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Molecular Mechanics Investigation of Diffrent Temperature Effects on *Bacillus licheniformis* α-amylase: A Computational Study

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Among the wide variety of amylolytic enzymes synthesized by microorganisms, α-amylases are the most widely used in industry. α-amylase (α-1,4-glucan-4-glucanohydrolase, E.C.3.2.1.1) is alternatively known as 1,4-α-D-glucan glucanohydrolase or glycogenase and releasing α-anomeric products. These enzymes randomly cleave the α-1,4-glycosidic linkages in starch, generating maltose and malto-oligosaccharides. Amylases constitute a class of industrial enzymes representing approximately 30% of the world enzyme production. Thermostable enzymes are therefore highly attractive and have increasing attention because of their potential use in biotechnological processes. The purpose of this study was to evaluate the activity of the enzyme α-amylase at different temperatures. For this purpose was used HyperChem 8.0.8 software. HyperChem is a powerful program that enables us to do high quality molecular calculations. HyperChem puts more molecular modeling tools at your fingertips than any other windows program. Temperature was chosen at five levels 298, 310, 318, 333 and 358 K. Results of this study for Epot in Amber for five levels temperatures 298, 310, 318, 333 and 358 K was 1151.84, in bio obtained 5881.64, 6019.84, 6294.26, 6558.46 and 7026.43 and in opls obtained -18610, -18432, 18303, 15603 and -17880. Total energy for Amber, bio and opls respectively, were included 1151.843, 11457.08 and -8369.65 Kcalmol^{-1}.

Key word: α-amylase, Molecular mechanics, Thermostable enzyme.

α-amylase (α-1,4-glucan-4-glucanohydrolase, E.C. 3.2.1.1) is alternatively known as 1,4-α-D-glucan glucanohydrolase or glycogenase [1]. This enzyme catalyzes the hydrolysis of α-D-(1,4)-glucosidic linkages in starch, glycogen, and various malto-oligosaccharides, releasing α-anomeric products. á-amylase has been studied extensively from various aspects, including structure and function, secretion, and industrial application. Amylases constitute a class of industrial enzymes representing approximately 30% of the world enzyme production α-amylase is also the most widely studied member of the glycosyl hydrolase family 13 [2].

In general, the enzymes usually get denatured and lose their activities at temperatures over 50–60°C. However, thermostable enzymes allow a higher operation temperature which is advantageous because of higher reactivity (higher reaction rate, lower diffusional restrictions), higher stability, higher process yield, lower viscosity, and fewer contamination problems. Thermostable enzymes are therefore highly attractive and have

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increasing attention because of their potential use in biotechnological processes. Besides, these enzymes also help in ascertaining the major attributes and mechanisms of how proteins achieve extreme thermostability[3].

A large number of bacteria, fungi and yeasts produce extracellular enzymes that degrade starch in different environmental niches. Variety of polysaccharide hydrolyzing enzymes that are suitable for various industrial applications have emerged in the last few decades, leading to the screening of these enzymes for novel properties[4].

The molecular weights of microbial α-amylases range from 12.5 to 160 kDa. The acidic α-amylase from *Aspergillus niger* has a lower molecular weight than that of acidic α-amylase from other molds. Most acid-stable α-amylases are high molecular weight enzymes as reported in *Bacillus stearothermophilus* US 100, *Lactobacillus manihotivorans*, *G.thermoleovorans*, *Bacillus* sp. KR8104 and *Bacillus acidicola*[5-8]. In general, a survey shows that the molecular weight (MW) of α-amylase from *Bacillus* spp. varies between 50 and 60 kDa with some exceptions. Thermostable α-amylase from *Bacillus licheniformis*, a monomeric enzyme with molecular mass of 55.2 kDa (483 amino acid residues) showed remarkable heat stability. Liu *et al*. found that the MW of a thermostable amylase from *B.licheniformis* was 53.13 kDa. The extracellular *á*-amylase produced by another strain (44MB82-A) of *B.licheniformis* was 58 kDa as judged by SDS-PAGE, but extremely smaller size of amylase (31 kDa) was demonstrated from another strain of *B.licheniformis*[9]. Most of these enzymes amylase are thermostable. As a result, their combined use for starch hydrolysis at a given temperature reduces their overall effectiveness due to the differences in optimum working temperature conditions[10].

Among the fungi, most amylase production studies have been done with mesophilic fungi within the temperature range of 25–37°C. *Bacillus amyloliquefaciens*, *B.subtilis*, *B.licheniformis* and *B.stearothermophilus* are among the most commonly used *Bacillus* sp. reported to produce α-amylase at temperatures 37–60°C [11,12]. *Bacillus licheniformis* ATCC 6346 purified α-amylase showed 8 min at pH 7.0 and 85°C[13].

MATERIALS AND METHODS

In this study was used HyperChem.8.0.8 Software. HyperChem is a sophisticated molecular modeling environment that is known for its quality, flexibility, and ease of use. Uniting 3D visualizationand animation with quantum chemical calculations, molecular mechanics, and dynamics, HyperChem puts more molecular modeling tools at your fingertips than any other Windows program[14].

The first structure of *Bacillus Licheniformis* α-amylase was taken from site PDB with PDB ID: **1BLI**, that containing 483 amino acid. This protein Classification is Hydrolase. Gene Names is amyS, amyL.

This software based on the ratio of the molecular dynamics simulations. The enzyme was investigated by HyperChem software of two parts. The first part consists of the following steps:

1. From top menu select file and then switch open. The structure *Bacillus Licheniformis* α-amylase interest is with pdb format.
2. was used from molecular mechanics. Molecular mechanics are four force fields provide computationally convenient methods for exploring the stability and dynamics of molecular systems. Added flexibility of user defined atom types and parameters. Choose from MM+, a general purpose force field, and three specialized biomolecule force fields: Amber, BIO+, and OPLS. In study were used three parameter including Amber, BIO+, and OPLS.
3. At this stage by entering in Compute, selected montecarlo then with select average, selected ekin, epot, etot and temp option.
4. In the box Monte carlo, the number of steps was found 50 times and temperature was chosen at five levels 298, 310, 318, 333 and 358 K. The reason that the temperatures chosen, Comparison of enzyme activity *Bacillus Licheniformis* with The enzyme activity of the species that were studied in vitro. All these steps should be stored in separate files.

For the second stage as the first stage structure of the enzyme entered in the software structure.

1. In file selected start log.
2. Field of forces were selected from setup. 3. Single point was selected from Compute. The software did a brief calculation. The calculation of an output file that each file contains information. This include Total energy, Gradient, Bond, Angle, Dihedral, VdW and Electrostatic.
4. Finally, after all calculations chosen stop points.

RESULTS AND DISCUSSION

The first, we examine different models for this protein that the results were announced in Table 1. The table have been reported compares the main model with other two models based on RMSD and residues. These three models are shown in Figure 1. Features comparison of the three models are in Table 1.

Table 1. Comparison of the three models together by RMSD parameter

Comparison	Total RMSD	RMSD of final subset
1vjsA to 1bliA	1.6 Å // 469 residues	0.3 Å // 461/469 residues
1ob0A to 1bliA	0.1 Å // 481 residues	0.1 Å // 481/481 residues

Table 2. Energy Total, Potential and Kinetic in first step

	Amber			BIO			Opls		
temp	E Kin-amber	E Tot-amber	E Pot-amber	E Kin-bio	E Tot-bio	E Pot-bio	E Kin-opls	E Tot-opls	E Pot-opls
298	6732.234	7884.077	1151.843	6732.234	18189.32	11457.08	4581.721	-3787.93	-8369.65
310	7003.33	8155.173	1151.843	7003.33	18460.41	11457.08	4766.22	-3603.431	-8369.65
318	7184.062	8335.904	1151.843	7184.062	18641.15	11457.08	4889.219	-3480.432	-8369.65
333	7522.932	8674.775	1151.843	7522.932	18080.19	10557.26	5774.96	-2594.69	-8369.65
358	8087.717	9239.56	1151.843	8087.717	19544.8	11457.08	5504.215	-2865.435	-8369.65

Table 3. Energy Total, Potential and Kinetic in last step

	Amber			BIO			Opls		
temp	E Kin-amber	E Tot-amber	E Pot-amber	E Kin-bio	E Tot-bio	E Pot-bio	E Kin-opls	E Tot-opls	E Pot-opls
298	6732.234	9865.836	3133.603	6732.23	12613.88	5881.641	4581.721	-13996.14	-18577.86
310	7003.33	10249.82	3246.49	7003.33	13023.17	6019.84	4766.22	-13665.91	-18432.13
318	7184.062	10671.73	3487.672	7184.06	13478.32	6294.261	4889.219	-13413.97	-18303.19
333	7522.932	11238.12	3715.183	7522.93	14081.39	6558.462	5774.96	-9827.572	-15602.53
358	8087.717	12419.75	4332.037	8087.72	15174.81	7087.097	5504.215	-12375.42	-17879.64

Table 4. Single point parameters

Parameter Force field	Elec Fm^{-1}	Vdw $J \cdot m^3 (kmol^2)^{-1}$	Dihedral $Kcalmol^{-1}$	Angle Kcal (mol per radian^2)$^{-1}$	Bond Kcal (mol perA°^{2})$^{-1}$	Gradient $Kcalmol^{-1}$ Ang^{-1}	Total Energy $Kcalmol^{-1}$
Amber	-5672.01	-284.1081	4219.61	1348	1340.33	21.078	1151.84287
Bio	-4705.29	11980.7	1673.67	1400.3	1107.73	455.4	11457.0837
Opls	-21259.7	10975.1	779.807	722.46	412.693	559.29	-8369.6504

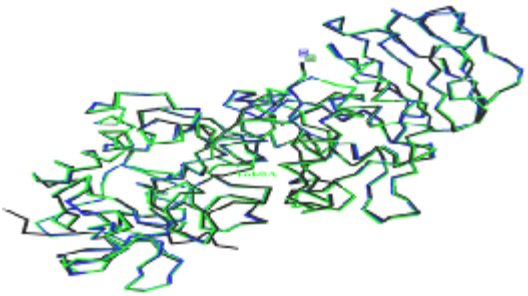

Fig. 1. Three models have been proposed for simulation (model 1: blue, model 2: green, model 1: black)

Fig. 2. *Basillus Licheniformis* α-amylase

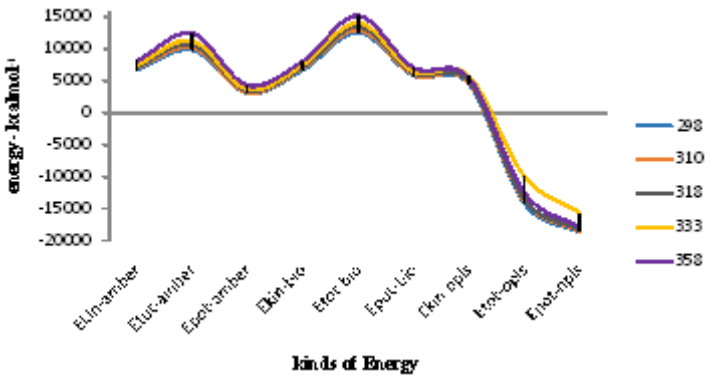

Fig. 3. Energy Total, Potential and Kinetic in last step in different temperatures

The models calculations were performed according to this structures:

a) PDB ID: 1vjsA: Structure of α-amylase precursor

b) PDB ID: 1BLI: Bacillus Licheniformis α-amylase

c) PDB ID: 1ob0A: Kinetic stabilization of Bacillus licheniformis α-amylase through introduction of hydrophobic residues at the surface

Discussion

In diagram 1 observe that this protein is different in the three field of force OPLS, BIO, AMBER. No difference in all the studied properties. There are many common in some properties such as gradients and bands and angle. Of course, the parameter Dihidral in the three field of force is slightly different. But the three parameters of the total energy, van der Waals and electrical energy in the three field of forces are significantly different from each other that shown in figure 1.

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