

Modelling of Bet v 1 Binding to Lipids

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Abstract

As birch pollen allergen enters epithelium of allergic patients via lipid rafts and caveola we began to analyse its putative amphiphilic and lipid ligands on atomic level using molecular modelling and computational ligand docking. We carry out 3D modelling docking with both experimentally verified Bet v 1 ligands as well as larger lipid molecules for which experimental affinity studies were not available. The results suggest that the hydrophobic cavity of Bet v 1 has different binding sites for different ligands and groups of ligand type-specific amino acids can be defined. Bet v 1 proteins may also be able to bind and transport more complex amphiphilic molecules like ceramides and sphingomyelins known to be enriched on caveolae/lipid rafts. Furthermore, the suggested binding mode, where the hydrophobic tail groups of lipids locate inside Bet v 1, while the polar head group may remain solvent accessible, would allow Bet v 1 to bind glycolipids, e.g. gangliosides, also rich on caveolae/lipid rafts. Taken together, this *in silico* work suggests that Bet v 1 bind to amphiphilic and lipid ligands present on the caveolae/lipid rafts and thus could provide a molecular mechanism for the pollen entry to epithelial tissue of allergic patients.

Introduction

Type I hypersensitivity, i.e. acute allergic reactions, is caused by allergens cross-linking with specific anti-IgE molecules on mast cells in immunologically sensitized patients. Most of the previous work in type I pollen allergies has focused on aberrant immunoresponses [1–4]. Bet v 1 proteins from *Betula verrucosa* is the main allergenic component causing the birch pollen allergy [3–5]. We have shown caveolar-dependent uptake and transport of pollen allergen Bet v 1 not only in respiratory epithelium from allergic patients but their healthy controls [6, 7]. As lipid is a major constituent of these caveola/lipid rafts we began to analyse the interactions of Bet v 1 and its putative lipid ligands in atom level with molecular modelling and computational ligand docking.

Bet v 1 family consist of small 17-kDa proteins which are coded by 13 gene loci and are expressed as over 20 different isoforms [8]. Several allergenic as well as non-allergenic Bet v 1 isoforms have been characterized and even the structure of Bet v 1/IgE complex has been solved [9]. Cross-allergenic proteins from several other plants are also known. However, fairly little is known about the actual biological function of the Bet v 1 proteins. Bet v 1 belongs to the family of *pathogenesis-related proteins 10* (PR-10) which are expressed in several plants

in stress conditions like pathogen infection or cold. Bet v 1 proteins were first reported to have some ribonuclease activity [10], but more recently these proteins have been shown to have affinity for a wide section of amphiphilic molecules including cytokinins, sterols and fatty acids [11, 12]. They are also able to interact and associate with lipid vesicles which induce large conformational rearrangements to the structure of Bet v 1 [13].

The structure of Bet v 1 has been determined by X-ray crystallography and nuclear magnetic resonance (NMR) [9, 12, 14, 15] and it consists of two short and one long alpha-helix and one 7-stranded beta sheet that wraps around the long C-terminal alpha helix. The most characteristic feature in the Bet v 1 structure is the large hydrophobic cavity inside the protein. In the Bet v 1 NMR structures the volume of the cavity varies between 557 and 930 Å³. The cavity is not completely closed but three tunnels connect the cavity to the surface of protein (the tunnels are referred as P, H1 and H2). In most of experimentally determined Bet v 1 structures, the cavity was filled with water and only in one case a ligand, deoxycholate that was used in a sample preparation, was found in the cavity [12].

Sequence region Glu45-Lys54 is strongly conserved among Bet v 1 and other closely related PR-10 proteins. This region contains a P-loop motif (GXGGXG) which

has been found to form many nucleotide binding proteins [16]. This sequence region is located on the surface of the protein forming a loop linking the first two strands of the beta sheet. It also locates next to one of the tunnels leading to the hydrophobic cavity (the P tunnel). Interestingly, the amino acids forming the hydrophobic cavity are not as strictly conserved [12].

The Bet v 1 proteins have remarkably high structural similarity to the StAR-related lipid-transfer (START) domains [17] which are able to associate to the lipid membranes [18]. Even though Bet v 1 shows no sequence similarity to the START domains the analogous secondary structural elements between the two protein types can be easily determined. The START domains act as lipid or sterol binding and transfer units in several proteins. Experimentally determined structures exist for several START domain containing proteins, such as phosphatidylcholine transfer protein (PCTP) [19] and MLN64 [20]. Like Bet v 1 proteins, also START domains are able to associate to the lipid membranes [18].

In this study we have used molecular modelling and computational ligand docking to get an insight to the interactions of Bet v 1 and its ligands at the atom level. In addition to the molecules whose binding has been experimentally shown, we have docked also larger lipid molecules for which experimental affinity studies are not available. The results suggest that the hydrophobic cavity of Bet v 1 has different binding sites for different kinds of ligands, and groups of ligand type-specific amino acids can be defined. The results further suggest that Bet v 1 proteins may be able to bind and transport more complex amphiphilic molecules like ceramide and sphingomyelin. This result has an interesting link to our recent observation showing that respiratory epithelial cells and tissue from birch pollen allergic patients but not from healthy controls take up pollen allergen Bet v 1 in a caveolae-dependent manner [6, 7].

Material and methods

The ligand docking was performed using the CDOCKER algorithm [21] included in the Discovery Studio molecular modelling program, (Accelrys Inc, San Diego, CA, USA). The CDOCKER algorithm is based on the CHARMM force field engine. In the beginning of the docking procedure, a set of alternative ligand conformations was created by running a short molecular dynamics (MD) simulation for the ligand model and a set of conformations from the simulation trajectory was picked. Then each of the ligand conformations was positioned into the selected docking site of the receptor protein. Random rotations were made for the ligand and the amount of bad van der Waals contacts was evaluated. If the van der Waals energy was better than a given threshold value, a number of subsequent simulated annealing MD simulations were run for the

ligand–receptor complex. The receptor was kept fixed during the simulation and a soft-core energy function was used instead of normal Lennard-Jones type van der Waals function. The simulated annealing protocol combined with soft-core potentials allowed effective sampling of the conformational space around the binding site. After the simulated annealing phase, the ligand conformation was optimized using Lennard-Jones type van der Waals function. The resulting set of different ligand conformations was finally ranked based on the interaction energy between the ligand and receptor.

This normal CDOCKER procedure described above was used for testing and for small molecules. We noticed that the selection of the initial docking site had a significant impact to the results for large ligands. To circumvent this effect we modified the procedure so that instead of positioning the ligand conformation always to the same starting point we added up to 8 Å randomness to the initial docking position. Another modification made to the basic CDOCKER procedure was that after the run the best-ranking conformations were optimized together with those receptor residues, which was located closer than 5 Å from the ligand. After this the structures were ranked again using the interaction energy between the ligand and the protein.

The parametrization of the protein and the ligand models was carried out using the CHARMM27 and CHARMM all atom force field included in the Discovery Studio 2.0 program. CHARMM27 force field was used for those ligands for which the parameterized templates were available [cholesterol, 1,2 dilauroyl-DL-phosphatidylethanolamine (DLPE), 1-palmitoyl-2-oleoyl-SN-glycero-3-phosphatidylethanolamine (POPE), 1,2 dimiristoyl-SN-glycero-3-phosphorylcholine (DMPC), deoxylysophosphatidylcholine (LPPC)]. For those ligands for which the well-parameterized templates were not available we used the CHARMM force field and the automatic parametrization tools of the Discovery Studio 2.0 program. In these cases the ligands were first sketched and parameterized with the Discovery Studio program. Thereafter the partial charges were modified to correspond the values used in the lipid models of CHARMM27 force field. For Bet v 1 protein the docking was carried out for all 20 NMR structures included in the protein database (PDB) entry 1btv and for a structure that was derived from the Bet v 1-deoxycholate complex (PDB entry 1fm4) [22]. Thus for each ligand 21 parallel docking experiments were performed to take account the flexibility of the protein.

Results

Testing the docking procedure

In only few studies, the binding mode of cholesterol has been predicted using molecular modelling [20, 23].

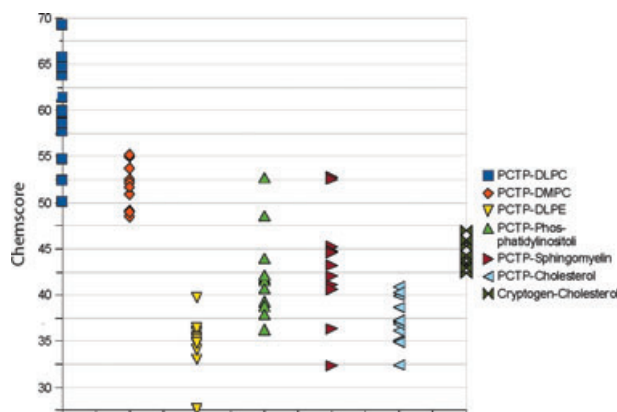


Figure 1 The Chemscore results for cryptogeen cholesterol and several PCTP-ligand complexes. The figure displays Chemscore values for the 10 structures that were ranked best by the CDOCKER method.

CDOCKER was chosen as the main docking tool in this study as we anticipated the force field based approach to provide the best results for flexible ligands [24]. To evaluate the method we redocked lipids into two known protein–lipid complexes: cryptogeen (PDB entry 1lri) [25] and PCTP (PDB entry 1ln1) [19]. We tested several methods to rank the resulting conformations (including generalized Born energies, ChemA score scoring function and astex statistical potential (ASP) scoring function). The best results were obtained when the structures were ranked using the force field based protein–ligand interaction energy. Cryptogeen is a small 10 kDa sterol carrier protein. PDB entry 1lri [25] contains a high resolution structure of this protein complexed with cholesterol. Our redocking experiment with CDOCKER produced very good results (Fig. 1). All the 10 best cholesterol conformations had less than 1.5 Å RMS deviation compared to the crystal structure.

Phosphatidylcholine transfer proteins promoting phosphatidylcholine transfer belong to the StAR protein

family and they are structurally very similar to Bet v 1 [26]. PCTP binds specifically phosphatidylcholines with the internal cavity. For the docking experiments we used the PCTP-1,2-dilinoleoyl-SN-glucero-3-phosphocholine (DLPC) complex (PDB entry: 1ln1). Several CDOCKER runs were carried out with slightly different sampling parameters (Table 1, Fig. S1). In most of the cases DLPC occupied the same space in the PCTP binding site as the native structure.

The fatty acid tails of DLPC conformations occupied the same space as in the crystal structure but adopted large distribution of different orientations so that the heavy atom required minimum distribution values were compared with the crystal structure varied between 3 and 6 Å. This docking experiment showed that CDOCKER was able to find reasonable ligand conformations for those parts of the lipid where a specific experimentally verified structural binding site existed.

Bet v 1 docking results

After showing the applicability of the available methods we docked a wide variety of ligands to the hydrophobic cavity of Bet v 1 (Table 2). Experimental information about the binding affinities was available for only a part of these putative ligands.

The only putative Bet v 1 ligand for which a crystal structure was available was deoxycholate, a bile acid structurally related to cholesterol. When deoxycholate was docked to Bet v 1, the 10 best-ranked ligand conformations had the same general orientation and location as the crystal structure with the C-3 bound hydroxyl group pointing inwards and the side chain carboxyl group interacting with the C-terminal alpha helix (Fig. 2A, B). The heavy atom root mean square (RMS) deviations compared with the crystal structure were, however, relatively large, i.e. 2.6–4.8 Å, but here the crystal and docking were generated with different Bet v 1 isoforms.

Rank	Interaction energy	Heavy atom RMSD	Phosphocholine RMSD	Chemscore value and rank	ASP score value and rank
1	−172.3	6.07	1.37	63.92 (7)	71.69 (44)
2	−171.82	3.73	1.27	61.43 (13)	77.89 (4)
3*	−171.16	1.09	1.18	69.29 (1)	76.63 (9)
4	−168.87	5.9	1.23	57.84 (42)	71.01 (54)
5	−168.81	3.26	1.22	52.46 (155)	72.95 (28)
6	−168.13	6.06	1.41	64.76 (6)	73.65 (25)
7	−167.68	3.73	1.38	58.63 (34)	71.77 (41)
8	−167.27	5.82	1.19	65.78 (4)	72.9 (29)
9	−167.18	6.04	1.02	50.12 (205)	71.83 (38)
10	−166.44	5.69	1.36	54.74 (106)	69.95 (73)
11	−166.04	3.77	1.42	53.41 (134)	74.45 (21)

Table 1 Energies and RMS deviations (RMSD) of the best-ranked PCTP-DLPC complexes.

ASP, astex statistical potential; PCTP, phosphatidylcholine transfer protein; DLPC, 1,2-dilinoleoyl-SN-glucero-3-phosphocholine; RMS, root mean square deviations.

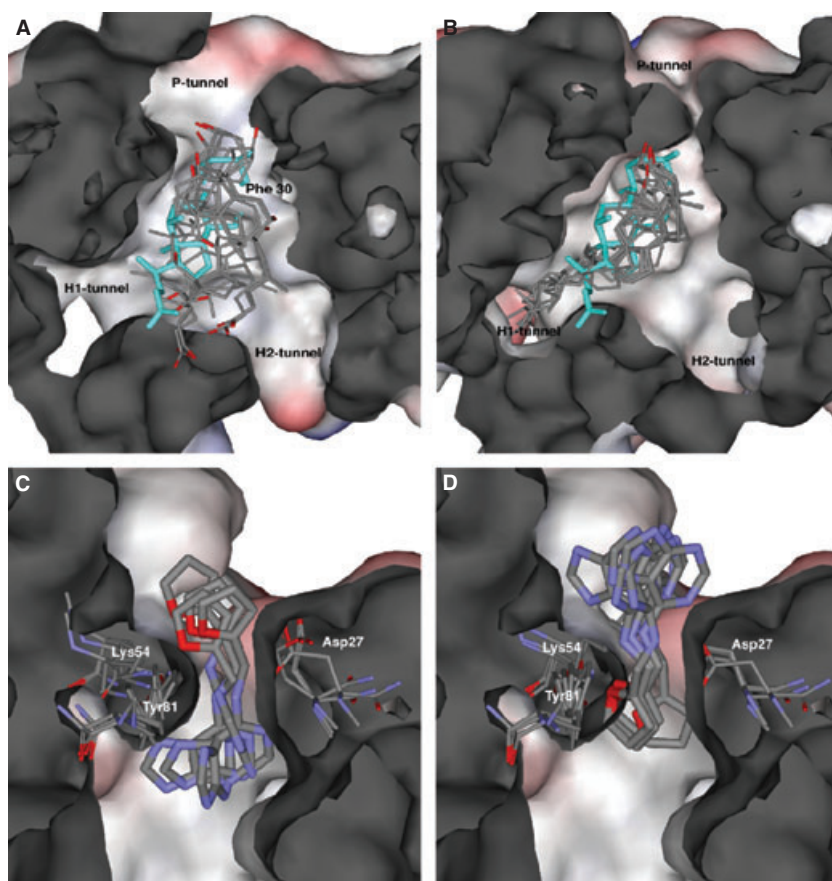
The optimized crystal structure is marked with asterisk.

Table 2 Docking results for Bet v 1 ligands.

Name	Systematic name	Interaction energy range, kcal/mol		Chemscore range		IC50 [μ M]
Deoxycholate	3 α ,12 α -Dihydroxy-5 β -cholanate	-155.1	-142.4	39.5	16.4	–
Cholesterol	Cholest-5-en-3 β -ol	-86.3	-79.2	46.4	40.0	–
Kinetin	N6-furfuryladenine	-55.5	-53.4	15.2	8.3	84
Capric acid	Decanoic acid	-73.6	-70.4	22.8	13.5	160
Palmitic acid	Hexadecanoic acid	-132.9	-127.9	36.2	19.4	7.7
Arachidic acid	Eicosanoic acid	-91.5	-83.8	37.4	18.7	27
Oleic acid	9Z-Octadecenoic acid	-151.0	-138.3	38.1	31.8	3.9
SDS	Sodium dodecyl sulphate	-102.9	-97.1	27.8	18.9	7.7
Sphingosine	Sphing-4-enine	-155.1	-135.5	31.8	23.0	–
LPPC	Deoxylyphosphatidylcholine	-182.2	-167.2	41.0	21.8	–
Ceramide	N-(octadecanoyl)-sphinganine	-142.2	-123.5	53.3	46.1	–
Sphingomyelin	N-(octadecanoyl)-sphing-4-enine-1-phosphocholine	-442.1	-343.1	52.4	13.1	–
Galactocerebroside		–	–	–	–	–
DMPC	1,2-Dimiristoyl-SN-glycero-3-phosphorylcholine	-147.2	-120.7	43.5	28.1	–
POPE	1-Palmytoil-2-oleoyl-SN-glycero-3-phosphatidylethanolamine	-135.2	-109.1	43.7	25.6	–
DLPE	1,2-Dilauroyl-DL-phosphatidylethanolamine	-163.5	-151.4	44.6	26.4	–

The table presents the force field based interaction energy range for the 10 best ligands conformations. Experimentally determined IC50 values (the indirect values) are indicated for those ligands that were studied by Mogensen *et al.* [11].

Figure 2 Deoxycholate and cholesterol docking results are presented as a thick stick model that is coloured using element based colour schema (grey = C, red = O, blue = N, orange = P). Hydrogen atoms are not displayed. The summary of the 10 best ranked (A) deoxycholate and (B) cholesterol structures are presented as thin stick models. The crystal structure of deoxycholate complexed with the one isoform of Bet v 1 is presented as cyan stick model. The figures show the summary of the five best ranking conformations where (C) purine is in the hydrophobic cavity of Bet v 1 and (D) five best ranking conformations where purine is on the surface of Bet v 1. The heavy atoms of kinetin molecules are presented as thick stick models while the Asp27, Lys54 and Tyr81 residues are presented as thin stick models. A clipped Bet v 1 surface model is presented for one Bet v 1 conformation.



The chemical structure of cholesterol was very similar to deoxycholate and thus it was not a surprise that the 10 best-ranked cholesterol structures was located in the same region as deoxycholate. The carbon atoms of the cholesterol ring structure had about 3 Å RMS deviation

compared with the corresponding atoms in deoxycholate crystal structure. This overall orientation also resembled the results that were obtained in the cholesterol-MNL64 docking study performed by Murcia *et al.* [23]. The C-3 linked hydroxyl group formed hydrogen bonds to Asp69,

Lys54 and Tyr81. The cholesterol ring structure had a large contact area with Tyr83 and Phe30 side chains. The aliphatic tail of cholesterol was located in the tunnel formed by Ser136, Met139, Val67 and Phe58 (referred here as the H1-tunnel). The Chemscore values of the best ranking cholesterol conformations varied between 40 and 46 suggesting that Bet v 1 would have nearly as high binding affinity to cholesterol as cryptogin, which was a well-established cholesterol-binding protein [25].

The Bet v 1 ligand binding studies performed by Mogensen *et al.* and Fujimoto *et al.* [11, 27] showed that kinetin (N6-furfuryladenine), a member of the cytokinin family, had moderate binding affinity for Bet v 1. These binding studies also indicated that Bet v 1 was able to bind kinetin and dehydroergosterol simultaneously without significant interference. This suggests that kinetins bind to a different site in Bet v 1 than sterols. When kinetin was docked to Bet v 1 with CDOCKER, the highest binding energies were obtained for a structure that located around the P-loop adjacent tunnel (referred here as the P-tunnel). Two different binding modes were detected among the best scoring ligand conformations: (i) conformations where the kinetin purine ring was inside the hydrophobic cavity and (ii) conformations where the purine ring was in the protein surface near the P-loop. In both cases the CH₂-NH group, joining the purine and furan rings was located in the narrow P-tunnel formed by Ile23, Leu24, Asp27, Lys54 and Tyr81.

The predicted kinetin-binding site overlapped partly with the deoxycholate/sterol-binding site. Especially, those kinetin conformations where the purine ring was located in the hydrophobic cavity significantly overlapped with the docked cholesterol conformations. Thus the fact that kinetin binding did not affect the dehydroergosterol binding would suggest that a biologically relevant binding mode would be the one where the purine ring was located in the protein surface. Also Mogensen *et al.* [13] suggested that the kinetin binding site would locate near the P-loop (Fig. 2C, D).

Bet v 1 has affinity for fatty acids and Sodium dodecyl sulphate (SDS) [13] but the atom level details of the binding modes have not been studied experimentally. We could detect in all the best-ranked conformations the negatively charged carboxyl head group that was hydrogen bonded to Lys137 and Asn118. In addition to these two amino acids, also Tyr83, Asn100, Tyr120 and Ser136 formed hydrogen bonds to the lipid head group in several cases. This head group binding site located in the vicinity of the H2-tunnel was formed by Thr7, Thr9, Lys137 and Glu141.

The docking results of SDS resembled the results of negatively charged fatty acids. In all the 10 best-ranking conformations the negatively charged sulphate group formed hydrogen bonds to Asn118 and Lys137. The two other phosphate oxygens formed hydrogen bonds to

Asn100 and Ser136 or Tyr 83 and Thr7. The aliphatic tail located in the hydrophobic cavity was pointing most frequently towards the H1 tunnel.

There is no experimental information about the binding affinity of Bet v 1 to more complex lipids like sphingolipids or glycerophospholipids and thus we performed a docking analysis with such structures (Table 2). To filter out functionally clearly artificial conformations we required that the distance between the last carbon atoms of the aliphatic tails must be less than 20 Å in length and that the centre of the mass of the lipid should not be further than 8 Å from the centre of the mass of Bet v 1.

Only few biologically important lipids have positive formal charge, of which sphingosine was used as a sample case. In all the 10 best scoring sphingosine conformations produced by CDOCKER, the positively charged ammonium group was hydrogen bonded to Asp27 in the P-tunnel region (Fig. 3). Two slightly different binding modes could be detected: in the first one the ammonium group coordinating site was the backbone carbonyl group Ile23 while in the second mode the Asp69 side chain participated in the coordination. The long aliphatic tail fitted well to the hydrophobic cavity and could adopt a rather straight conformation, pointing either towards the H1- or H2-tunnel.

In the case of ceramide, two frequently existing binding modes could be detected after the filtering. In both cases the polar head region was located in the hydrophobic cavity near the P-tunnel forming hydrogen bonds to Lys54, Asp69 and Tyr81 while the sphingosine tail was located in the H1 tunnel and the fatty acid tail in the H2 tunnel. The two binding modes differed in the orientation of the polar head group. In one binding mode, the ceramide NH was hydrogen bonded to Tyr81 side chain and the C3 bound hydroxyl group was hydrogen bonded to Asp69 (Fig. 4A). In the other binding mode, the orientation was flipped around; the C3 bound hydroxyl group was hydrogen bonded to Tyr81 and the NH group was hydrogen bonded to Asp69.

In sphingomyelin, a polar phosphocholine group is attached to the C-1 hydroxyl group of ceramide. In 5 of the 10 best-ranked ligand conformations poses the phosphocholine group located in the P-tunnel (Fig. 4C). The RMS deviation of the phosphocholine group backbone within these conformations varied between 1.6 and 4.0 Å. The phosphate group formed hydrogen bonds to Lys54 while the choline group pointed towards the Asp27 side chain. It is noteworthy that in this binding mode the phosphocholine head group fitted exactly to the P-tunnel. Further, this binding mode brought the phosphate group close to the P-loop region glycines in a way that was comparable with the phosphate locations in the P-loop containing nucleotide binding proteins. The orientation of the ceramide part of sphingomyelin resembled the second binding mode obtained by the plain ceramide docking experiment.

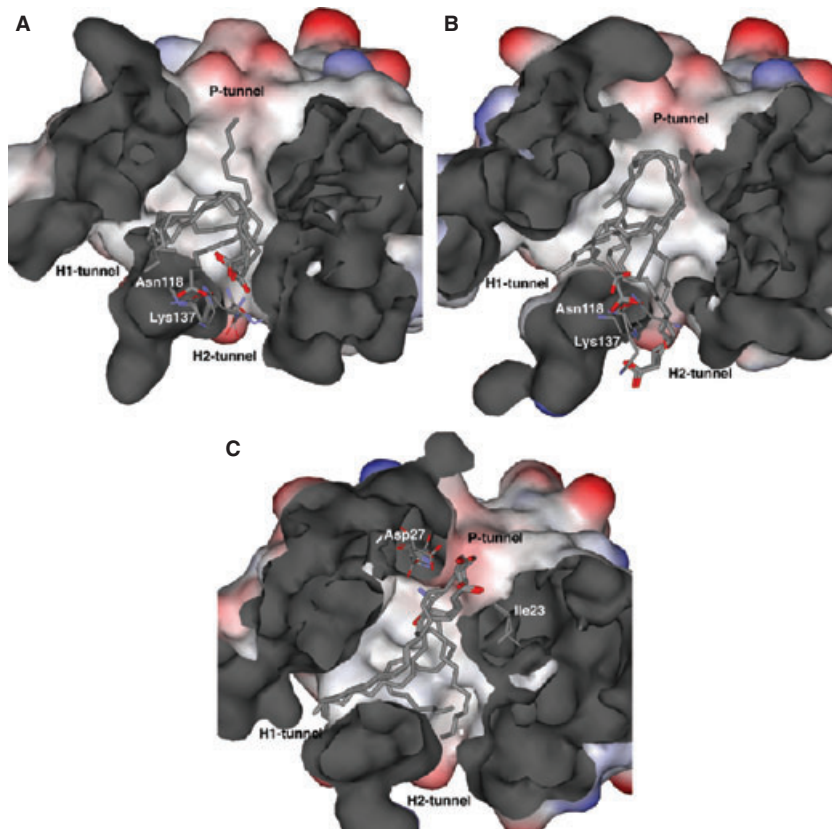


Figure 3 (A) The summary of the five best ranking conformations for palmitate (18 carbon atoms) (Lys137, Asn118), (B) arrachidate (20 carbon atoms) (Lys137, Asn118) and (C) sphingosine conformations (Ile 23, Asp 27).

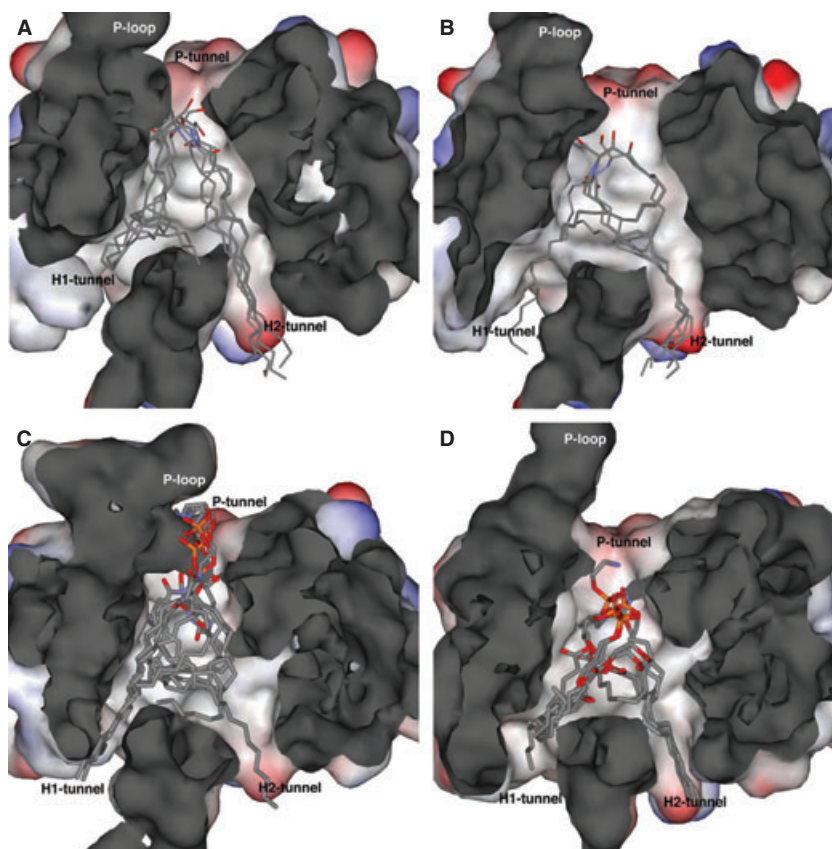


Figure 4 The summary of the five best ranking conformations ceramide conformations that have (A) b NH-Tyr81 hydrogen bonded binding mode, (B) NH-Asp69 hydrogen bonded binding mode. Of the 20 best scoring ceramide conformations, seven had adopted the binding mode (1,2,6,11,13,15,16) and five had adopted the b binding mode (3,4,5,7,12). In the rest conformations large areas of the aliphatic tails located outside of the hydrophobic cavity and the ceramide head group binding sites were distributed around the hydrophobic cavity. (C) Five examples of high scoring sphingomyelin conformations that have the phosphocholine head group in the P-loop tunnel. (D) Five examples of high scoring 1,2 dilauroyl-DL-phosphatidylethanolamine (DLPE) conformations that have the phosphocholine head group near the P-loop tunnel.

Galactocerebroside, in which a galactose is linked to the C-1 hydroxyl group of a ceramide, was used as a sample molecule for glycosphingolipids. Several CDOCKER searches were performed with slightly different parameter settings but no biologically reasonable conformations were obtained. In all cases large parts of the aliphatic tails were located outside the Bet v 1 protein. In the conformations that gave the best binding energies, galactose was located in the surface pocket next to the P-loop. In this position it was able to form a network of hydrogen bonds to the Bet v 1 surface residues. However, in these conformations, one of the aliphatic tails was located completely outside the Bet v 1 protein. After centre of mass and lipid tail distance-based filtering, only three conformations remained. In these cases the galactose group was located inside the hydrophobic cavity forming only few hydrogen bonds. The hydrophobic tails penetrated through P and H tunnels so that large areas of the tails were located outside of the protein (Fig. 4B). Thus also these conformations were considered as failures.

Deoxylysophosphatidylcholine was the simplest phosphatidylcholine model used. The polar head group of LPPC has zwitterionic nature as the phosphate group carried a negative charge while the choline group had a positive charge. Two binding modes could be identified from the group of 10 lowest binding energy LPPC conformations. In the first mode the polar head group was located in the H2-tunnel near Lys137. This is the same region where the polar head groups of negatively charged fatty acids bind. In the second binding mode the polar head group was located in the P-tunnel and formed hydrogen bonds to Lys54 and other nearby residues. Like in the case of sphingomyelin, this binding mode brought the phosphate group close to the P-loop.

Docking of DMPC to Bet v 1 produced only a small set of conformations. In several cases large regions of aliphatic chains were located outside of the protein. Thus, after centre of mass and tail distance filtering, only eight conformations remained. In the best-ranked structure the phosphocholine group was located in the P-tunnel while in the other structures the phosphocholine group was located near the H1-tunnel or inside the hydrophobic cavity near the P-tunnel.

In the DLPE docking experiments we found that in 8 out of 10 best short-listed conformations the phosphate group was located near the P-loop tunnel while the fatty acid tails occupied the H1 and H2 tunnels (Fig. 4D). The location of fatty acid tails varied a lot but in all eight structures the phospho and ethanolamine groups were concentrated in a small region so that the phospho group formed hydrogen bonds with Lys54 and Tyr81 and the amine group had interactions with the Asp27 side chain. In most cases the phosphate and ethanolamine groups did not locate in the P-loop tunnel but inside the hydrophobic cavity.

1-Palmytoil-2-oleoyl-SN-glycero-3-phosphatidylethanolamine differs from DLPE by having longer fatty acid tails (18 and 16 carbon atoms). The longer tail also had a *cis*-oriented double bond between C9 and C10, which decreased the conformational space of this molecule. Two binding modes could be detected. The first mode resembled the DLPE conformations with polar head group located near the P-tunnel and one of the hydrophobic fatty acid tails located in the H1 and H2 tunnels. In this case the positively charged amine group was hydrogen bonded to the backbone carbonyl of Ile23, while one of the phosphate oxygens was hydrogen bonded to Lys54 and one of the fatty acid carbonyls to Tyr83. In the second binding mode, the amine group was hydrogen bonded to the Gly88 backbone carbonyl while the phosphate group formed hydrogen bonds with Gln132 and Ser136. However, in these binding modes large parts of the fatty acid tails were located outside the Bet v 1 protein.

Discussion

Our docking studies with cholesterol, kinetin and fatty acids were well in line with the experimental data. The atom level models allow us to predict certain key residues that are important for binding different ligands. Kinetin is predicted to bind to the P-tunnel next to the conserved P-loop. Lys54, Asp69 and Tyr81 are presumably the most critical residues for the affinity in this case. The docking results suggest that these residues are probably important for binding cholesterol and other steroids, too. This seems contradicting as the binding experiments showed that kinetin binding did not affect the steroid affinity of Bet v 1. However, the predicted binding sites of kinetins and cholesterol overlapped only slightly especially in the cases where the kinetin purine ring was located on the protein surface.

Residues that are critical for kinetin and sterol binding are not likely to affect the binding affinity of negatively charged fatty acids and SDS. For these ligands the key binding residues are suggested to be Asn118 and Lys137 that is located near the H2 tunnel more than 10 Å away from the kinetin binding site. The orientation of the lipids suggests that the H1 tunnel will be the entrance route of these molecules when they move from a micelle or bilayer into the Bet v 1 hydrophobic cavity.

Docking experiments performed with large and flexible lipid molecules clearly reached beyond the capability of the computational docking tools used here. Due to the huge conformational space and the limited accuracy of the scoring methods the obtained results should be considered as just putative suggestions of the binding mechanisms of Bet v 1 to more complex lipid molecules. Even though the force field energies and Chemscore values presented in Table 2 in principle provide tools to estimate the relative affinity of different ligands, the accuracy

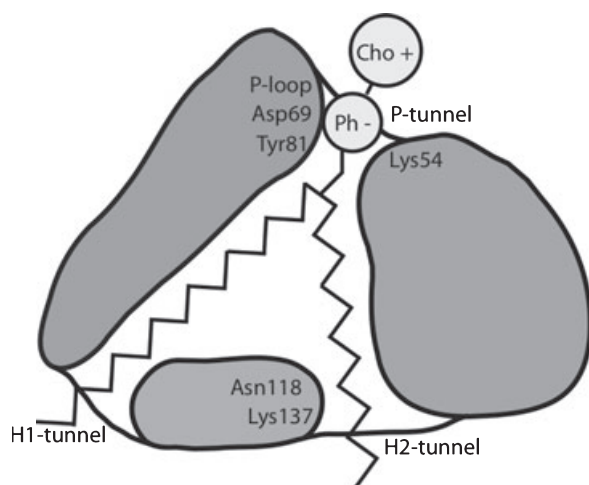


Figure 5 Schematic presentation of the hypothetical phospholipid-binding mode of Bet v 1 proteins.

of these values is probably very low. However, when we take a look into the docking results on a general level, we can detect interesting trends that provide possible explanations and hypotheses about the functionality of Bet v 1.

Recently, interactions with phospholipid vesicles were reported to induce large conformational rearrangements to Bet v 1. This observation suggests that Bet v 1 can bind large lipid molecules using the same, as yet still mostly unknown, mechanism that structurally analogous START domains use. At the moment there is no experimental data showing that Bet v 1 can bind large lipid molecules. Still, the docking experiments showed that there were no steric hindrances that would make the location of the lipids inside the hydrophobic pocket impossible, once the lipid had managed to enter to the hydrophobic cavity.

Even though several binding modes were present in the docking results, the best-scored conformations adopted in several cases a binding mode that is schematically presented in Fig. 5. This binding mode is biologically interesting as it provides a possible explanation for the tunnels that connect the hydrophobic cavity to the surface of the protein. The H1 and H2 tunnels on both sides of the long C-terminal helix provided an unspecific binding site for two hydrophobic tails of phosphatidates or sphingolipids, while the P-tunnel was able to accommodate the polar head group of the lipid (Fig. 5). This hypothesis would also provide a possible explanation for the highly conserved P-loop motif that could in this binding mode interact with the phospho group of the lipid head group.

According to the docking results, lipid binding was not specific; however, sphingomyelins and ceramides seemed to fit to the binding pocket better than phosphatidates. The fact that in the sphingomyelin model the phos-

phatidylcholine was located in the surface would also allow Bet v 1 to bind such lipids where additional groups have been linked to the polar head group of the lipid.

Our earlier experimental work provides evidence for the crucial role of caveola/lipid rafts in the early phases of allergic reactions as they transport the pollen allergens through the epithelial tissue to presensitized mast cells, which then burst and cause the symptoms of allergic reactions [6, 7]. This observation has an interesting link to the docking results we present here as sphingomyelins that are known to be present and enriched on caveola/lipid rafts [28] were found to be potential ligands for Bet v 1. Further, the suggested binding mode where the hydrophobic tail groups of lipids locate inside Bet v 1, while the polar head group may remain solvent accessible, would in principle allow Bet v 1 to bind glycolipids, e.g. gangliosides, too.

Author statement

Both authors planned and executed experiments and wrote the manuscript together.

Declaration of commercial interest

The authors have no commercial interests in this work.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. PCTP-DLPC complexes. The crystal structure of PCTP bound DLPC lipid molecule is presented as a thick stick model that is coloured using element based colour schema (grey = C, red = O, blue = N, orange = P). Hydrogen atoms are not displayed. The best scoring DLPC conformation, produced by the CDOCKER programs is presented as a green stick model where only P and N atoms are coloured according the element type. The surrounding PCTP protein is represented as spliced surface model. The choline and phosphate groups of the docked DLPC model align well with the corresponding atoms in the crystal structure while fatty acid tails have a larger deviation compared with the crystal structure. DLPC, 1,2-dilinoleoyl-SN-glucero-3-phosphocholine; PCTP, phosphatidylcholine transfer protein.

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