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DOCKING AND MOLECULAR DYNAMICS SIMULATIONS FOR DISCOVERY OF POTENTIAL INHIBITORS OF SOLUBLE ACID INVERTASE IN SUGARCANE

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ABSTRACT

Inversion of sucrose, impairing sugar recovery from harvested sugarcane leads to reduction in sugar production. The inversion or sucrose catabolism in sugarcane stalks mostly is due to the enzymatic activity of soluble acid invertases. With an objective to save sucrose from degradation to glucose and fructose, virtual screening, docking and molecular dynamics simulations were implemented to identify potential soluble acid invertase inhibitors. Soluble acid invertase 3D structure (PM0076107) was prepared in protein preparation wizard of Maestro v9.2. A set of 372 structural analogues of sucrose were obtained from one million compounds of ligand info Meta database. Ligands were prepared using LigPrep to generate fully customized ligand dataset of 2,520 conformations. Using Glide v5.7, the dataset was docked with in the active site of soluble acid invertase. Out of the docked ligands, 64 lead molecules with better XPGscore than sucrose were identified. The 15 leads selected based on clustering were re-docked through quantum polarized ligand docking. Based on results of the both docking protocols, 15 leads were proposed as potential inhibitors of soluble acid invertase. Lead 1, showed the best Gscore (XP: -12.07 kcal/mol; QPLD: -13.07 kcal/mol) and interactions with residues that are important for sucrose binding. Furthermore, Prime/MM-GBSA calculation of soluble acid invertase-lead1 complex obtained after QPLD showed lowest free energy of ligand binding ("G = -166.02 kcal/mol). The conformational and interaction stability of soluble acid invertase – lead1 docking complex was stable during 10ns molecular dynamic simulations. Therefore, lead1 was proposed as potential competitive inhibitor to sucrose.

KEYWORDS: Sugarcane, sucrose, soluble acid invertase inhibitors, virtual screening, molecular dynamics simulations.

INTRODUCTION

Sugarcane (*Saccharum* spp.) is the basic raw material for sugar production contributing 75 per cent to the total sugar pool globally apart from sugar beet and other sources. Sixty to seventy per cent of the cane produced in India is used for sugar production and this is steadily increasing to meet ever increasing demand. The requirement of sugar by 2030 is projected as 36 million tones in India. To achieve this target, sugarcane production should be about 500 million tons from the current 350 million tons. The increased sugar production has to come from higher sugarcane productivity and sugar recovery as increasing the area might not be possible. Increasing productivity from the current 68 t ha^{-1} to 100 t ha^{-1} or beyond is possible with intervention of production technologies in sugarcane growing states of India as we already have an example of the state of Tamilnadu where the productivity is 105 t ha^{-1}. On the other hand, sugar recovery ranged from 9.5 per cent to 10.5 per cent in India over several decades and an increase beyond 10.5 per cent might be a difficult target to achieve.

Several factors that can reduce sucrose content in sugarcane crop such as the nature of cultivars and their inversion behavior, atmospheric conditions, flowering, lodging of the crop, water logging, drought and infestation of microbes (*Leuconostoc spp.*) and pests are categorized as causes for pre-harvest sucrose losses which are managed in various proportions in field conditions. Whereas time lag after harvest and external temperature are the most important factors that are responsible for post-harvest sucrose losses which determine the rate of sucrose loss through inversion, dextran formation and respiration (Solomon, 2000). Post harvest sucrose loss in the field or in the factory has become an alarming issue (Eggleston, 2002; Solomon, 2009). In India, the time lag between harvesting to milling of canes ranges between 3 and 7 days, which entails 10 to 15 per cent loss in recoverable sugar (Solomon *et al*., 2001) translating to a minimum loss of 2 million tons of sugar annually.

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The inversion of sucrose to its monosaccharides, glucose and fructose, is catalyzed by three isoenzymes of invertases (â fructofuranosidase, E.C. 3.2.1.26) which are categorized into 2 groups based on pH optima (Wyse & Dexter, 1971). Soluble acid invertase is high in apoplast and vacuoles of young internodes but virtually absent in mature tissue with pH optimum 4.4 and Km = 1.3 × 10 2 M. Bound acid invertase present in cell wall is bounded in all aged tissues with pH optimum 3.8 and Km = 8 × 10 3 M. Neutral invertase occurs in cytoplasm at low concentrations in young tissue and greater concentrations in mature tissue having pH optimum 7.0 and Km = 3 × 10 4 M (Hawker and Hatchi, 1965; Glasziou and Gayler, 1972). Acid invertases have been hypothesized to be key enzymes for sucrose catabolism in sugarcane stalks (Singh *et al*., 2008). They exhibit greatest activities in the pH range of 3.5 5.5 and occur as soluble enzyme (soluble acid invertase, SAI) in the vacuole or insoluble enzyme (cell wall bound acid invertase, CWI) in the cell wall. A sharp increase in acid invertase leads to increases in reducing sugars and a subsequent drop in commercial cane sugar (CCS) (Solomon *et al*., 1997). It has been proposed that the endogenous invertases are activated due to loss of moisture and lack of any physiological and biochemical control mechanism soon after harvest (Solomon *et al*., 1990).

The soluble acid invertase, occurring in the vacuole and apoplastic space of elongating internodes, disappeared when internode growth ceased by application of growth inhibitor, glyphosate (Su *et al*., 1992) and reappeared when growth increased with application of gibberillic acid (Gayle and Glasziou, 1972). Thus growth promoters have short term effect and can be used to control pre harvest but not post harvest sucrose losses. Some inhibitors of sugarcane invertases have been reported such as iodide, lead, mercury, arsenic, tungsten, (Alexander, 1965), tris (hydroxymethyl) aminomethane (Hatch *et al.,* 1963) and sodium metasilicate (Alexander, 1968a). The latter compound completely inhibited purified invertase at 3 4 mM inhibitor concentration and also lowered the activity of several hydrolytic and oxidative enzymes in sugarcane (Alexander, 1968b). They were found to be effective in preserving sucrose in crude cane juice but not in the standing crop or harvested sugarcane piled in the field or factory. Inhibiting the endogenous invertase, the target protein, appears to be a promising option in arresting to certain extent pre and to greater extent post harvest sucrose losses in sugarcane wherein an inhibitor would act as a competitive binder to sucrose which binds with invertase and arrests the cleavage of sucrose into glucose and fructose akin to the principle of drug designing in medicine. Homology modeling, virtual screening, docking and molecular dynamics simulations were implemented in the present study to identify potential competitive inhibitors of soluble acid invertase of sugarcane.

MATERIAL AND METHODS

Homology modeling

Homology models are useful in structure based drug designing applications, especially when a crystallographic or NMR structure is unavailable (Sivasubramanian *et al*., 2009). In our previous work, a homology model of soluble acid invertase was constructed in complex with sucrose (Hemanthkumar and Umamaheswari, 2012). The model was validated using GA341 score, DOPE score, Procheck, ProSA, ProQ etc. and was deposited in protein model database (PMDB) (Hemanthkumar and Umamaheswari, 2012). The homology model of soluble acid invertase was retrieved from the PMDB (Hemanthkumar and Umamaheswari, 2012; Castrignano *et al.,* 2006).

Geometry based high throughput screening

The Ligand.Info meta-database tool retrieves structural analogues for the queried small molecule by implementing 2D geometry search techniques from eight renowned small molecule databases such as Havard's ChemBank, ChemPDB, KEGG Ligand, Drug likeliness National Cancer Institute (NCI), Anti-HIV NCI, Unannotated NCI, AkoS GmhB, Asinex Ltd. *etc*. Sucrose structure was imported to Ligand.Info meta-database tool and an in-house library of structural analogs was compiled (Grotthuss *et al*., 2003; Umamaheswari *et al*., 2010a; Umamaheswari *et al*., 2010b; Priyadarshini *et al.,* 2011; Sandeep *et al*., 2012).

Virtual screening through molecular docking

Tertiary structure of soluble acid invertase and in-house library of sucrose structural analogs were imported to Maestro v9.4, Schrodinger LLC, 2013, for molecular docking to investigate binding affinity of the ligand dataset towards soluble acid invertase. The soluble acid invertase structure was preprocessed with the protein preparation workflow in Maestro v9.4. All hydrogens were added to soluble acid invertase and energy was minimized using OPLS 2005 force field in impact

molecular mechanics engine setting the maximum root mean square deviation (RMSD) of 0.30 Å (Umamaheswari *et al*., 2010a; Umamaheswari *et al*., 2010b; Priyadarshini *et al*., 2011; Sandeep *et al*., 2012). Minimization was performed constraining the heavy atoms with the hydrogen torsion parameters turned off to allow free rotation of the hydrogen atoms.

LigPrep (Brooks *et al*., 2008) is an application tool in Schrödinger software suite that combines tools for generating 3D structures from 1D (SMILES) and 2D (SDF) representation, ionization states using Epik (Shelley *et al*., 2007) and searching for tautomers and steric isomers from a single input structure. The sucrose structural analog library ligands were prepared to expand protonation and tautomeric states at 7.0±2.0 pH units using LigPrep with Epik. Post LigPrep evaluations discarded high-energy ionization / tautomer states.

The grid for molecular docking of soluble acid invertase and ligand dataset was generated centered on active site residues after ensuring that the protein and ligands were in the accurate form for docking. The grid box size was set to 20 Å × 20 Å ×20 Å. Van der Waal radii of receptor atoms were scaled to 1.00 Å with a partial atomic charge of 0.25 to soften the potential for non-polar parts of the receptor. Glide extra precision (XP) docking method was applied to rank the ligands based on their binding affinities towards soluble acid invertase and to study interaction of the best lead (Priyadarshini *et al.*, 2011; Sandeep *et al*., 2012; Friesner *et al*., 2004; Friesner *et al*., 2006; Navya *et al*., 2012). The XP docking method generates 10000 poses for each ligand during docking and reports the best pose based on the energy term Emodel. The XP docking method being highly accurate, the best poses of each ligand were ranked based on XP Gscore. The cutoff XP Gscore parameter for XP docking was set at 0.0 kcal/mol and a constraint was set to discard ligands with positive XP Gscore from the final docking output.

Quantum polarized ligand docking

The 64 leads obtained through XP docking were clustered using Canvas v1.5. Fifteen leads selected based on clustering along with sucrose were re-docked to soluble acid invertase using quantum polarized ligand docking (QPLD) (Du *et al*., 2011).

Molecular dynamics simulations

The simulations of soluble acid invertase – lead1 docking complex was carried out for 10 ns using Desmond v3.0 (Shan *et al*., 2011; Santiago *et al*., 2011; Jatana *et al*., 2011; Priyadarshini *et al*., 2013; Pradhan *et al*., 2014). The system for MD simulation was built by embedding SPC (single point charge) model to describe the water molecules around the docking complex. MD simulation methodology was applied to soluble acid invertase-sucrose complex for comparative analysis with soluble acid invertase-lead1 complex.

RESULTS AND DISCUSSION

Homology modeling

Computational methodologies have become a crucial component of drug discovery programmes, from target identification to lead optimization and beyond. Virtual screening method predicts binding affinities between drug target and ligands through molecular docking and ranks them in decreasing order. Along with binding affinity it also predicts accurate binding modes and molecular interactions between protein and ligand, hence, became immensely important to carry out initial steps of drug discovery prior to experimental validation.

Tertiary structure of the protein is initial requirement for structure based inhibitor design. In the absence of an experimentally determined structure, homology modeling is an efficient method for structure prediction and to obtain quick experimental design. In our previous studies, we have modeled 3D structure of soluble acid invertase, and deposited at the protein model database (PMDB ID: PM0076107) (Hemanthkumar and Umamaheswari, 2012). The model validation report (Table 1) showed that soluble acid 3D structure was reliable for lead discovery based on computational docking (Hemanthkumar and Umamaheswari, 2012). The amino acids *viz*., Asn32, Gln49, Trp57, Ile61, Trp93, Thr94, Gln118, Arg154, Arg157, Asp158 Glu217 and Tyr300 were determined as active site residues (Hemanthkumar and Umamaheswari, 2012). The active site residues were validated through sucrose incorporation into the homology model from the structural template, 2ADD, during homology modeling. Additionally sequence alignment of target and template showed that the active site residues were 100% conserved (Hemanthkumar and Umamaheswari, 2012). Therefore, discovery of lead molecules that would block these residues by interacting with better binding affinity

Table 1. Comparison of target and template structures

Validation method	Target	Template
GA341	1.0	1.0
DOPE Score	-58380.85 kcal/mol	-65962.19 kcal/mol
ProQ	4.026	5.892
PROCHECK		
Most favorable region	85%	84.4%
Additionally allowed region	12.5%	14.9%
Generously allowed region	1.8%	0.4%
Disallowed region	0.6%	0.2%
RMSD		
C-alpha	0.27 Å	---
Overall	0.62 Å	---

Table 2. Proposed leads for soluble acid invertase

Lead No.	Gscore (kcal/mol)		ΔG (kcal/mol)	
	XP	QPLD	XP	QPLD
1	-12.07	-13.77	-74.05	-166.02
2	-12.03	-12.24	-98.11	-152.83
3	-12.01	-11.91	-85.89	-147.49
4	-11.71	-11.75	-72.61	-130.16
5	-11.59	-10.85	-47.21	-137.61
6	-11.54	-11.62	-109.68	-129.29
7	-8.11	-11.11	-87.12	-129.25
8	-11.54	-10.87	-73.48	-128.39
9	-11.47	-11.90	-76.29	-122.09
10	-11.4	-12.42	-69.31	-115.98
11	-11.39	-12.66	-71.92	-114.69
12	-9.46	-10.72	-83.43	-116.99
13	-8.84	-11.71	-59.89	-112.49
14	-11.66	-11.89	-52.35	-105.44
15	-10.31	-10.97	-69.40	-101.95
Sucrose	-9.06	-10.71	-43.37	-111.64

compared to sucrose would be ideal step forward towards inhibitor designing against soluble acid invertase.

Molecular docking

Accurate binding affinity prediction between protein and ligand through molecular docking requires careful optimization of their 3D structures. Therefore, the protein was optimized in protein preparation wizard applying OPLS-2005 force field. The structure was optimized to add hydrogen atoms, remove water molecules and remove steric classes in 3D structure. Further, energy was minimized applying OPLS 2005 force field to obtain a structure with lower energetic conformation. Structural analog search for sucrose led to compile an in-house library of 374 ligands. 2560 conformations were generated during ligand preparation. The ligand preparation in LigPrep ensured all ligands at their lower energetic conformation.

The 2560 conformations of 374 ligands were docked into the active site of soluble acid invertase. 366 unique ligand conformations were docked with soluble acid invertase. The docked compounds were ranked based on XP Gscore. The lowest XP Gscore of a compound represents comparatively higher binding affinity of the particular ligand towards protein. Sixty four ligands were observed to have lowest XP Gscore compared to sucrose. Hierarchical clustering of 64 leads led to identify 15 representing lead molecules which could act as potent competitive inhibitors to sucrose. The 15 leads also docked well in QPLD method (Table 2). The result

showed significant improvement of binding affinity of lead1 towards soluble acid invertase after accurate charge calculation for the ligand through quantum mechanics method.

Lead1 showed the lowest Gscore (XP: -12.066 kcal/mol; QPLD: -13.77 kcal/mol), lowest free energy of ligand binding ("G = -166.02 kcal/mol) compared to other proposed leads and sucrose (Table 2), hence, represents the highest binding affinity towards soluble acid invertase. The docking interaction showed a strong intermolecular hydrogen bond network with 11 hydrogen bonds (Fig. 1). The intermolecular hydrogen bonds (Fig. 1) are comparable to intermolecular hydrogen bonds between soluble acid invertase and sucrose reported earlier (Hemanthkumar & Umamaheswari, 2012). The molecular interactions of docking complex of soluble acid invertase - lead1 showed that the residues such as Asn32, Gln49, Arg154, Asp155, Arg157, Asp180, Met215, Glu217 (2 hydrogen bonds) and Asp261 (2 hydrogen bonds) were involved in intermolecular hydrogen bonding. The residues; Trp30, Asn32, Gln49, Ile56, Trp57, Ile61, Trp93, Thr94, Gln118, Arg154, Asp155, Arg157, Asp158, Asp180, Glu182, Gly214, Met215, Glu217, Asp261, Lys298, Tyr300, Trp318, Gly320, Glu321, Ala334 and Asp328 showed good van der Waal interaction with lead1 (Fig. 1). The result revealed that lead1 would be a potent competitive inhibitor of soluble acid invertase of sugarcane.

MD simulation studies

The binding orientations of lead molecules obtained after simulations showed better correlation to their biologically active states as MD simulations are carried out closer to the physiological environment condition with the system embedded with water molecules, temperature and pressure. The energy of the system was stable throughout the simulations period. The analysis of the RMSD plot for soluble acid invertase and lead1 showed that after a small rearrangement from the initial conformation, the complex was stable during entire MD simulation period. The RMSD of protein and ligand remained below 4Å in all 2084 trajectories. The root mean square fluctuations (RMSF) of a given residue in the MD trajectories were calculated by averaging over all the atoms of the given residue. RSMF of backbone and side chain residues were within the limit of 3Å. Very few fluctuations exceeded the 3Å limit. The lower atomic fluctuations indicated smaller conformational changes. The energy plot, RMSD plot and RMSF plot showed that the docking complex was conformationally stable and well in line with energy plot, RMSD plot and RMSF plots of soluble acid invertase – sucrose MD simulation complex.

The hydrogen bonds observed in the docking complex were monitored in all trajectories. Similar binding interactions were noticed during MD simulation as observed in docking complex. The amino acid residues

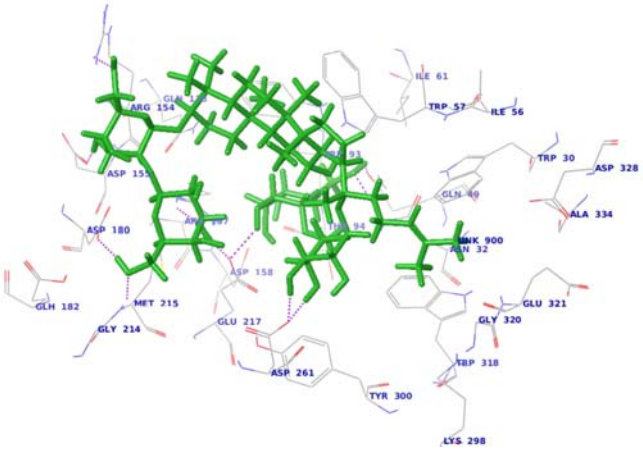

Fig. 1. Docking interaction of lead1 in soluble acid invertase active site

such as Asn32, Gln49, Arg154, Asp155, Arg157, Asp180, Met215, Glu217 (2 hydrogen bonds) and Asp261 (2 hydrogen bonds) were involved in eleven intermolecular hydrogen bonds in the docking complex. These hydrogen bonds were monitored in all 2084 trajectories recorded during 10ns MD simulations. The result revealed that Asn32, Gln49, Arg154, Arg157, Asp180, Met215, Glu217 and Asp261 were involved in intermolecular hydrogen bond formation during MD simulations (Fig.2). Moreover, water mediated hydrogen bonds were also observed in all 2084 trajectories. This analysis revealed that the interaction predicted between soluble acid invertase and lead1 were stable. Further, similar interactions were also revealed in soluble acid invertase-sucrose complex during 10 ns MD simulations (Fig. 3). Therefore, lead1 could be proposed as a potential soluble acid invertase inhibitor.

CONCLUSION

The knowledge of tertiary structure of soluble acid invertase would be useful in designing structure based virtual screening protocols for rational inhibitor design. Competitive inhibitor was predicted through structure

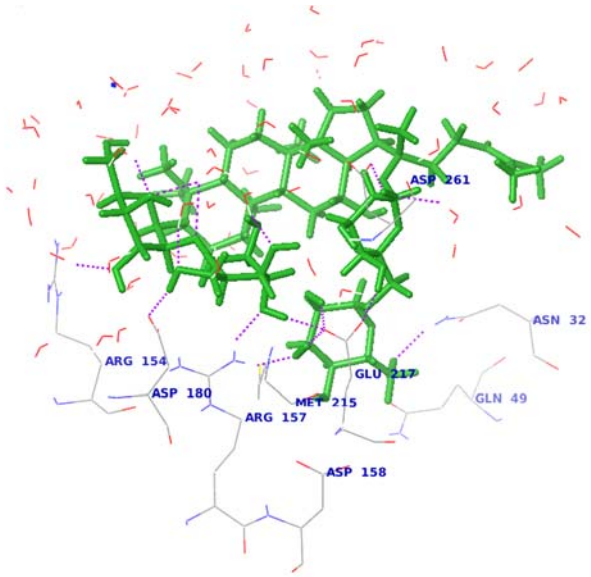

Fig. 2. Interactions of lead1 and soluble acid invertase after 10 ns MD simulations

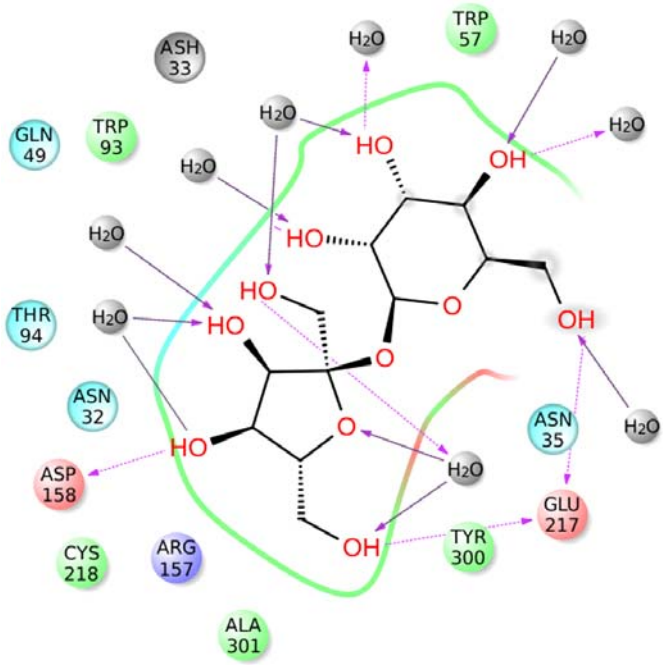

Fig. 3. Interactions of soluble acid invertase – sucrose complex during 10 ns MD simulations

based virtual screening approach by exploring the proposed homology model of soluble acid invertase. The results revealed that the binding interactions between lead1 and the active site residues of soluble acid invertase were stronger when compared with that of sucrose. Molecular dynamics simulations of soluble acid invertase and lead1 complex also revealed that the interactions predicted during docking analysis were stable. Thus, lead1 was proposed as competitive inhibitor binding with soluble acid invertase thereby preventing the binding of sucrose with the enzyme. Along with lead 1, the 14 other potential leads obtained in the study could further be analyzed for potential binding affinity with active site residues of soluble acid invertase through experimental verification *in vitro* and *in vivo*.

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