunits (Fig. 2). This region is a suitable candidate for IgE epitope. The regions of Api m 3 predicted to be determinants of allergy (Figs. 1 and 2) are located on the surface of the protein molecule, in positions suitable for interaction with other molecules such as IgE.

The allergenic Api m 3 contains a C-terminal extension of 33 residues in comparison with the non-allergenic phosphatases HPAP and RPAP. The programs used for prediction of allergenicity classified this region as “allergen”. It contains also the sequence XEEY (res. 355–358) characteristic for a number of IgE epitopes

[28]. The extension can play a role in the interaction between the honeybee acid phosphatase and IgE.

The program of Kolaskar and Tongaonkar [24] predicted 14 peptides from the polypeptide chain of the honeybee venom phosphatase as possible linear antigenic determinants. However, only three of them (res. 24–30, 290–303, and 308–320, Fig. 1) cover the criteria for the antibody binding regions and 3-D epitopes. Some of the predicted sequences of the Api m 3 molecule are situated in the region of the dimer interface (Pro 25–Pro 36; Met 85–Asn 98, and Pro 111–Arg 118). Other are not enough accessible to the solvent and can not play the role of 3-D antigen binding sites. All three antigenic sites, predicted by the method mentioned above and using the 3-D model, contain exposed hydrophobic residues and are in position suitable for binding antibodies.

Api m 3 is an important component and major allergen of the honeybee venom. The model revealed a very likely mechanism of hydrolytic action and putative functional sites. Serious anaphylactic-like reactions can occur to patients after honeybee stings but the allergenic and antigenic determinants of the acid phosphatase have not yet been experimentally determined. The data about the predicted epitopes can be used for site-directed mutagenesis experiments of Api m 3 mutants with reduced IgE binding capacity. Mutants with reduced allergenicity are a suitable tool for the allergen-based immunotherapy [34]. The predicted antigenic sites can be used for the design of immunological experiments.

Acknowledgments

D.G. and N.G. thank the PLS-Design GmbH, Hamburg, Germany, for the financial support. This work was also supported by the Deutsche Forschungsgemeinschaft via the Project BE 1443/18-1. Ch. B. acknowledges the RIS Project for providing financial support.

References

- [1] F. Forestier, M. Palmer, Bee venom in rheumatology—an experiment performed with 1600 cases, *Apiacta* 19 (1984) 19–22.
- [2] C.M. Kim, Bee venom therapy for arthritis, *Z. Rheumatol.* 41 (1989) 67–72.
- [3] D.K. Klinghardt, Bee venom therapy for chronic pain, *J. Neurol. Orthopedic MedSurg.* 11 (1990) 195–197.
- [4] M.C. Thaller, S. Schippa, G.M. Rossolini, Conserved sequence motifs among bacterial, eukaryotic, and archaeal phosphatases that define a new phosphohydrolase superfamily, *Protein Sci.* 7 (1998) 1647–1652.
- [5] G. Schneider, Y. Lindqvist, P. Vihko, Three-dimensional structure of rat acid phosphatase, *EMBO J.* 12 (1993) 2609–2615.
- [6] C.G. Jakob, K. Lewinski, R. Kuciel, W. Ostrowski, L. Lebiada, Crystal structure of human prostatic acid phosphatase, *The Prostate* 42 (2000) 211–218.
- [7] A.R. Hayman, Tartrate-resistant acid phosphatase (TRAP) and the osteoclast/immune cell dichotomy, *Autoimmunity* 41 (2008) 218–223.
- [8] V. Calderone, C. Forleo, M. Benvenuti, M.C. Thaller, G.M. Rossolini, S. Mangani, A structure-based proposal for the catalytic mechanism of the bacterial acid phosphatase AphA belonging to the DDDD superfamily of phosphohydrolases, *J. Mol. Biol.* 355 (2006) 708–721.
- [9] V. S. Reddy, A. K. Singh, R. Rajasekharan, The *Saccharomyces cerevisiae* PHM8 gene encodes a soluble magnesium dependent lysophosphatidic acid phosphatase, *J. Biol. Chem.* PMID (2008) 18234677.
- [10] G. Schenk, T.W. Elliot, E. Leung, L.E. Carrington, N. Mitic, L.R. Gahan, L.W. Guddat, Crystal structures of a purple acid phosphatase, representing different steps of this enzyme's catalytic cycle, *BMC Struct. Biol.* 8 (2008) 6.
- [11] D.R. Hoffman, Hymenoptera venom allergens, *Clin. Rev. Allergy Immunol.* 30 (2006) 109–128.
- [12] D.R. Hoffman, E.T. Weimer, R.H. Sakell, M. Schmidt, Sequence and characterization of honeybee venom acid phosphatase, *J. Allergy Clin. Immunol.* 115 (2005) S107.
- [13] E. Barboni, D.M. Kemeny, S. Campos, C.A. Vernon, The purification of acid phosphatase from honey bee venom (*Apis mellifica*), *Toxicon* 10 (1987) 1097–1103.
- [14] L. März, C. Kühne, H. Michl, The glycoprotein nature of phospholipase A2, hyaluronidase and acid phosphatase from honey-bee venom, *Toxicon* 21 (1983) 893–896.
- [15] T. Grunwald, B. Bockisch, E. Spillner, J. Ring, R. Bredehorst, M.W. Ollert, Molecular cloning and expression in insect cells of honeybee venom allergen acid phosphatase (Api m 3), *J. Allergy Clin. Immunol.* 117 (2006) 848–854.
- [16] C. Venclovas, M. Margelevicius, Comparative modeling in CASP6 using consensus approach to template selection, sequence-structure alignment, and structure assessment, *Proteins* 61 (S7) (2005) 99–105.
- [17] S.F. Altschul, W. Gish, W. Miller, W. E.W. Myers, D.J. Lipman, Basic alignment search tool, *J. Mol. Biol.* 215 (1990) 403–410.
- [18] M.C. Petisch, Computer modelling of biological molecules, *Biochem. Soc. Trans.* 24 (1996) 274–279.
- [19] R.A. Laskowski, M.W. MacArthur, D.S. Moss, J.M. Thornton, PROCHECK: a program to check the stereochemical quality of protein structures, *J. Appl. Crystallogr.* 26 (1993) 283–291.
- [20] B. Lee, F.M. Richards, The interpretation of protein structures: estimation of static accessibility, *J. Mol. Biol.* 55 (1971) 379–400.
- [21] Collaborative Computational Project, Number 4, The CCP4 Suite: program for protein crystallography, *Acta Crystall.* D50 (1994) 760–763.
- [22] W. Kabsh, A solution for the best rotation to relate two sets of vectors, *Acta Crystall.* A32 (1976) 922–923.
- [23] S. Saha, G.P.S. Raghava, AlgPred: prediction of allergenic proteins and mapping of IgE epitopes, *Nucleic Acids Res.* 34 (2006) W202–W209.
- [24] A.S. Kolaskar, P.C. Tongaonkar, A semi-empirical method for prediction of antigenic determinants on protein antigens, *FEBS Lett.* 276 (1990) 172–174.
- [25] M.A. Marti-Renom, A.C. Stuart, A. Fiser, R. Sanchez, F. Melo, A. Sali, Comparative protein structure modelling of genes and genomes, *Annu. Rev. Biophys. Biomol. Struct.* 29 (2000) 291–325.
- [26] K. Ostanin, E.H. Harms, P.E. Stevis, R. Kuciel, M.-M. Zhou, R.L. Van Etten, Overexpression, site-directed mutagenesis, and mechanism of *Escherichia coli* acid phosphatase, *J. Biol. Chem.* 267 (1992) 22830–22836.
- [27] K. Gehlhar, K.R. Rajashankar, E. Hofmann, Ch. Betzel, W. Weber, S. Werner, A. Bufer, Lysine as a critical amino acid for IgE binding in Phl p 5b C terminus, *Int. Arch. Allergy Immunol.* 140 (2006) 285–294.
- [28] D.H. Beezhold, V.L. Hickey, G.L. Sussman, Mutational analysis of the IgE epitopes in the latex allergen Hev b 5, *J. Allergy Clin. Immunol.* 107 (2001) 1069–1076.
- [29] E.J. Mullaney, A.H.J. Ullah, Identification of a histidine acid phosphatase (phy A)-like gene in *Arabidopsis thaliana*, *Biochem. Biophys. Res. Commun.* 251 (1998) 252–255.
- [30] R.L. Van Etten, Human prostatic acid phosphatase: a histidine phosphatase, *Ann. N. Y. Acad. Sci.* 390 (1982) 27–51.
- [31] R.L. Van Etten, R. Davidson, P.E. Stevis, H. MacArthur, D.L. Moore, Covalent structure, disulfide bonding, and identification of reactive surface and active site residues of human prostatic acid phosphatase, *J. Biol. Chem.* 266 (1991) 2313–2319.
- [32] K.S. Povari, A.M. Herrala, R.M. Kurkela, P.A. Taavitsainen, Y. Lindqvist, G. Schneider, P.T. Vihko, Site-directed mutagenesis of prostatic acid phosphatase. Catalytically important aspartic acid 258, substrate specificity and oligomerization, *J. Biol. Chem.* 269 (1994) 22642–22646.
- [33] D.H. Beezhold, V.L. Hickey, J.E. Slater, G.L. Sussman, Human IgE-binding epitopes of the latex allergen Hev b 5, *J. Allergy Clin. Immunol.* 103 (1999) 1166–1172.
- [34] P. Rabjohn, C.M. West, C. Connaughton, H.A. Sampson, R.M. Helm, A.W. Burks, G.A. Bannon, Modification of peanut allergen Ara h 3: effects on IgE binding and T cell stimulation, *Int. Arch. Allergy Immunol.* 128 (2001) 15–23.