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Full Length Research Paper

Molecular docking study of tripeptides VPP and IPP inhibiting angiotensin I converting enzyme to alleviate high altitude pulmonary edema

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High altitude pulmonary edema (HAPE) remains the major cause of death related to high altitude exposure with a high mortality in absence of emergency treatment. Due to the deprival of oxygen in higher altitudes, the blood vessels constricts and squeezes blood in the vessels. This makes pressure to go up which in turn forces blood into air pockets in lungs that can kill people with HAPE. Angiotensin Converting Enzyme (ACE) is found in lung capillaries. It catalyses conversion of angiotensin I to angiotensin II which is a potent vasoconstrictor and inactivates bradykinin which is a potent vasodilator thereby causing HAPE. Tripeptides Val-Pro-Pro (VPP) and Ile-Pro-Pro (IPP) act as ACE inhibitors that reduce vasoconstriction and facilitate vasodilation, so that they can be used in the treatment of HAPE. Enzyme-inhibitor docking is performed between Angiotensin I Converting Enzyme ACE and two natural inhibitors, tripeptides Val-Pro-Pro (VPP) and Ile-Pro-Pro (IPP) using the Patchdock Software. The docking study provided a quantitative energetic measure (Atomic Contact Energy) of 262.56 and 147.73 for ACE inhibition by VPP and IPP respectively. This ensures that the tripeptides Val-Pro-Pro (VPP) and Ile-Pro-Pro (IPP) are able to inhibit the activity of the ACE which in turn can induce decreased formation of Angiotensin II and decreased inactivation of bradykinin thereby it can alleviate HAPE.

Key words: High Altitude Pulmonary Edema (HAPE), angiotensin converting enzyme, Val-Pro-Pro, Ile-Pro-Pro, patchdock.

INTRODUCTION

High Altitude Pulmonary Edema (HAPE) is a life-threatening form of non-cardiogenic pulmonary edema that occurs in otherwise healthy mountaineers at altitudes above 2,500 m (Roach et al., 2002). In some people it might also occur at lower altitudes that is between 1500 and 2500 m. It remains the major cause of death related to high altitude exposure with a high mortality in absence of emergency treatment. It is caused by the shortage of oxygen which occurs due to the lower air pressure at high altitudes. Due to the deprival of oxygen the lung reacts in the same way that blood vessels constrict. The blood in these vessels gets squeezed and the pressure goes up forcing fluid out of blood and into air pockets that can kill people with HAPE (Kenneth et al., 2006). Even though the mechanisms by which the shortage of oxygen causes HAPE are poorly understood there are two processes that are believed to be important causative factors. One factor is increased pulmonary arterial and capillary pressures (pulmonary hypertension) secondary to hypoxic pulmonary vasoconstriction (Bärtsch et al., 1991). Another factor is an idiopathic non-inflammatory increase in the permeability of the vascular endothelium (Swenson et al., 2002). It is considered that genetic basis can be a cause to this condition. Also, there is an involvement of a gene for Angiotensin Converting Enzyme (ACE) causing this condition.

ACE

Angiotensin I Converting Enzyme (ACE) is an exopeptidase. It is found mainly in lung capillaries. It catalyses conversion of angiotensin I to angiotensin II which is a potent vasoconstrictor. It is involved in the inactivation of

bradykinin, a potent vasodilator. These two actions of ACE make it an ideal target in the treatment of conditions such as high blood pressure, heart failure and diabetic nephropathy type 2 diabetes mellitus. Since inhibition of ACE by usage of ACE inhibitors results in decreased formation of Angiotensin II and decreased inactivation of bradykinin, it can be used as an ideal tar-get for HAPE.

ACE Inhibitors

ACE inhibitors are a group of pharmaceuticals that are used primarily in treatment of hypertension and congestive heart failure. ACE inhibitors reduce vasoconstriction and facilitate vasodilation, thereby it can lead to a way to treat HAPE. There are a number of chemicals like captopril, zofenopril, enalapril, ramipril, quinapril, perindopril, lisinopril, benazepril, and fosinopril function as ACE inhibitors. Also, naturally occurring ACE inhibitors include casokinins and lactokinins are breakdown products of casein and whey (FitzGerald et al., 2004).

Tripeptides VPP and IPP

Apart from the chemical and natural inhibitors of ACE, there are tripeptides Val-Pro-Pro (VPP) and Ile-Pro-Pro (IPP) produced by the probiotic *Lactobacillus helveticus* have been shown to have ACE-inhibiting and antihypertensive functions (Nakamura et al., 1995; Aihara et al., 2005). It is also shown that *L. helveticus* fermented milk had an antihypertensive effect after oral administration to spontaneously hypertensive rats (Nakamura et al., 1995).

VPP and IPP are associated with the antihypertensive effect of *L. helveticus* fermented milk (Masuda et al., 1996). Efficacy of *L. helveticus* fermented milk containing VPP and IPP has been tried in hypertensive patients by ingesting fermented milk drink on a daily basis for 8 weeks (Hata et al., 1996). It is thought to be more effective to consume the fermented milk as part of the diet during the early stages of a therapeutic plan for hypertension or for prevention of the incidence of hypertension. Studying the efficacy and safety of powdered *L. helveticus* fermented milk tablets on subjects with hypertension revealed that, foods containing powdered *L. helveticus* fermented milk could be used in dietary approaches to the primary prevention of hypertension (Aihara et al., 2005). Tripeptides Val-Pro-Pro (VPP) and Ile-Pro-Pro (IPP) can reduce vasoconstriction and facilitate vasodilation, thereby it can lead to a way to treat HAPE.

Objective

The objective of this work is to perform insilico molecular docking study of the tripeptides Val-Pro-Pro (VPP) and Ile-Pro-Pro (IPP) with the angiotensin I converting enzyme (ACE) and to derive an intermolecular complex. This work is of vital importance for the development of new therapeutics to decrease formation of Angiotensin II and to decrease inactivation of bradykinin as a means of inhibiting ACE, thereby it can alleviate HAPE.

METHODOLOGY

PDB

PDB is one of the repositories for 3-D structural data of proteins and nucleic acids. The PDB database is operated by the research collaboratory for structural bioinformatics (RCSB). As at March 3, 2009 PDB had 56217 macromolecular structure data entries.

Retrieval of angiotensin I converting enzyme (ACE)

Crystal structure of angiotensin I converting enzyme (ACE) from the organism *Homo sapiens* with the PDB ID 1O8A (Figure 1) is retrieved from the Protein Data Bank (PDB).

MarvinSketch

MarvinSketch is an advanced, Java based chemical editor for drawing chemical structures, queries and reactions which supports wide range of file types MOL, MOL2, SDF, RXN, RDF (V2000/V3000), SMILES, SMARTS/SMIRKS, MRV, InChi, CML, PDB etc.

Sketching structures of tripeptides VPP and IPP

Tripeptides (Figures 2a and b) Val-Pro-Pro (VPP) and Ile-Pro-Pro (IPP) have been drawn using MarvinSketch in MOL format and converted to PDB format using MolConverter.

Molecular docking

Binding of a small molecule (ligand) with a large molecule (protein) is called docking. Docking is the process by which two molecules fit together in 3D space. The objective of computational docking is to determine how two molecules will interact which will aid the interaction studies in bio-molecules. Molecular docking is often employed to aid in determining how a particular drug lead will interact to form a binding pocket.

Patchdock

Patchdock algorithm (Duhovny et al., 2002) is inspired by object recognition and image segmentation techniques that are used in computer vision. Given two molecules, their surfaces are divided into patches according to the surface shape. All possible patches concave, convex or flat surface patches (Figure 3) which can be visually seen are detected using segmentation algorithm. The patches are then filtered, so that only patches with hot spot residues are retained.

Once the patches are identified, they are superimposed using shape matching algorithm. Shape matching algorithm uses hybrid of the geometric hashing (Schneidman et al., 2003) and pose clustering matching techniques to match the patches detected by segmentation algorithm. Concave patches are matched with convex patches and flat patches with any type of patches to obtain complexes. All the candidate possible complexes are examined.

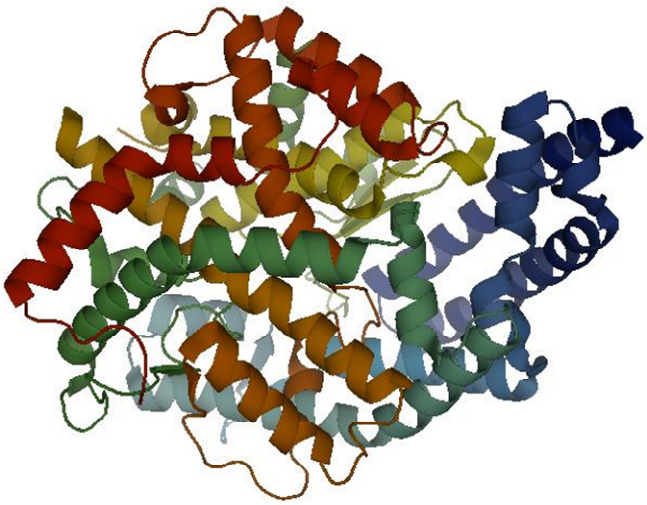


Figure 1. Angiotensin I converting enzyme (ACE).

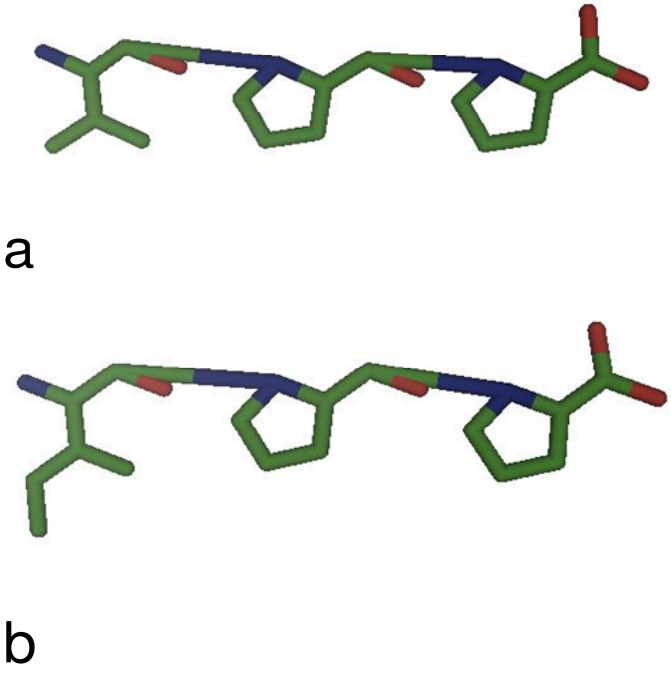


Figure 2. Tripeptides VPP and IPP targeting Angiotensin I converting enzyme (ACE). a) Val-Pro-Pro (VPP) b) Ile-Pro-Pro (IPP).

Complexes with unacceptable penetrations of the atoms of the receptor to the atoms of the ligand are discarded. Remaining candidate complexes are ranked according to a geometric shape complementarity score.

Docking of ACE and ACE Inhibitors - Tripeptides VPP and IPP

Angiotensin I converting enzyme (ACE) is the receptor molecule. ACE inhibitor - Tripeptides VPP and IPP are ligand molecules.

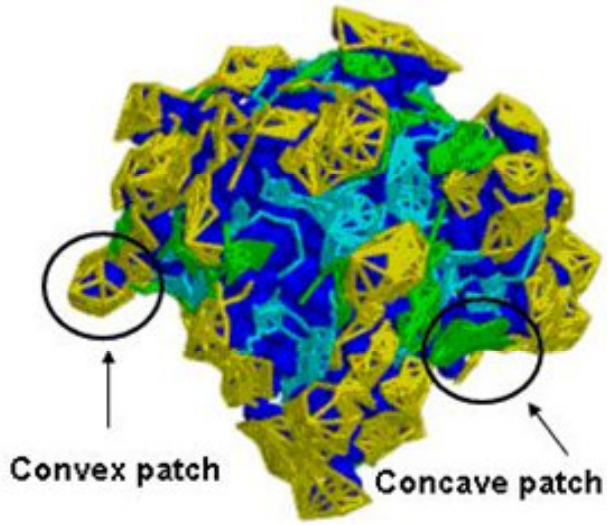


Figure 3. Patches.

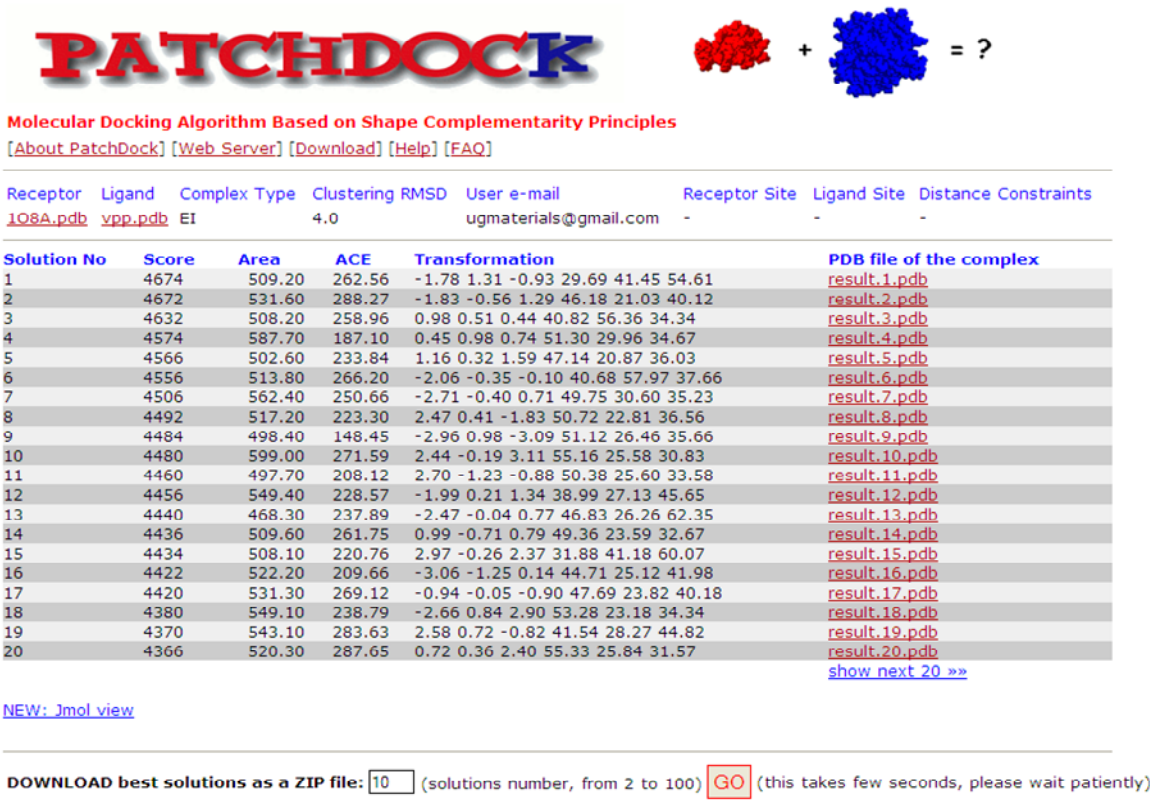
Receptor and ligand molecule VPP is uploaded in PDB format in Patchdock server, an automatic server for molecular docking. Clustering RMSD is chosen as 4.0 Å. E-mail address to retrieve the result is given. Complex type is chosen as enzyme – inhibitor type. Then the docking job is submitted to the Patchdock server. Same steps are repeated for the ligand molecule IPP. Another docking job is submitted to the Patchdock server. Results are obtained through the e-mail address provided and the docked complex structures of ACE-VPP and ACE-IPP are downloaded.

RESULTS AND DISCUSSION

The ouput of PatchDock is a list of candidate complexes between receptor (ACE) and ligand VPP (Figure 4) and IPP (Figure 5) molecules. The list is presented in the format of a table. Each table line represents one candidate complex. Solution No. represents the number of the solution. Score correspond to geometric shape complementarity score (Duhovny et al., 2002). The solutions are sorted according to this score. Area stands for the approximate interface area of the complex. ACE indicates Atomic Contact Energy (Zhang et al., 1997). Transformations represented are 3D transformations that include 3 rotational angles and 3 translational parameters. These transformations are applied on the ligand molecule. PDB file of the complex denotes the predicted complex structure in PDB format. High scoring solution structure of ACE-VPP and ACE-IPP are downloaded.

ACE-VPP and ACE-IPP complexes

ACE-VPP complex structure (Figure 6a) has a complementarity score value of 4674 with an Atomic Contact Energy (ACE) of 262.56. Closer view of VPP inside the binding pocket of ACE is shown in Figure 6b. ACE-IPP complex structure (Figure 7a) has a comple-mentarity score value



PATCHDOCK + = ?

Molecular Docking Algorithm Based on Shape Complementarity Principles

[About PatchDock] [Web Server] [Download] [Help] [FAQ]

Receptor	Ligand	Complex Type	Clustering RMSD	User e-mail	Receptor Site	Ligand Site	Distance Constraints
1O8A.pdb	vpp.pdb	EI	4.0	ugmaterials@gmail.com	-	-	-

Solution No	Score	Area	ACE	Transformation	PDB file of the complex
1	4674	509.20	262.56	-1.78 1.31 -0.93 29.69 41.45 54.61	result.1.pdb
2	4672	531.60	288.27	-1.83 -0.56 1.29 46.18 21.03 40.12	result.2.pdb
3	4632	508.20	258.96	0.98 0.51 0.44 40.82 56.36 34.34	result.3.pdb
4	4574	587.70	187.10	0.45 0.98 0.74 51.30 29.96 34.67	result.4.pdb
5	4566	502.60	233.84	1.16 0.32 1.59 47.14 20.87 36.03	result.5.pdb
6	4556	513.80	266.20	-2.06 -0.35 -0.10 40.68 57.97 37.66	result.6.pdb
7	4506	562.40	250.66	-2.71 -0.40 0.71 49.75 30.60 35.23	result.7.pdb
8	4492	517.20	223.30	2.47 0.41 -1.83 50.72 22.81 36.56	result.8.pdb
9	4484	498.40	148.45	-2.96 0.98 -3.09 51.12 26.46 35.66	result.9.pdb
10	4480	599.00	271.59	2.44 -0.19 3.11 55.16 25.58 30.83	result.10.pdb
11	4460	497.70	208.12	2.70 -1.23 -0.88 50.38 25.60 33.58	result.11.pdb
12	4456	549.40	228.57	-1.99 0.21 1.34 38.99 27.13 45.65	result.12.pdb
13	4440	468.30	237.89	-2.47 -0.04 0.77 46.83 26.26 62.35	result.13.pdb
14	4436	509.60	261.75	0.99 -0.71 0.79 49.36 23.59 32.67	result.14.pdb
15	4434	508.10	220.76	2.97 -0.26 2.37 31.88 41.18 60.07	result.15.pdb
16	4422	522.20	209.66	-3.06 -1.25 0.14 44.71 25.12 41.98	result.16.pdb
17	4420	531.30	269.12	-0.94 -0.05 -0.90 47.69 23.82 40.18	result.17.pdb
18	4380	549.10	238.79	-2.66 0.84 2.90 53.28 23.18 34.34	result.18.pdb
19	4370	543.10	283.63	2.58 0.72 -0.82 41.54 28.27 44.82	result.19.pdb
20	4366	520.30	287.65	0.72 0.36 2.40 55.33 25.84 31.57	result.20.pdb

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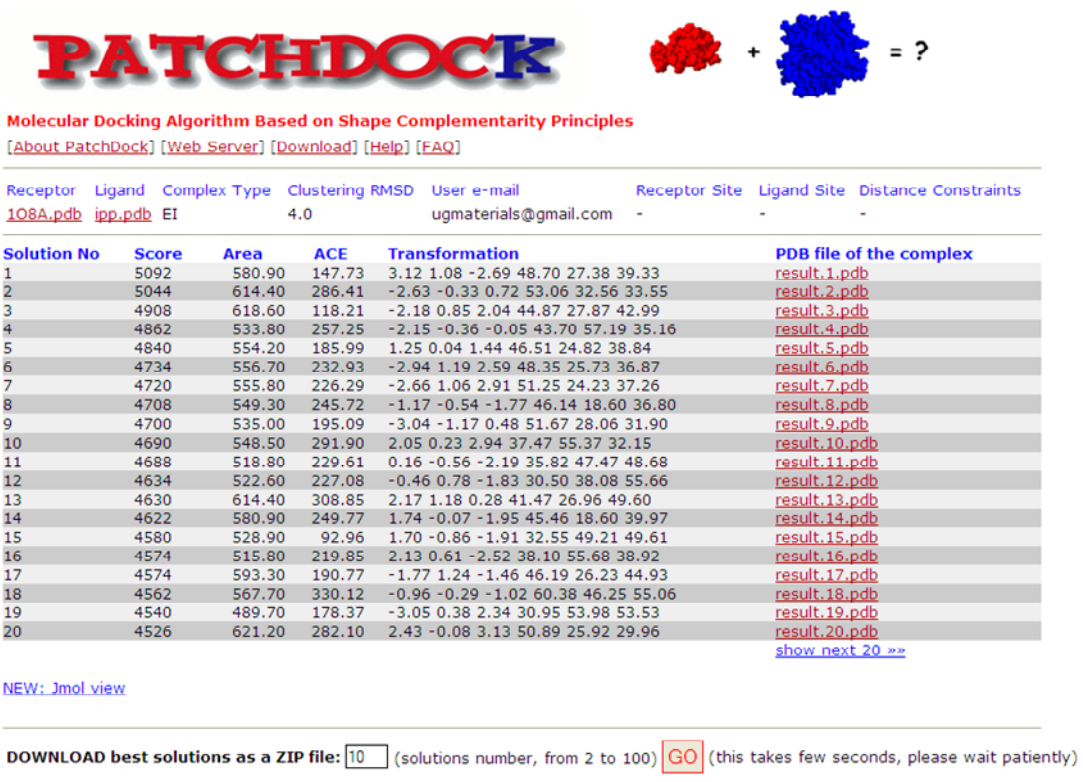
Figure 4. Candidate complexes of ACE – VPP.



PATCHDOCK + = ?

Molecular Docking Algorithm Based on Shape Complementarity Principles

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Receptor	Ligand	Complex Type	Clustering RMSD	User e-mail	Receptor Site	Ligand Site	Distance Constraints
1O8A.pdb	ipp.pdb	EI	4.0	ugmaterials@gmail.com	-	-	-

Solution No	Score	Area	ACE	Transformation	PDB file of the complex
1	5092	580.90	147.73	3.12 1.08 -2.69 48.70 27.38 39.33	result.1.pdb
2	5044	614.40	286.41	-2.63 -0.33 0.72 53.06 32.56 33.55	result.2.pdb
3	4908	618.60	118.21	-2.18 0.85 2.04 44.87 27.87 42.99	result.3.pdb
4	4862	533.80	257.25	-2.15 -0.36 -0.05 43.70 57.19 35.16	result.4.pdb
5	4840	554.20	185.99	1.25 0.04 1.44 46.51 24.82 38.84	result.5.pdb
6	4734	556.70	232.93	-2.94 1.19 2.59 48.35 25.73 36.87	result.6.pdb
7	4720	555.80	226.29	-2.66 1.06 2.91 51.25 24.23 37.26	result.7.pdb
8	4708	549.30	245.72	-1.17 -0.54 -1.77 46.14 18.60 36.80	result.8.pdb
9	4700	535.00	195.09	-3.04 -1.17 0.48 51.67 28.06 31.90	result.9.pdb
10	4690	548.50	291.90	2.05 0.23 2.94 37.47 55.37 32.15	result.10.pdb
11	4688	518.80	229.61	0.16 -0.56 -2.19 35.82 47.47 48.68	result.11.pdb
12	4634	522.60	227.08	-0.46 0.78 -1.83 30.50 38.08 55.66	result.12.pdb
13	4630	614.40	308.85	2.17 1.18 0.28 41.47 26.96 49.60	result.13.pdb
14	4622	580.90	249.77	1.74 -0.07 -1.95 45.46 18.60 39.97	result.14.pdb
15	4580	528.90	92.96	1.70 -0.86 -1.91 32.55 49.21 49.61	result.15.pdb
16	4574	515.80	219.85	2.13 0.61 -2.52 38.10 55.68 38.92	result.16.pdb
17	4574	593.30	190.77	-1.77 1.24 -1.46 46.19 26.23 44.93	result.17.pdb
18	4562	567.70	330.12	-0.96 -0.29 -1.02 60.38 46.25 55.06	result.18.pdb
19	4540	489.70	178.37	-3.05 0.38 2.34 30.95 53.98 53.53	result.19.pdb
20	4526	621.20	282.10	2.43 -0.08 3.13 50.89 25.92 29.96	result.20.pdb

show next 20 »»

NEW: Jmol view

DOWNLOAD best solutions as a ZIP file: 10 (solutions number, from 2 to 100) GO (this takes few seconds, please wait patiently)

Figure 5. Candidate complexes of ACE – IPP.

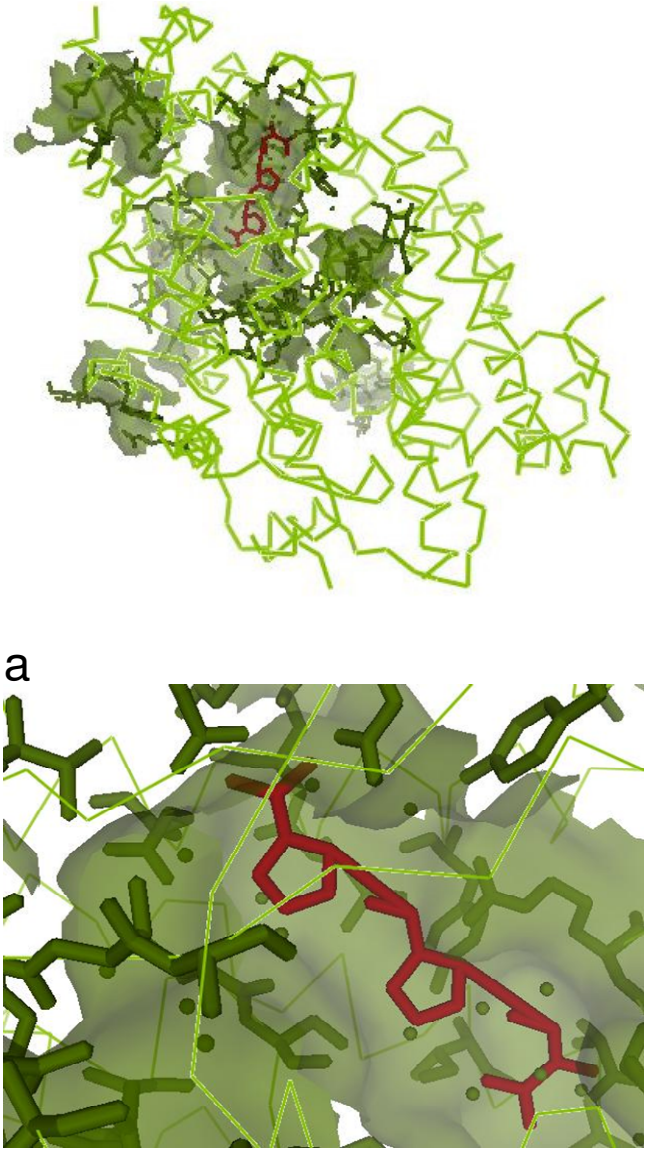

Figure 6. PYMOL Visualized complex structure of ACE-VPP. (a) ACE-VPP (b) VPP inside binding pocket of ACE.

of 5092 with an Atomic Contact Energy (ACE) of 147.73. Closer view of IPP inside the binding pocket of ACE is shown in Figure 7b.

Conclusion

Enzyme-inhibitor docking is performed between angiotensin I converting enzyme ACE and two natural inhibitors, tripeptides Val-Pro-Pro (VPP) and Ile-Pro-Pro (IPP) using the Patchdock Software. Prediction of the intermolecular complex where drug binds to the receptor there by altering the chemical behavior of the receptor macromolecule is of vital importance for the analysis of the

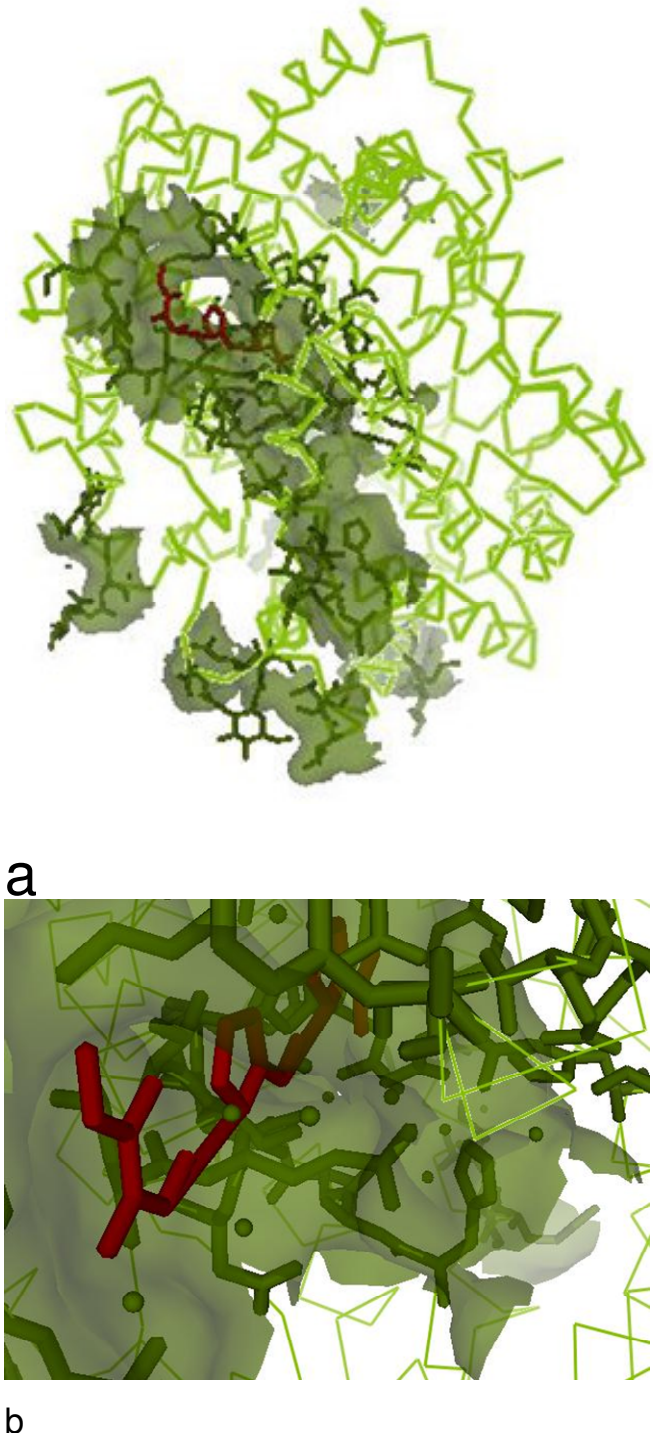

Figure 7. PYMOL Visualized complex structure of ACE-IPP. (a) ACE-IPP (b) IPP inside binding pocket of ACE.

therapeutics. The docking study provide a quantitative energetic measure that ensures that, the tripeptides Val-Pro-Pro (VPP) and Ile-Pro-Pro (IPP) are able to inhibit the activity of the ACE which in turn can induce decreased formation of Angiotensin II and decreased inactivation of bradykinin thereby can alleviate HAPE.

Dairy peptides VPP and IPP are small peptides that are formed when casein (milk protein) is broken down into smaller pieces. They can be manufactured using a naturally obtained enzyme preparation to break casein down into hydrolyzed casein powder. They can also be made by fermentation using lactic acid bacteria instead of an enzyme preparation and can be used to inhibit ACE which in turn can alleviate HAPE.

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