



***In Silico* Evaluation of the Structural Dynamics of Beta-Amylase from Sweet Potato (*Ipomoea batatas*)**

David Akintayo Obe^{1*} and Toluwase Hezekiah Fatoki²

¹Department of Biochemistry, Faculty of Basic Medical Sciences, College of Medicine, University of Ibadan, Oyo State, Nigeria.

²Department of Biochemistry, Federal University Oye, PMB 373 Oye-Ekiti, Ekiti State, Nigeria.

Authors' contributions

This work was carried out in collaboration between both authors. Author DAO analyzed and wrote the first draft of the manuscript. Author THF reviewed and edited the manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

Background: Sweet potato tubers are invaluable crop that could serve both dietary and industrial purposes owing to its high β -amylase content. β -amylases play essential role in plant carbohydrate metabolism as well as in many industrial applications such as the malting process in the brewing and distilling industries.

Aim: This study aims at better understanding of the evolutionary and molecular properties, and structural dynamics of β -amylase of sweet potato using *in silico* approach.

Methodology: 16 of the 250 sequences that are at least 69% identity to the query sequence (P10537) were manually selected from UniProt database for further analysis.

Result: It has theoretical isoelectric point of 4.97 and molecular weight of 56 kDa. The root-mean-square fluctuation (RMSF) of sweet potato β -amylase showed possible conservation of the amino acid residues 105-130 and 260-345, with highest fluctuation in C-terminal loop (residues 443-498). The catalytic role of Glu187 and Thr344 in β -amylase of sweet potato has been elucidated, and it provided the missing link in the previously available mechanisms, while Cys96 is essential for the inactivation of enzyme activity.

*Corresponding author: E-mail: obedavidakintayo@yahoo.com;

Conclusion: Elucidation of molecular mechanisms of expression and catalytic activity, together with the understanding of physicochemical properties of β -amylase from sweet potato will help in development of useful applications that are of industrial importance.

Keywords: Sweet potato; *Ipomoea batatas*; β -amylase; *in silico* properties; dynamics; RMSF.

1. INTRODUCTION

Sweet potato (*Ipomoea batatas*) is among the important food crops in the world [1]. It is a sweet, starchy root vegetable that originated from Central America but now widely grown in various parts of the world [2]. It belongs to the family Convolvulaceae. Sweet potato is a rich source of carbohydrates, calcium, iron, vitamins and minerals [3,4]

Sweet potato been a higher plant, stores starch synthesized from its leaves chloroplasts for energy metabolism. The signaling for the breakdown of starch to maltose is one of the essential metabolic pathway in all higher plants [5]. The breakdown of starch is a hydrolytic process of linear glucan that involves the combined action of enzymes such as amylases, glucosidases, and disproportioning enzymes such as D-enzyme and glucan transferases [6].

Sweet potato has three major types of amylases; α -amylase, β -amylase and starch phosphorylase. Of these three, β -amylase is the most abundant as it accounts for 5% of the total soluble protein present in it, making sweet potato a rich source of β -amylase when compared to other sources like cereals, grains and wheat [3,7]. Also, the sweet taste it has after cooking is due to the abundance of maltose over other forms sugars indicating that β -amylase catalyzed reaction occurs the most in sweet potato [8].

β -Amylases (EC 3.2.1.2) are hydrolytic exoenzymes that cleave α -1,4 glucosidic bonds at the non-reducing end of polyglucan chains to produce maltose [6]. β -amylases (BAMs) are found in eukaryotes and bacteria, and they play a key role in plant biology as well as in many industrial applications, such as the malting process in the brewing and distilling industries [9]. According to Kaplan and Guy, the expression and activity of BAMs show a complex regulation by light, sugars, phytohormones, proteolytic cleavage, and abiotic stresses such as osmotic, salt, cold and heat stress [10].

BAM originated over 400 million years ago and expanded together with the differentiation of plants into organisms of increasing complexity [9], through both paralogous and orthologous evolutionary processes. Based on structural and functional similarity, BAMs are classified as members of the glycoside hydrolase 14 family (GH-14) [11]. The GH-14 family is sub-divided into GH-14A (β -amylases present in bacteria) and GH-14B (β -amylases found in plants) [12]. The three-dimensional structure of BAM has been determined for organisms such as sweet potato (*Ipomoea batatas*), barley (*Hordeum vulgare*), soybean (*Glicine max*), *Arabidopsis thaliana*, *Baculus polymyxa* and *Bacillus cereus* [13,14,15,16,17]. In all cases, β -amylase exhibits a well conserved (β/α)₈ barrel fold in the core domain and an active site in the cleft of the barrel [6,9,13]. The study carried out by Thomas et al. on recombinant soybeans (*Glicine max*) β -amylases (SBA) was able to establish the role of the carboxylic acid (pKa of 3.7) and imidazole (pKa of 7.0) groups present at the active site of the enzyme through the study of pH against activity studies of the enzyme [18]. Analysis of the crystal structure of SBA in complex with maltose suggested that Glu186 and Glu380 present at the active site of the enzyme may work through general acid-base mechanism of catalysis [16,19,20]. These two residues positions remain unchanged before and after the binding of the sugar molecules [6]. SBA inverts and cleaves starch from the non-reducing end [19]. A loop that has a conserved sequence of GGNVGDIV found in all known β -amylases has been suggested to be important substrate binding and product release. Val99 (found in the middle of the loop) and Asp101 interact with the glucose residues of the substrate by van der Waals contacts, confirming the vital role of this loop [6,16]. Although, many research works have elucidated the importance of the enzyme's active site residues in its catalysis in some organisms [6,21]. But the postulation of different mechanisms of action for β -amylases from these studies suggested that our understanding of starch degradation in plants is incomplete due to the fact that there was a disruption in allosteric communication between the putative secondary binding site and the active site [21].

Protein dynamics is central to all biological events such as bio-catalysis, cellular regulation and signal transduction [22]. The dynamics of protein structures defines their biological functions. Because the experimental investigation of protein flexibility is often difficult or impossible, computational approaches play a significant role in this field [23]. This work make use of computational methods to better understand the evolutionary and molecular properties, and structural dynamics of β -amylase of sweet potato.

2. MATERIALS AND METHODS

2.1 Pathway and Sequence Retrieval

The pathway of action of β -amylase in an organism metabolism was obtained from www.kegg.jp. The sequence of β -amylase from sweet potato was downloaded from Uniport with accession number of P10537 (AMYB_IPOBA) and 499 amino acid residues. Homologs that are of 68.5% minimum identity was extracted by Blastp from the Uniprot database and the FASTA format of the sequences was downloaded and saved in MS-word for further analysis.

2.2 Sequence Alignments and Phylogenetic Analyses

Evolution Distance Analysis was conduct according to the method of Fatoki [24]. The sequence ID retrieved from UniProt, was entered into the query box of NCBI Blastp and ran against SWISS-PROT database. The BLAST Tree View for Fast Minimum Evolution was obtained. Phylogenetic analysis involved the retrieved BAM sequences which were aligned using ClustalO multiple sequence alignment tool (<https://www.ebi.ac.uk/Tools/>) at default setting. The newick format of the generated phylogenetic tree on EMBL-EBI was viewed using phylo.io.

2.3 Enzyme Subcellular Localization

The enzyme domain was predicted by using ScanProsite tool from expasy (<http://expasy.org/tools/scanprosite/>). Subcellular localization of β amylase of *Ipoma batata* was predicted by using WoLF PSORT (<https://wolfpsort.hgc.jp/>) [25]. Four different subcellular compartments appeared in the output with numerical scores, the one with the highest value was picked and considered valid as the predicted compartment [26].

2.4 Sequence Properties Prediction

The enzyme sequence was analyzed by using the prediction server (SignalIP, NetOGlyc and NetNGlyc) available at the <http://www.cbs.dtu.dk/services/> to identify the presence of a signal peptide and number and site of N- and O-glycosylation, in the enzyme respectively. The protein properties such as theoretical molecular weight, isoelectric point, and hydropath plot of β amylase was obtained using EMBOSS Pepstat and EMBOSS Pepwindows, at default settings. The sequence of P10537 (AMYB_IPOBA) that was retrieved from UniProt was run against PDB. The structure obtained was viewed and modeled with Cn3D viewer.

2.5 Structure Flexibility Dynamics

The structural dynamics of β -amylase of sweet potato (PDB: 5WQU, chain A) which was prepared with PyMol software was investigated. The protein was then subjected to fast simulation of structural flexibility using CAB-flex 2.0 server at default settings [23]. The contact map and root-mean square fluctuations (RMSF) of amino acid residues in the server-analyzed protein were obtained.

3. RESULTS AND DISCUSSION

β -Amylase (EC 3.2.1.2) is an enzyme that produces maltose by hydrolyzing the alpha-1,4-glycosidic bond in starch or glycogen molecules. Fig. 1 is a segment of carbohydrate metabolism in an organism, showing the position of β -amylase in the pathway. An initial search for β -amylase (*Ipomea batata*) on UniProt database returned two hits. P10537 (AMYB_IPOBA) was picked for this analysis because it has been curated. The accession number ran on UniProt blast generated 250 sequences, out of which 16 sequences that are at least 69% identity to the query sequence were manually selected.

The multiple sequence alignment (MSA) showed the presence of three highly conserved regions that are found in all known β amylases. The conservation of a loop motif "His-Gln-Cys-Gly-Gly-Asn-Val-Gly-Asp" close to the N-terminal observed in the sequences, Thr345, and two catalytic glutamic residues (Glu188 (proton donor) and Glu383 (proton acceptor) in sweet potato β -amylase were observed in all the species [6].

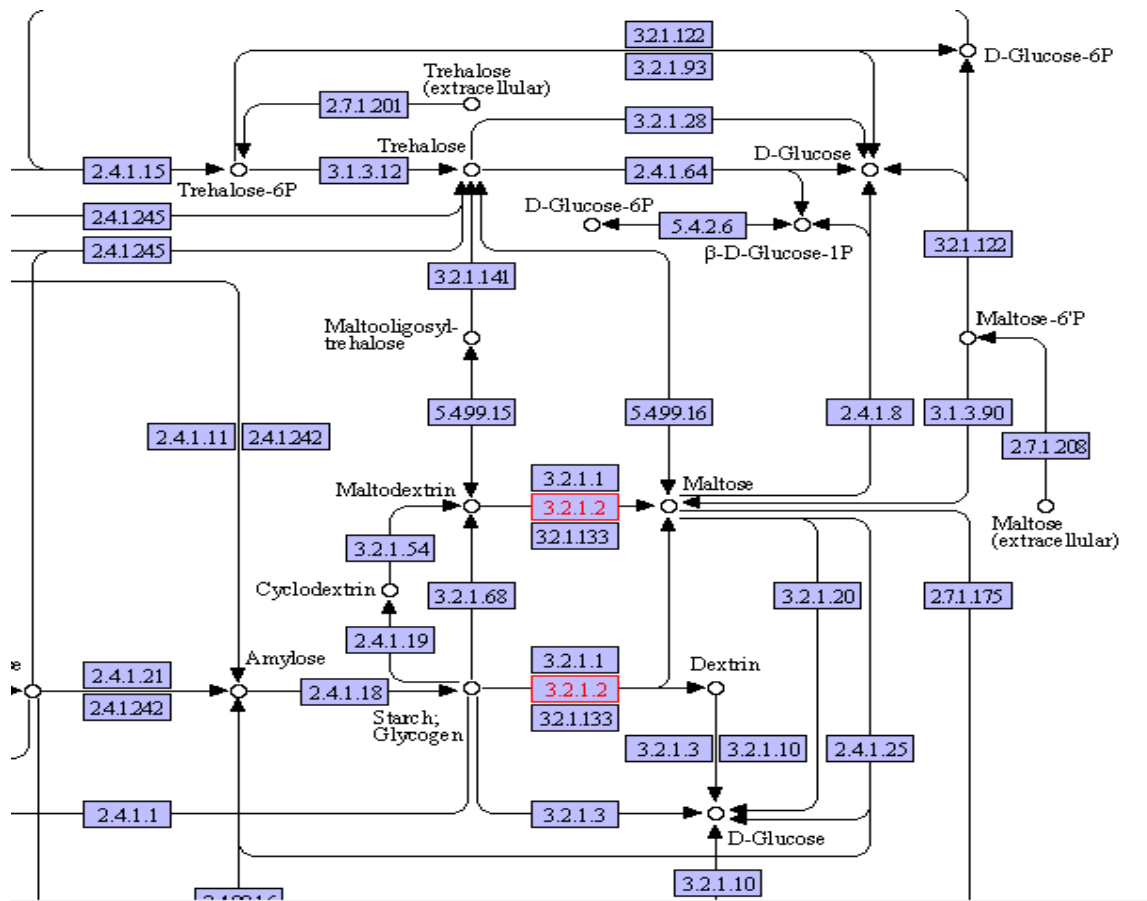


Fig. 1. Pathway showing the action of B-amylase (3.2.1.2) on starch, glycogen and maltodextrin (www.kegg.jp)

sp P10537 AMYB_IP0BA	DELKQVKAGGCDGVMVDWMGII EAKGPKQYDWSAYRSLFQLVKKCGLKIQA IMSF	HQC GGNVGD
tr A0A068VI38 A0A068VI38_COFCA	KQLKKLKEIGVDGIMVDWMGII ENKGPKEWAAAYRSLFELVVKIGLKIQA IMSF	HQC GGNVGD
tr A0A0J8EGY4 A0A0J8EGY4_BETVU	RQLYKLEAEVDGVMVDWMGII ESKGPKQYDWKAYRSLFQVIKDCGLKVA IMSF	HQC GGNVGD
tr A0A0J88BR4 A0A0J88BR4_BETVU	RQLYKLEAEVDGVMVDWMGII ESKGPKQYDWKAYRSLFQAIKDCGLKVA IMSF	HQC GGNVGD
tr A0A4U5QID5 A0A4U5QID5_POPAL	KQLKDLRAAIDGVMVDWMGII EAKGPKQYDWSAYRSLFELVNRCDLKIQA IMSF	HQC GGNVGD
tr A0A2K1X2Z8 A0A2K1X2Z8_POPTR	KQLKDLRAAGIDGVMVDWMGII EAKGPKQYEWASAYRSLFELVVKCDLKIQA IMSF	HQC GGNVGD
tr A0A067J5U2 A0A067J5U2_JATCU	KQLNELRAAGVDGVMVDWMGII EVGKPEQYDWSAYRSLFELIQCCDLKIQA IMSF	HQC GGNVGD
tr A0A445E0P5 A0A445E0P5_ARAHY	KQLLQLEAGVDGVMVDWMGII ELNGPKQYDWTYRSLFQVQECGLKVA IMSF	HQC GGNVGD
tr I1LRU3 I1LRU3_SOYBN	EQLLQLEAAGVDGVMVDWMGII ELKGPKEWAAAYRSLFQVQECGLTQA IMSF	HQC GGNVGD
tr A0A445HN72 A0A445HN72_GLYSO	EQLLQLEAAGVDGVMVDWMGII ELKGPKEWAAAYRSLFQVQECGLTQA IMSF	HQC GGNVGD
tr A0A445KFZ4 A0A445KFZ4_GLYSO	EQLLQLEAAGVDGVMVDWMGII ELKGPKEWAAAYRSLFQVQECGLTQA IMSF	HQC GGNVGD
tr Q42795 Q42795_SOYBN	EQLLQLEAAGVDGVMVDWMGII ELKGPKEWAAAYRSLFQVQECGLTQA IMSF	HQC GGNVGD
tr F6HIM2 F6HIM2_VITVI	KQLKELRAAGVDGVMVDWMGII ESKGPKQYDWTAYRSLFQAVQERGLKVA IMSF	HQC GGNVGD
tr A0A5E4G1X6 A0A5E4G1X6_PRUDU	KELKELRAAGVDGVMVDWMGII ESKGPKQYDWTAYRSLFQAVQERGLKVA IMSF	HQC GGNVGD
tr A0A5E4G1J1 A0A5E4G1J1_PRUDU	KELKELRAAGVDGVMVDWMGII ESKGPKQYDWTAYRSLFQAVQERGLKVA IMSF	HQC GGNVGD
tr A0A251Q3X9 A0A251Q3X9_PRUPE	KELKELRAAGIDGVMVDWMGII ESKGPKQYDWTAYRSLFQAVQECGLKVA IMSF	HQC GGNVGD

Fig. 2. A Segment of Multiple Sequence Alignment of P10537 and other homologs (Red = small, hydrophobic, aromatic. Blue = acidic. Magenta= basic. Green = hydroxyl, amine, amide, basic. Gray = others. "*" = identical, "." = conserved substitutions (same colour group), "-" = semi-conserved substitution (similar shapes). The red box shows the conservation of a loop motif "His-Gln-Cys-Gly-Gly-Asn-Val-Gly-Asp" close to the N-terminal observed in the sequences.

Since orthology does not define evolutionary distance, the Fast Minimum Evolution provides the overview of the evolutionary similarity as shown in Fig. 3A. The β amylase of sweet potato (*Ipomoea batata*) belongs to the taxonomic lineage of *Eudicots*. P10537 showed evolutionary relationship with the proteins from monocots and firmicutes (phylum of gram-positive bacteria) suggesting that the enzyme might have evolved from bacteria origin [6]. The cladogram of the tree data from phylogenetic analysis of multiple sequence alignment gave the same result as that viewed in phylo.io but was more comprehensive than that viewed at NGPhylogeny.fr. The phylogenetic tree obtained from phylo.io (Fig. 3B)

revealed three clusters, of which β amylase of sweet potato is grouped in the same cluster with *Populus trichocarpa* (A0A2K1X2Z8), *Populus alba* (A0A4U5QID5), *Jatropha curcas* (A0A067JSU2), *Coffea canephara* (A0A068VI38), *B Vulgaris subsp. Vulgaris* (A0A0J8EGY4 and A0A0J8BBR4). *Coffea canephara* is an ortholog of β amylase (AYMB_IPOBA).

The WoLFPSORT prediction showed that β -amylase of sweet potato (*Ipomoea batata*) is an extracellular enzyme as also confirmed in by other scientific researches [6,8,27]. Amino acid sequence VNADN was predicted as the signal

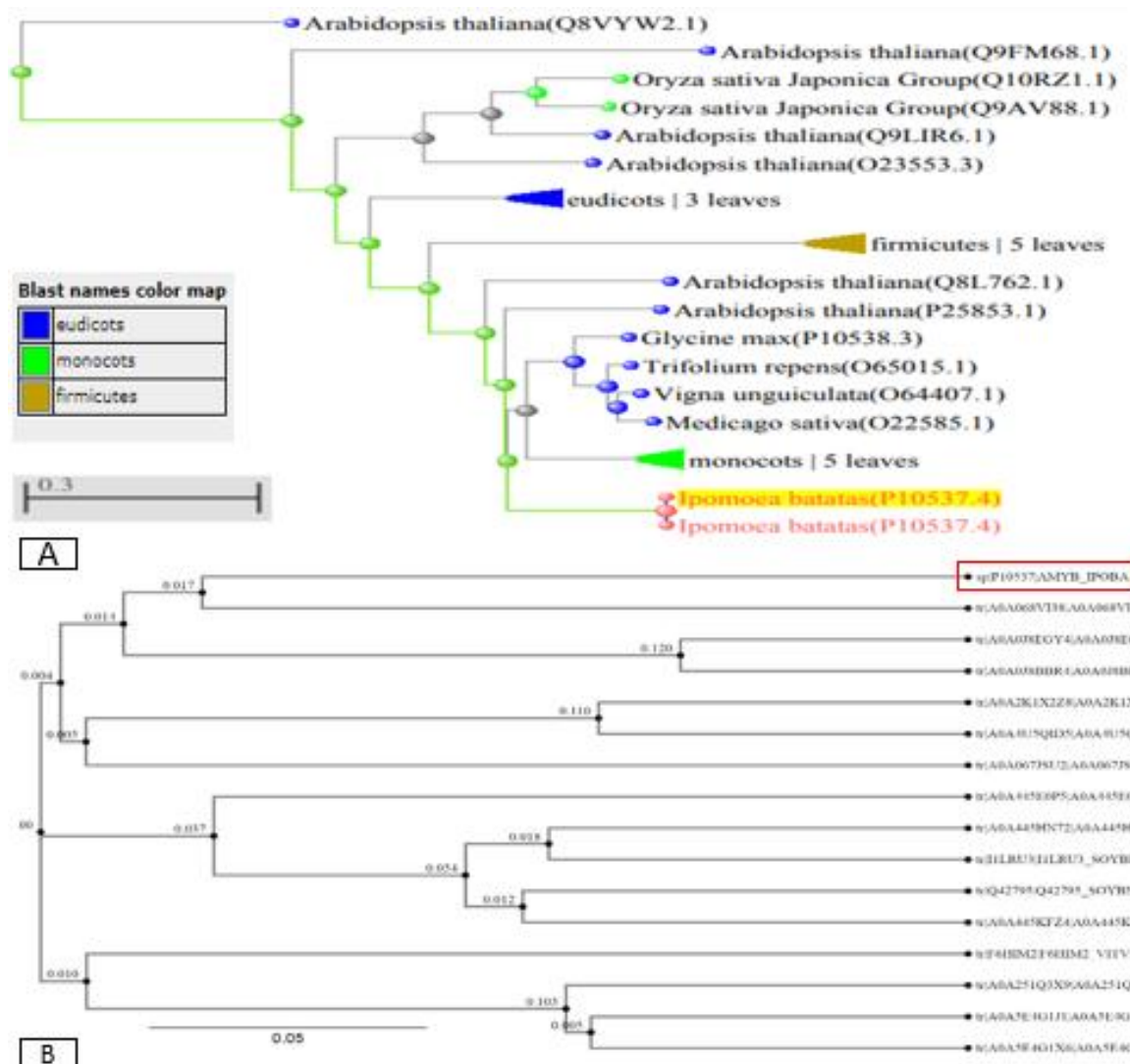


Fig. 3. (A) P10537 Fast Minimum Evolution by BLAST Tree View. (B) Phylogeny visualized at phylo.io. Both showing sweet potato β -amylase in red

peptide with the 0.7408 likelihood of having a cleavage at the 27th amino acid from the N-terminal of the protein. This may explain why the protein is found predominantly in the extracellular and not targeted to a subcellular location. The enzyme has five (5) predicted possible O-glycosylation positions and three (3) N-glycosylation sites. Although, it has not been reported that β -amylase undergoes any glycosylation for its activities. Experimental evidence from UniProt showed that P10537 undergoes a posttranslational modification that result in the remover of the first amino acid (methionine).

The sequence statistics and hydropathy plot is shown in Table 1 and Fig. 4 respectively. Most of the amino acid residues tend toward hydrophobic (negative) region. This plot also suggest that the enzyme is not a membrane bound enzyme but an enzyme free with the cytosol. In protein purification techniques such as 2D gel electrophoresis, X-ray crystallography, capillary isoelectric focusing and liquid chromatography-mass spectrometry, isoelectric point (pI) of a protein is an important factor to consider for the success of this analytical techniques in biochemistry. Isoelectric point occurs when the pH of a solution or molecule is said to be zero [28,29]. The optimum pH of a protein is dependent on the composition of the amino acids in its active site. Since protein-protein interaction is favoured over protein-water interaction, pI is

the determinant of the protein's minimum solubility level [30,31]. Careful observation of 9596 structures obtained from the PDB showed a relationship between pI and pH of a protein at which it would crystallize. The analysis showed that acidic proteins will crystallize at pH 0-2.5 above their isoelectric point while basic proteins crystallized 0.5–3.0 pH units below their pI [28,32]. This agrees with the data used in the purification of β amylase from sweet potato in most researches [2,6].

The apo structure of P10537 retrieved from PDB (5WQA_A) and viewed in Cn3D (Fig. 5) agrees with the observation of Cheong and colleagues that each subunit of sweet potato β -amylase is composed of a large $(\alpha/\beta)_8$ core domain and 15 loops connecting the alternating β -strands α – helix [33].

The value of RMSF is a measure of protein's residue flexibility over the entire time of simulation [34]. The higher RMSF value, the higher the flexibility of the protein. The more flexible a protein is, the less stable the protein. The RMSF of sweet potato β -amylase showed possible conservation of the amino acid residues 105-130 and 260-345 (Fig. 6). The regions with low RMSF indicate a rigid and stable structure. Overall, β -amylase of sweet potato has high fluctuating residues, with highest fluctuation in C-terminal loop (residues 443-498).

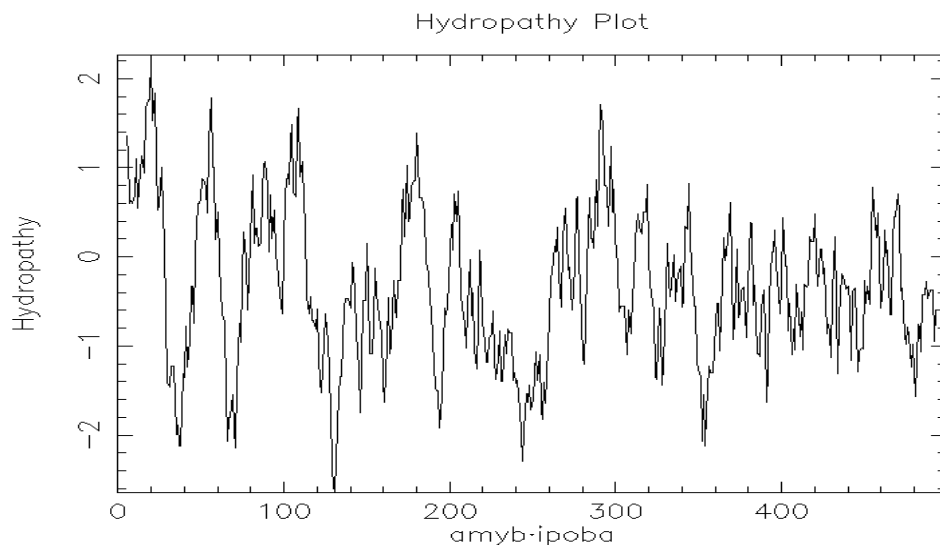


Fig. 4. Hydropathy plot of β amylase of sweet potato



Fig. 5. Cn3D view of β amylase of sweet potato

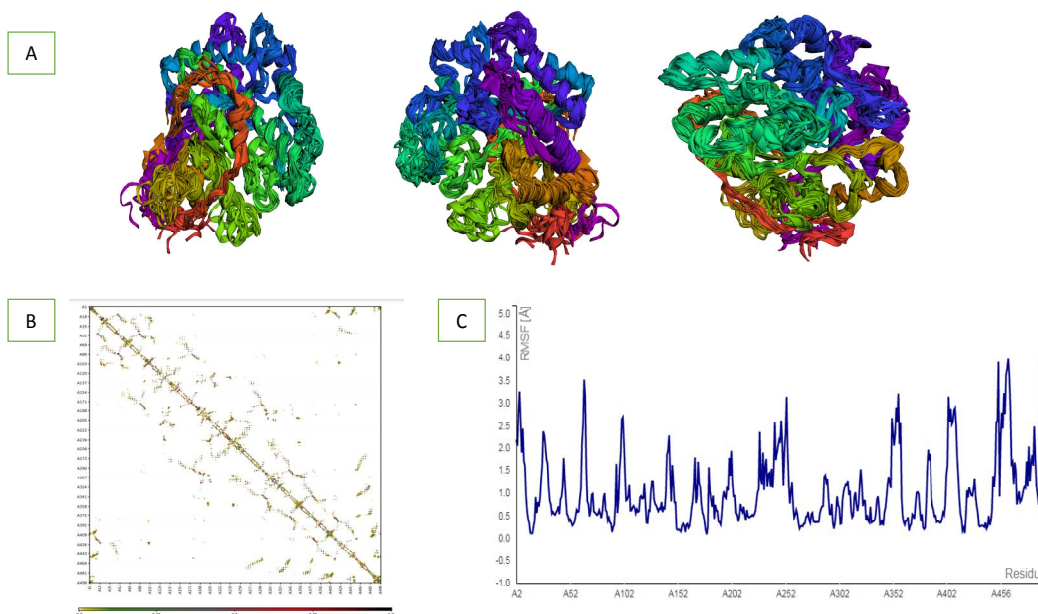


Fig. 6. Structural flexibility dynamics of β -amylase (PDB ID: 5WQU) (A) Cluster of 10 model structures (B) Contact map (C) Fluctuation plot

Table 1. Pepstats of P10537

Theoretical physicochemical properties	P10537
Amino acid Residues	499
Molecular weight	56KDa
Average Residue Weight	112.384
Net Charge	-10.0
Isoelectric Point	4.9669
A ₂₈₀ Molar Extinction Coefficients (reduced/cystine bridges)	98780/99155
A ₂₈₀ Extinction Coefficients 1mg/ml (reduced/cystine bridges)	1.761/1.768
Improbability of expression in inclusion bodies	0.577

It has been reported that the regions directly involved in the catalytic reactions of the soybean BAM includes Glu186, Thr342, and Glu380, while the flexible loop which involve amino acids 96–103, is essential for binding of the glucan chain and enzymatic activity [16,17]. There are five amino acid residues (Met51, Glu87, Asp176, Glu178, and Asn340 in soybean BAM) that are conserved in the higher plants [16]. To convert the optimum pH of soybean β -amylase (pH 5.4) to that of the bacterial type enzyme (pH 6.7), three mutants of soybean β -amylase, M51T, E178Y, and N340T, were constructed such that the hydrogen bond networks were removed by site-directed mutagenesis, and these three residues are located near Glu380 and are considered as candidates for the control of the pKa of Glu380 [34].

However, the catalytic role of Thr344 in β -amylase of sweet potato has been elucidated, and it provided the missing link in the previously available mechanisms obtained from soybean and barley [6]. Moreover, Glu187 was also found to play catalytic role while Cys96 is essential for the inactivation of enzyme activity [13].

4. CONCLUSION

Sweet potato tubers are invaluable crop that could be used as both dietary and industrial purposes owing to its high β amylase content. β -amylases play essential role in plant carbohydrate metabolism as well as in many industrial applications, such as the malting process in the brewing and distilling industries. Elucidation of molecular mechanisms of expression and catalytic activity, together with the understanding of physicochemical properties of β -amylase from sweet potato will help in development of useful applications that are of industrial importance.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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