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#### Original



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#### ABSTRACT

The major aim of this study is to introduce bimolecular structures from a bioinformatic perspective and informs about the bioinformatic softwares in this field. Each protein has a specific chemical or structural function, strongly suggesting that each has a unique three-dimensional structure. In this study, we investigated  $\alpha$ -amylases in Aspergillus oryzae. Among starch converting enzymes, the  $\alpha$ -amylases (endo-1, 4- $\alpha$ -D-glucan glucanohydrolase [E.C.3.2.1.1]) are of special importance and extensively studied. PDB and NCBI databases and Chimera, Predict Protein and Multalign softwares used for this research. By using these softwares, multiple analyses, including determination of residue composition, secondary structures, conserved regions, ligand binding site done. Starch-hydrolyzing enzymes such as amylases, pullulanases and glucoamylases play an important role in food, chemical, and pharmaceutical industries. In the certain applications like detergent and bakery industries the properties of  $\alpha$ -amylase needed is quite challenging Using of bioinformatic softwares improves our understanding about proteins 3D structures and their functions.

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## Introduction

The most known amylolytic enzymes are  $\alpha$ -amylase (EC 3.2.1.1),  $\beta$ -amylase (EC 3.2.1.2) and glucoamylase (EC 3.2.1.3) that are, however, quite different from each other. They don't only differ in their primary and tertiary structures, but also in their machineries and catalvtic reaction mechanisms employed. They have therefore been classified into different GH families: GH13- $\alpha$ -amylases, GH14- $\beta$ -amylases, and GH15–glucoamylases<sup>1</sup>. Among starch converting enzymes, the  $\alpha$ -amylases (endoglucanohydrolase 4-α-D-glucan 1. [E.C.3.2.1.1] are of special importance<sup>2</sup> and studied starch-hvdrolvzing extensively enzymes such as amylases<sup>3</sup>, pullulanases and glucoamylases play an important role in food. chemical and pharmaceutical industries<sup>4</sup>. In the certain applications like detergent and bakery industries the properties of α-amylase needed is quite challenging<sup>5</sup>. Fungal amylases have been used to manufacture starches, starch derivatives starch saccharification and products. The most important fungi used for  $\alpha$ -amylase production is Aspergillus oryzae, A. niger, and Rhizopus  $oryzae^{6,7}$ . Threedimensional structures of amylases shown in Figure 1.

### **Materials and Methods**

In this study the three-dimensional structure of the alpha-amylase enzymes in *Aspergillus oryzae* was studied in various aspects. At the first step, amino acid sequences of *Aspergillus oryzae*  $\alpha$ -amylase or Taka-Amylase A (PDB ID: 2TAA) was taken from the Protein Data Bank website. The amino acid sequence of this enzyme in several strains from NCBI databases were obtained and aligned by Multalign software. Three-dimensional structure of the enzyme was carried out with the help of Chimera UCSF software. Predict protein software was used for prediction of amino acid

content of the enzyme and its secondary structure.

#### **Results and Discussion**

Protein structure analysis

The Taka-Amylase A (EC=3.2.1.1) has three chains A, B and C that each of them has 479 amino acids in monomer structure with signal domain, and its molecular weight is 54,810 (Da). Amino acid sequence analysis of Taka-Amylase A for each chain (www.uniprot.org) shown in Table 1.

The application forms that conserved regions in the sequence of the enzyme determined by Multalign software that shown in Figure 2.

Secondary structure and solvent accessibility prediction for chain

PROFsec predicts secondary structure elements and solvent accessibility using evolutionary information from multiple sequence alignments and a multi-level system. Three states of secondary structure predicted: helix (H; includes alpha-, pi- and 3 10-helix), (beta-) strand (E = extended strand in beta-sheet conformation of at least two residues in length) and loop. Secondary structure predicted by a system of neural networks with an expected average accuracy of more than 72%. Evaluation of enzyme molecules indicated that the active form of the enzyme is composed of three chains in the same sequence and secondary structure of the enzyme is related to the structure shown in the following diagram. The secondary structure of the enzyme has 59% loop, 20.9 % helix and 20.09 % strand. Based on the sequence diagram of the bulk structure of the enzyme is made of loops and the amount of helix and strand is approximately equal. Amino acid and secondary structure, composition shown in Figures 3 and 4, respectively. The structure of the enzyme also



British Biomedical Bulletin examined in terms of solvent accessibility shown in Figure 5.

The NetPhos 2.0 server produces neural network predictions for serine, threonine and tyrosine phosphorylation sites in eukaryotic proteins. By this software phosphorylation site on enzymes identified. Figure 6 that shown phosphorylation site on enzyme structure.

This enzyme has three calcium ions in each of the three chains at position 479 that shown in Figure 7. The position of the essential calcium ion may be assumed to be near both site C and the maltose residual. The calcium ion may play a role in tightening the catalytic sites suitable for binding amylase and/or for supplying water molecules to the reaction.

## Conclusions

The illustration of the whole chain folding indicates some characteristic features of enzyme structure which have so far been recognized in general. The molecule is composed of two domains: the main and the C-terminal domains which are connected to each other by a single polypeptide chain. In fact, a molecular model of Taka-amylase A ( $\alpha$ -amylase) from *A. oryzae* by Matsuura et al suggests that both His and Asp in these homologous areas functions as active sites, while Asp and Lys in region 2 and His in region 4 may function as a substrate-binding sites. Since another active site of Glu and the neighboring substrate-binding sites of Val, Leu and Asp between regions 2 and 4 have been suggested<sup>8</sup>, the sequences were examined for an alignment containing these four amino acid residues. Understanding the three-dimensional structure of proteins is its function. Combining essential for Bioinformatics and biochemistry helps in our understanding of the biochemical model.

Today with the increasing volume of information, familiarity with Bioinformatics software is important to achieve desired results quickly and the accurately.

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No.	Feature key	Position (s)	Length	Description			
1	Signal peptide	1 – 21	21				
2	Chain	21 – 499	479	Alpha-amylase Atype-1/2			
3	Active site	227	1	Nucleophile			
4	Active site	251	1	Proton donor			
5	Binding site	56, 104, 143 225, 255, 317 365	1, 1, 1 1, 1, 1 1	Substrate			
6	Site	318	1	Transition state stabilizer			

**Table 1.** Amino acid sequence analysis of Taka-amylase a for each chain (www.uniprot.org)



	1	10	20	30	40	50	60	70	80	90	100	110	120	130
gi  2307541pdb  2TAAIA gi  403132761db j1BAD0 gi  3936598481db j1BAH gi  3583751521db j1GAA gi  5255800151gb  EPS2 Consensus	nnvau nvau nvau	wslflygl Irslflygl nnMilffl kflglaal	ATI qvAapalaATI qvAapalaATI sfvvsalaATI flAqtvaglTa aaTj	PADHRSQSIYFL PADHRSQSIYFL PADHRSQSIYFL PAEHRSQSIYFL PAEHRSQSIYFL PA#HRSQSIYFL	L TORFARTO L TORFARTO L TORFARTO L TORFARTO .MTORF&RTO .\$TORF&RTO	gSTTALCNTa gSTTALCNTa gSTTALCNTg NSTTASCD1s NSvTASCNTn nStTAsC#t.	DqkYCGGTH DqkYCGGTH DRkYCGGTH aRqYCGGsH DRvYCGGTH dr ,YCGGtH	QGIIDKLDYIQ QGIIDKLDYIQ QGIIDKLDYIQ QGIINQLDYIQ QGIINQLDYIQ QGII#qLDYIQ	SMGFTAINIT SMGFTAINIT SMGFTAINIT SMGFTAINIT SMGFTAINIT SMGFTAINIT	IPYTaQLPQDc IPYTaQLPQL1 IPYTaQLPQL1 IPYTEQ1PQD1 IPYTEQLPQD1 IPYTEQLPQD1 IPYTEQLPQdt	a <mark>ygdayl</mark> gyi aygdayhgyi aygdayhgyi gygqayhgyi gdgeayhgyi gdgeayhgyi aggfaayhgyi	4QtDIYslNef 4QQDIYslNef 4QQDIYslNef 4QQDayalNsf 4QQEIYnvNnf 4QqEIYnvNnf	IYGTADDLKA IYGTADDLKA IYGTADDLKA IYGTADDLKA IYGTADDLKA IYGTADDLKA	LSSALHE LSSALHE LSSALHE Lasalhs Lsgalhs Lsgalhs
	131	140	150	160	170	180	190	200	210	220	230	240	250	<b>26</b> 0
gi   230754  pdb   2TARIA gi   40313276  db ji BADO gi   393659848  db ji BAH gi   358375152  db ji GAA gi   525580015  gb   EPS2 Consensus	RGHYL RGHYL RGHYL RGHYL RGHYL RGHYL	HYDYYANH HYDYYANH HYDYYANH HYDYYANH HYDYYANH HYDYYANH	HGYdGAGsSVI HGYdGAGsSVI HGYdGAGsSVI HGhnGtGASVI HGYaGAGNtVI HGyaGAGNtVI	DYSYFKPFSSQC DYSYFKPFSSQC DYSYFKPFSSQC DYSYYrPFnSQL DYSYFKPFSSsc DYSYFKPFSSsc DYSYZkPFsSq	IYFHPfCfIq IYFHPfCfIq IYFHsfClIq IYFHnlCwIS IYFHpyClIS IYFHp,C,Is	NYEDQTQYED Nyedqtqyed Dyedqtqyen Nyenqtnyed Dysnqtnyed Hy. #qt#yed	CHLGDNTYS CHLGDNTYS CHLGDNTYS CHLGDNTYa CHLGDNTYS CHLGDNTYS	LPOLDTTkdvYI LPOLDTTkdvYI LPOLDTTkdeYI LPOLDTTrtdYI LPOLDTT1ssYa LPOLDTT1ssYa	KNeHYDHYg9 KNeHFDHYg9 KNeHYDHYgt KNeHYDHYe9 It i HYNHYsd Kn , HY#HY, s	EL VSNYSIDGL SL VSNYSIDGL L VSNYSIDGL SL VSNYSVDGL IL VSNYSIDGL SL VSNYSIDGL	RIDTYKHYQK RIDTYKHYQK RIDTYKHYQK RIDTYKHYQK RIDTYKHYEK RIDTYKHYEK	Kd <b>fhpgynka</b> f Kdfhpgynkaf Kdfhpgynkaf Knfhpgynna: Ksfhpgynaf K <b>.fhpgy#</b> , ar	IGVYCIGEVI IGVYCIGEVI IGVYCIGEVI GVYCIGEVF IGVYCVGEVF IGVYC!GEVF	JGDPAYT DGDPAYT DGDPAYT DGDaAYT SGDPAYT dGDpAYT
	261	270	280	290	300	310	320	330	340	350	360	370	380	<b>39</b> 0
gi 12307541 pdb 12TAR1A gi 140313276 ldb j1BAD0 gi 1393659848 ldb j1BAD gi 1358375152 ldb j1GAA gi 15255800151 gb 1EPS2 Consensus	CPYQN CPYQN CPYQC CPYQC CPYQN CPYQ4	IVHDGVLNY IVHDGVLNY IVHDGVLNY Idldgvlny Igldgvlny Igldgvlny	PIYYPLLnAFI PIYYPLLnAFI PIYYPLLnAFI PnYYPLLnAFi PIYYqLLgAFi PiYYpLL.AFi	KSTSGSndDLY) KSTSGSndDLY) KSTSGSnnDLY) ESTNGSTSDLY) KSTSGSTSSLY) KSTSGSTSSLY)	IMINTYKSDC IMINTYKSDC IMINTYKSDC IMINTYKSLC IMINSYASDC IMINCYKSdC	POSTLLGTFY POSTLLGTFY POSTLLGTFY POSTLLGTFY aDPTLLGnFI .DsTLLGtF!	enhdnprfa Enhdnprfa Enhdnprfa Enhdnprfa Enhdnprfa Enhdnprfa	SYTNDiaLAKN SYTNDiaLAKN SytnDiaLAKN NYTSDnSLAKN SYTSDySqAKN SYTSD,slAKN	/AaFIILnDG /AaFIILnDG /AaFIILnDG aALFLILaDG /isFIfLsDG va.FiiL.DG	IPITYAGQEC IPITYAGQEC IPITYAGQEC IPITYAGQEC IPIYYAGQEC IPIYAGQEC	IHY aGGNDPAN IHY aGGNDPAN IHY aGGNDPAN IHY SGGNDPAN IHY SGGNDPAN IHY SGGNDPAN	IREATHLSGY IREATHLSGY IREATHLSGY IREATHLSGY IREATHLSGY IREATHLSGY	)TdSELYk1I )TdSELYk1I )TdSELYk1I (TtSELYk1I (TtSELYtHI sknaQLYqHI ,t,s#LY,hI	ASANAIR ASANAIR ASANAIR ASSNAIR ASSNAIR ASSNAIR ASSNAIR
	391	400	410	420	430	440	450	460	470	480	490	500	510	<b>52</b> 0
gi 12307541 pdb 12TAA1A gi 140313276 ldb j1 BADO gi 13936598481 db j1 BAD gi 13583751521 db j1 GAA gi 15255800151 gb 1EPS2 Consensus	nyAI9 nyAI9 nyAI9 thAI9 slAI9 AI9	KDtGFvTY KDtGFvTY KDtGFvTY GDsGY1TY KDanYiTs KD.g%.Ty	KN-PyikDdt KNwPiYkDdt KNwPiYkDdt KNyPiYqDts KNnafYtDsn KN.p.y.D	TIANRKGLOGS TIANRKGLOGS TIANRKGLOGS TIANRKGynGLU TIANRKGysGSU TIANRKGGSU	)IVTILSNKG )IVTILSNKG )IVTILSNKG )UTTVLSNIG )VVTVLSN-G ).IT!LSN.G	ASGdSYTLSL AsgdSYTLSL AsgdSytlsl AsgSSYTLSL SSGSSYTLSL AsgSSYTLSL	SGasYTAGQ SGaGYTAGQ SGaGYTAGQ pGLGYTAGQ SGsGYeAGL SGsgYtAGq	qLtEvigCTtY qLtEvigCTtY qLtEvigCTtY einEiYTCsn1 kLvEnYTCTaY .1.E.ytCt.v	TVgSDGNVPV TVgSDGNVPV TVgSDGNVPV TVDSNGsVPV TVDSNGNTav TVDSNGNTav	'PMagGLPRV- 'PMagGLPRV- 'PMagGLPRV- 'PMkSGLPRI- 'SMLSGLPRVf 'PM.sGLPR!	LYPteKLa-( LYPteKLa-( LYPteKLa-( LYPadKLvn( Mlassacslo \$ypkl;	iSkiCSds5 iSkiCSss iSkiCSss iSSfCS iSSaCS1c5sa iSSaCS1c5sa iSSaCS1c5sa	acsatattlk	tttatat
	521	530	540	550	560	570	580	590	600	610	620	630		
gi   230754  pdb   2TAA   A gi   40313276   db j   BADO gi   393659848   db j   BAM gi   358375152   db j   GAA gi   525580015   gb   EPS2 Consensus	sctqa	talpvlfk	dtvatsygqsi	ylagsisqlgs	sunaanaval	sadkytssnp	luyatutlp	vgtsfqykfikl	ktsssgsvt.	vesdpnrsytv	ptgcvgstat	.vtatur		

**Figure 2**. Multiple sequence alignment by Multalign software. Accession numbers: Taka-Amylase A (2TAA|A), alpha-amylase [*Aspergillus awamori*] (BAD06002.1), alpha-amylase [*Aspergillus sojae*] (BAM28635.1), alpha-amylase [*Aspergillus kawachii* IFO 4308] (GAA91738.1), alpha-amylase Amy13A [*Penicillium oxalicum* 114-2] (EPS26265.1)



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#### Figure 6. Phosphorylation sites on enzymes structure



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