Short Research Article

Computational analysis reveals that dual point mutation in rice SBEIIb leads to decrease in starch binding affinity

Abstract

Aim: To check the effect of mutation on the binding affinity of Starch branching enzyme II with maltopentaose substrate

Place and Duration of Study: Department of Bioinformatics, Centre for Plant Molecular Biology and Bioinformatics, Tamil Nadu Agricultural University, Coimbatore, between May 2022 and July 2022

Methodology: The wild type rice SBEIIb and mutated rice SBEIIb protein were modelled using SWISS MODEL and subjected to PROCHECK for validation of the modelled protein. These protein structures were docked with ligand maltopentaose using Auto dock vina of PyRx software. The docked structures were visualized in BIOVIA Discovery studio.

Results: There was a decrease in binding affinity after mutating the protein at 2 of the maltopentaose binding sites (Y178A & F234A). The binding energy of wild type rice SBEIIb protein was -7.5kcal mol^{-1} whereas after mutation it decreased to -5.8 kcal mol^{-1}. The number of hydrogen bonds also decreased from 8 to 4 respectively.

Conclusion: Double mutation of binding site residues resulted in binding affinity as well as interactions. *In silico* analysis prior to wet lab experiments lead to rational choice of mutations that may lead to the production of rice grains with reduced amylopectin content.

Keywords: Starch branching enzyme, Maltopentaose, Docking, Starch

Introduction

Diabetes is a chronic metabolic disorder which has become a fast-growing global problem. Type 2 diabetes is the most common of diabetes prevailing in most of the population. 537

million adults (20-79 years) have been reported as diabetes patients in 2021 and this number is predicted to rise to 643 million by 2030 and 783 million by 2045 [1]. Glycemic index (GI) is a number that measures the rate at which blood glucose level increases in our body after consumption of food. The food with higher GI tends to raise the blood sugar level higher and faster. So, food with low GI may help people suffering with diabetes in controlling the blood glucose levels.

Resistant starch consumption is known to decrease the glycemic index, as it is a kind of starch that is not digestible – not absorbed in the small intestine and is passed directly to the large intestine. High amylose content is related with the more resistant starch. The digestion of starch depends on the reassociation rate of amylose and amylopectin. On cooling, amylose content rapidly re-associates, forms a precipitate which resists digestion whereas amylopectin re-associates slowly and thus complexes formed are easily digestible [2]. Cereal crops with high amylose content are not widely available. Thus, there is an increasing demand to develop cereal crops with higher amylose content thereby high resistant starch to meet the nutritional requirements.

Rice is the world's second most important cereal crop (following only corn) with production of nearly 510 million metric tons of milled rice in 2020-21.It is a major staple food for more than 3.5 billion people in the world, particularly in Asia, parts of Africa and Latin America. Rice starch consists of 20% amylose and 80% amylopectin, approximately [3, 4]. Starch is the major storage polysaccharide produced in the plastids of higher plants, composed of two distinct types of glucose polymers, amylose and amylopectin [3]. It is osmotically inert, water insoluble and functions as both short- and long-term reserve carbohydrate [5]. Amylose is the linear polymer of glucose with α (1→4) glycosidic bonds and amylopectin is a branched polymer containing α (1→4) and α (1→6) linked glucose units. Biosynthesis of starch (amylose and amylopectin) is catalysed via cascade of enzymes. The two polymers are synthesized via two different pathways involving different enzymes – the amylose synthesis requires granule bound starch synthase enzyme (GBSS), ADP- glucose pyrophosphorylase (AGPase) and amylopectin synthesis pathway involves combined activity of several enzymes with different isoforms - AGPase, starch synthase (SS), starch branching enzymes (SBEs), starch debranching enzymes (SDBEs), pullulanase (PUL), and phosphorylases (Phos) [6].

Starch branching enzyme (SBE) is the only enzyme that introduces α (1→6) glycosidic bonds and increases the branching density. SBEs catalyze the transglycosylation of α (1→4)

glycosidic linkages resulting in the formation of α (1→6) branch points within α (1→4) glucans [7]. There are two isoforms of SBE found in cereals- SBEI and SBEII. SBEI and SBEIIb are known to be expressed specifically in endosperm while SBEI expresses in every tissue in rice [8]. Biochemical analyses conducted by Satoh *et al.,* (2003) for the deficient mutants depicted that reduction in BEIIb causes a specific decrease in short chains with a degree of polymerization DP >13, with the greatest decrease occurring in chains with DP 8–11, whereas a lack of BEI caused the synthesis of amylopectin to contain fewer medium-sized ($16 < DP < 23$) and long ($DP > 37$) chains [9].

Molecular docking facilitates virtual screening of molecules in short time for facilitating the structure-based drug design or to know the interaction of protein with the ligand and to reduce the screening time [10]. Based on the advances in the field of molecular docking, molecular simulation and release kinetics, biomolecules can be tested against the potential protein targets to know the binding interactions. Thus, advances in computational biology tools led to the foundation of *in silico* study of biomolecules with proteins prior to the wet lab experiments.

Further, molecular docking is an efficient bioinformatics tool primarily used for identification of mode of action of small molecules against potential protein targets for the development of novel drug molecules or assessing the efficacy of biomolecules for testing the inhibiting or activating the target proteins based on the behaviour of small molecules to binding pockets of target proteins [11]. Similarly, molecular docking can be used for other aspects like studying interactions of protein-protein, protein-ligand, protein-DNA.

Three-dimensional crystal structure of BEI in complex with maltopentaose at a resolution of 2.2 Å with molecular replacement was reported by Chaen *et al.,* (2002) [12]. Maltopentaose was shown to be linked to a hydrophobic pocket created by the N-terminal helix, CBM48, and α-amylase domain, while glucose moieties may be localised at different surfaces on the N-terminal helix (α2) and CBM48. Three sites were reported in interactions observed in the crystal structure of the BEI–maltopentaose complex, naming Site 1 (maltopentaose), Site 2 & 3 (glucose). The amino acid residues reported at these sites were reported to be conserved in most isoforms of branching enzyme in various crops. Li *et al.,* studied the effect of 5 single mutations (Y235F, E399D, S232F, R342K and R246K; numbering as in SBEI) in corn SBEIIa which were reported to be catalytic active in SBEI and are highly conserved in a variety of α-amylase enzymes [13]. Go *et al.,* (2015) rationalized the above observations in

terms of structural model by using combination of molecular dynamics (MD) simulation and docking approaches and examined the effect of mutations R342K, E399D, Y235F on the binding of maltopentaose [14].

In this study, molecular modelling and docking study was carried out to understand the binding of maltopentaose in the wild and mutated starch branching enzyme of rice (SBEIIb).

Materials and Methods

Selection and molecular modelling of protein targets

The experimentally validated 3D structures for rice SBEIIb protein were not present so computational modelling was performed using a bioinformatic workspace. The UniProt (https://www.uniprot.org) database was utilised to retrieve the protein target sequences of *Oryza sativa indica* SBEIIb enzyme. The Uniprot ID of the rice SBEIIb protein is A2X5K0**.** The FASTA format of the protein was submitted to NCBI's web BLASTp for performing similarity search against PDB database. Query coverage of >80% and sequence identity of >30% was used as criteria to select the template protein for modelling.

SWISS-MODEL (Method: Rigid-body assembly) was used to model both the wild and mutated protein. Three-dimensional structure of wild and mutated SBEIIB were modelled by blasting against PDB, followed by HHblits in the homology modelling methodology [15].

Model Validation of protein targets

Structural Analysis and Verification of protein server (SAVES), the PROCHECK programme (https://saves.mbi.ucla.edu/) was used for analysing the energy and stereo-chemical geometry of the protein structures. Ramachandran plot was built for both normal and modified protein using the PROCHECK tool to find whether the residues are in the energetically favoured region or not. The protein structure in PDB format was subjected to energy minimisation, followed by loop building for removal of amino acids residues present in the disallowed regions observed in Ramachandran plot with the help of SWISS-PDB Viewer.

Preparation of the Ligand and its analysis

Structure of the ligand maltopentaose was obtained in SDF format from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). The SDF format was converted to PDB format using Open Babel software.

Maltopentaose Binding sites

Maltopentaose binding site residues of BEIIb was identified by performing pairwise sequence alignment with BEI enzyme which are highly conserved. Binding site residues were identified as Y178, E179, W206, P208, E233, F234, D423, T424, W448, E449 and R452 respectively.

Molecular docking interactions and virtual screening

PyRx 0.8's AutoDock vina module was used for the molecular docking [16].'Make macromolecule' and 'Make ligand' options were used for the preparation of the protein and the ligand molecule in the PyRx programme. For the energy minimization of ligand,conjugate gradient first-order derivatives of an optimization procedure with 200 steps, and commercial molecular mechanics parameters-Unified Force Field (UFF) were used. The maltopentaosebinding sites for BEIIb were taken corresponding to the ones reported by Chaen *et al.* for BEI as the sites remain conserved [12]. AutoDock4 and autogrid4 parameter files were used for grid configuration and docking.During the execution of the docking technique,BIOVIA Discovery studio client 2021 (https://www.3ds.com/products-services/biovia/) was used to visualise the interactions of docked conformations of protein-ligand complexes. Different colours were assigned for distinguishing between the receptor, ligand, and interacting atoms. The docking study was performed with the protein structure modelled using 3aml Os06g0726400 protein (Starch Branching Enzyme I (BEI) from Oryza sativa L) as a template and maltopentaose as ligand molecule.

Protein-protein interaction

The STRING database was used to investigate the interactions of starch branching enzyme IIb with that of other enzymes in starch synthesis pathway [17]. The information about query

proteins and the protein domains with which they interact, in addition to the number of other interacting domains in the network that belong to a specific domain, were studied. Genes and proteins that interact with one other were used to build a degree-sorted network. In order to better comprehend the functional significance and association between the query protein and its interaction partners, the protein domains of all the interacting partners were examined. The network was built with a medium level of confidence of 0.40 and a minimum of 10 interacting partners.

Results

Modelling of the rice SBEIIb protein

Three-dimensional molecular modelling of the SBEIIb enzyme was done by using SWISS-MODEL software by using a template protein - Starch Branching Enzyme I (BEI) (PDB ID: 3AML, Locus ID: Os06g0726400) from Oryza sativa L The reported parameters such as Global Mean Quality Estimation (GMQE) score around 0.71, sequence identity percentage – 59.79%, similarity percentage – 49% and maximal query coverage – 82% were used to ensure the quality of modelled structures (Figure 1).

The sequence retrieved from Uniprot database of normal SBEIIb protein is as-

>SBEIIb

MAAPASAVPGSAAGLRAGAVRFPVPAGARSWRAAAELPTSRSLLSGRRFPGAVRVG
GSGGRVAVRAAGASGEVMIPEGESDGMPVSAGSDDLQVPALDDELSTEVGAEVEIE
SSGASDVEGVKRVVEELAAEQKPRVVPPTGDGQKIFQMDSMLNGYKYHLEYRYSL
YRRLRSDIDQYEGGLETFSRGYEKFGFNRSAEGVTYREWAPGAHSAALVGDFNNWN
PNADRMSKNEFGVWEIFLPNNADGSSPIPHGSRVKVRMETPSGIKDSIPAWIKYSVQA
AGEIPYNGIYYDPPEEEKYIFKHPQPKRPKSLRIYETHVGMSSTEPKINTYANFRDEVL
PRIKKLGYNAVQIMAIQEHAYYGSFGYHVTNFFAPSSRFGTPEDLKSLIDKAHELGLV
VLMDVVHSHASNNTLDGLNGFDGTDTHYFHSGSRGHHWMWDSRLFNYGNWEVLR
FLLSNARWWLEEYKFDGFRFDGVTSMMYTHHGLQVAFTGNYSEYFGFATDADAVV
YLMLVNDLIHGLYPEAITIGEDVSGMPTFALPVQDGGVGFDYRLHMAVPDKWIELL
KQSDESWKMGDIVHTLTNRRWSEKCVTYAESHDQALVGDKTIAFWLMDKDMYDF
MALDRPATPSIDRGIALHKMIRLITMGLGGEGYLNFMGNEFGHPEWIDFPRAPQVLP
NGKFIPGNNNSYDKCRRRFDLGDADYLRYRGMLEFDRAMQSLEEKYGFMTSDHQYI

SRKHEEDKMIIFEKGDLVFVFNFHWSNSYFDYRVGCLKPGKYKVVLDSDAGLFGGF
GRIHHTAEHFTADCSHDNRPYSFSVYSPSRTCVVYAPAE

(it is modified or change)

Modelling of the modified protein SBEIIb target using SWISS-MODEL

Mutated SBEIIb enzyme were also modelled using the same procedure mentioned above. The results were as following Global Mean Quality Estimation (GMQE) score around 0.70, sequence identity percentage – 60.57%, similarity percentage – 49% and maximal query coverage – 81% respectively was observed for the modelled structure.

The mutated sequence of SBEIIb protein is as-

>SBEIIb_Mutation_Y178A_F234A

MAAPASAVPGSAAGLRAGAVRFPVPAGARSWRAAAELPTSRSLLSGRRFPGAVRVG
GSGGRVAVRAAGASGEVMIPEGESDGMPVSAGSDDLQVPALDDELSTEVGAEVEIE
SSGASDVEGVKRVVEELAAEQKPRVVPPTGDGQKIFQMDSMLNGYKYHLEYRYSL
YRRLRSDIDQAEGGLETFSRGYEKFGFNRSAEGVTYREWAPGAHSAALVGDFNNWN
PNADRMSKNEAGVWEIFLPNNADGSSPIPHGSRVKVRMETPSGIKDSIPAWIKYSVQ
AAGEIPYNGIYYDPPEEEKYIFKHPQPKRPKSLRIYETHVGMSSTEPKINTYANFRDEV
LPRIKKLGYNAVQIMAIQEHAYYGSFGYHVTNFFAPSSRFGTPEDLKSLIDKAHELGL
VVLMDVVHSHASNNTLDGLNGFDGTDTHYFHSGSRGHHWMWDSRLFNYGNWEVL
RFLLSNARWWLEEYKFDGFRFDGVTSMMYTHHGLQVAFTGNYSEYFGFATDADAV
VYLMLVNDLIHGLYPEAITIGEDVSGMPTFALPVQDGGVGFDYRLHMAVPDKWIEL
LKQSDESWKMGDIVHTLTNRRWSEKCVTYAESHDQALVGDKTIAFWLMDKDMYD
FMALDRPATPSIDRGIALHKMIRLITMGLGGEGYLNFMGNEFGHPEWIDFPRAPQVLP
NGKFIPGNNNSYDKCRRRFDLGDADYLRYRGMLEFDRAMQSLEEKYGFMTSDHQYI
SRKHEEDKMIIFEKGDLVFVFNFHWSNSYFDYRVGCLKPGKYKVVLDSDAGLFGGF
GRIHHTAEHFTADCSHDNRPYSFSVYSPSRTCVVYAPAE

(it is change)

Model validation of the wild type protein

The protein model was analysed using SAVES server and from Ramachandran plot it was found that there were 88.5% residues in most favoured region, 10.5% in additional allowed regions, 0.8% in generously allowed region and 0.2% residue (ILE 285A) in disallowed

region respectively. This protein model was subjected to energy minimisation and loop building to refine the residues in the disallowed region. After building loop, there were 88.6% residues in most favoured region, 10.7% in additional allowed regions, 0.7% in generously allowed region and 0.0% residue in disallowed region respectively (Figure 2).

Model validation of the mutated protein

The protein model was analysed using SAVES server and from Ramachandran plot it was found that there were 88.9% residues in most favoured region, 9.9% in additional allowed regions, 1.0% in generously allowed region and 0.2% residues (ILE 285A) in disallowed region. This protein model was subjected to energy minimisation and loop building to refine the residues in disallowed region. After building loop, there were 88.1% residues in most favoured region, 11.1% in additional allowed regions, 0.9% in generously allowed region and 0.0% residue in disallowed region. (Figure 3).

Virtual screening and molecular docking

Maltopentaose

Docking studies were used to investigate the binding affinity of protein structure SBEIIb with the compound Maltopentaose. The binding affinity of Maltopentaose with the wild type protein target SBEIIb was -7.5 kcal/mol (H-bonds: TYR178, GLU179, LYS191, PHE234, ASP423, THR424, ASN444, ASN447) (Figure 4).

The binding affinity of Maltopentaose with the target SBEIIb was -5.8 kcal/mol (H-bonds: GLU179, ASN232, ARG452) (Figure 5).

Protein-protein interaction

The STRING database suggested 10 enzymes that interact with SBE enzyme (1,4-alpha-glucan branching enzyme). The interacting enzymes are Granule-bound starch synthase 1, Putative isoamylase-type starch debranching enzyme, Protein Disproportioning enzyme 2, Granule-binding starch synthase II, Granule-bound starch synthase II, 1,4-alpha-glucan-branching enzyme, 4-alpha-glucanotransferase DPE1, Soluble starch synthase 1, Alpha-1,4 glucan phosphorylase, Putative isoamylase-type starch branching enzyme ISO2. Table 3 provides the information regarding the interacting proteins and their functions (Figure 6).

Discussion

Molecular docking is the key tool in structural molecular biology for predicting the interaction between ligand and the protein. Docking is a technique for doing virtual screening on enormous chemical libraries, ranking the outcomes, and putting forward structural theories for how the ligands block the target. So, prior to in vitro studies the *in-silico* studies help us to predict the different interaction analysis between proteins or proteins with biomolecules or protein with DNA. Li *et al*.,(2015). reported five different single-mutations (Y235F, E399D, S232F, R342K and R246K; numbering in SBEI) in SBEIIa protein derived from corn [13]. The mutation Y235F, E399D, S232F reported an overall loss of branching activity, mutation R342K resulted in an increase of shorter branches (DP<3) and a modest decline in activity. R246K, on the other hand, which is located on the opposing branches of the enzyme facing away from the core binding area, had no effect on activity. Since, the mutations reported by Li *et al.,* were structurally conservative, Go *et al*., (2015) studied the effect of R234K, E399D, Y235F mutations on the binding of maltopentaose in SBEIIa to rationalise experimental observations [14]. In this study, we took the maltopentaose binding sites reported by Chaen *et al*., for SBEI protein and aligned the sequences of SBEI and SBEIIb to get the corresponding sites in SBEIIb and further carried out a dual mutation to check the interaction effect. The binding affinity of normal starch branching enzyme (target protein of rice) with the maltopentaose was found to be -7.5kcal/mol but after mutating amino acid residues Trp178Ala (Y178A) and Phe234Ala (F234A) the binding affinity of maltopentaose with the modified protein was -5.8kcal/mol which implies that there is decrease in binding affinity between the maltopentaose and starch branching enzyme which will lead to misfunctioning or malfunctioning of starch branching enzyme. Due to malfunctioning of the SBEIIb enzyme will lead to formation of longer straight chains with lesser branching, ultimately there will be high amylose content and low amylopectin content potentially resulting in slower digesting starch. The same work can be carried out using wet lab experiments to prove that due to mutation in starch branching enzyme leads to malfunctioning of this enzyme and the final product will be having more amylose content.

Conclusion

Limited progress has been achieved in attempts to create SBEs in order to change the structure and consequently the functional qualities of starches. This is due, in part, to a lack of fundamental knowledge on the interactions between SBEs and their substrates. In this study, we've demonstrated how docking techniques can be used to pinpoint specific residues for future mutagenesis studies, helping to provide basic mechanistic explanations crucial for the creation of starch with improved functional properties like high amylose content for the various metabolic disorders occurring due to high glycemic index and low fibre content of food. We have shown the interaction of maltopentaose with the normal and mutated SBEIIb protein through the dual mutation of Y178A & F234A causing decrease in the binding affinity and number of hydrogen bonds. These results can be subjected further to molecular simulation for providing the additional confidence in the validity of results. Overall, the research highlights the value of such computational methods for comprehending the fundamental biosynthetic processes at the atomic level and should make it easier to create the varieties containing desired starch with improved functional characteristics.

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Figure 1 3D Structure of ligand (Maltopentaose) and protein (Starch branching enzyme)

Figure 2, 3D and 2D Interaction of normal protein (SBEIIb) with the ligand (Maltopentaose)

Figure 3, 3D and 2D Interaction of mutated protein (SBEIIb) with the ligand (Maltopentaose)

Figure 4 Ramachandran Plot for Wild-type SBEIIb Protein

Figure 5 Ramachandran Plot for mutated SBEIIb Protein

Figure 6 Protein-protein interaction of SBEIIb

UNIPROT ID	PROTEIN TEMPLATE ID	IDENTITY %	SIMILARITY %	GMQE VALUE	COVERAGE %
A2X5K0	**3AML**	**59.79**	**49**	**0.71**	**82**

Tables

Table 1 Details of modelled wild type rice SBEIIb protein

Table 2 Details of modelled mutated rice SBEIIb protein

UNIPROT ID	**PROTEIN TEMPLATE ID**	**IDENTITY %**	**SIMILARITY %**	**GMQE VALUE**	**COVERAGE %**
A2X5K0	3AML (Os06g0726400 protein Starch Branching Enzyme I (BEI) from Oryza sativa L)	60.57%	49	0.70	81

Table 3 Details on protein interacting with SBEIIb in PPI (Protein-protein Interaction) Analysis

S.no	Enzyme	Function
1.	Granule-bound starch synthase 1	Required for the synthesis of amylose in

		endosperm
2.	Putative isoamylase-type starch debranching enzyme	Starch debranching enzyme that plays a role in the degradation of transitory starch during the night in leaf blades, facilitates the formation of spherical amyloplasts containing compound granules in the endosperm, and affects morphological characteristics of plastids, belongs to glycosyl hydrolase 13 family.
3.	Protein Disproportioning enzyme 2	Cytosolic alpha-glucotransferase essential for the cytosolic metabolism of maltose, an intermediate on the pathway by which starch is converted to sucrose in leaves at night.
4.	Granule-binding starch synthase II, Granule-bound starch synthase II	Belongs to the glycosyltransferase 1 family, bacterial/plant/glycogen synthase subfamily
5.	1,4-alpha-glucan-branching enzyme	Catalyzes the formation of the alpha-1, 6-glucosidic linkages in starch by scission of 1,4-alpha-linked oligosaccharide from growing alpha-1,4-glucan chains and the subsequent attachment of the oligosaccharide to the alpha-1, 6 position
6.	4-alpha-glucanotransferase DPE1	Chloroplastic alpha-glucanotransferase involved in maltotriose metabolism
7.	Soluble starch synthase 1	Synthesis of amylose
8.	Alpha-1,4 glucan phosphorylase	Phosphorylase is an important allosteric enzyme in carbohydrate metabolism. Enzymes from different sources differ in their regulatory mechanisms and in their natural substrates.

9.	Putative isoamylase-type starch branching enzyme ISO2	Starch-debranching enzyme involved in amylopectin biosynthesis in endosperm. Functions by removing excess branches or improper branches that interfere with the formation of double helices of the cluster chains of amylopectin and crystallization of starch.