International Journal of Biochemistry Research & Review
16(1): 1-12, 2017; Article no.IJBCRR.31097
ISSN: 2231-086X, NLM ID: 101654445

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Temperature Induced Conformational Entropy of α-Amylase with and without Additive

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Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

Article Information

DOI: 10.9734/IJBCRR/2017/31097
Editor(s):
(1) G. Padmaja, Central Tuber Crops Research Institute Sreekariyam, Thiruvananthapuram, India.
Reviewers:
(1) Gustavo Orlando Bonilla Rodriguez, Sao Paulo State University, Brazil.
(2) Bechan Sharma, University of Allahabad, Allahabad, India.
(3) Sadia Aslam, Govt. College Women University, Faisalabad, Pakistan.
(4) Emmanuel M. Papamichael, University of Ioannina, Greece.
Complete Peer review History: http://www.sciencedomain.org/review-history/18135

Original Research Article

Received 20th December 2016
Accepted 3rd March 2017
Published 9th March 2017

ABSTRACT

Aims: 1) To formulate models based on defined principle for the application of Fitter's model and 2) ultimately show that there are changes in the radius of an enzyme in solution and consequently conformational entropy change with temperature before and during catalytic activity.
Study Design: Theoretical and experimental.
Place and Duration of Study: Department of Chemistry and Biochemistry, Research Division, Ude International Concepts LTD (862217), B. B. Agbor, Delta State, Nigeria; Owa Alizomor Secondary School, Owa Alizomor, Ika North East, Delta State, Nigeria. The research was conducted between June and December, 2016.
Methodology: Bernfeld method of enzyme assay was used. Assays were carried out on diluted crude human salivary alpha amylase (HSαA). Data from assay were plotted in two ways viz: Velocity (v) vs thermodynamic temperature (T) and $1/v$ vs T. Proportionality constants were determined from slope and intercept of the linear regression. These were used to determine desired parameters.

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Results: The results showed that, hydrodynamic radius, r_{p0} (or positive Δr) was higher at higher temperatures and in the absence of calcium salt. Without calcium chloride but with raw starch and gelatinized starch, Δr values were in the range of 0.81 Å-2.30 Å and 4.5 Å-8.45 Å respectively. With calcium chloride and gelatinized starch at higher temperatures, Δr ranged from 3.71 Å-4.80 Å. Increasing values of r_{p0} corresponded with increasing values of conformational entropy change, ΔS_{conf}.

Conclusion: The derived models were theoretically/conceptually and experimentally compatible given that the hydrodynamic radius, r_{p0} (or positive Δr) increased with the increase in temperature whether or not the enzyme showed increasing activity. The activity of the enzyme decreases when physiological limit of ΔS_{conf} is exceeded. The values of Δr were higher at higher temperatures in the presence and absence of calcium chloride.

Keywords: Models; crude human salivary amylase; hydrodynamic radius; conformational entropy.

1. INTRODUCTION

Conformational entropy is very important factor affecting protein stability. Folding and unfolding are fundamental biological processes in cell and are important for the biological functions of proteins [1]. This implies that folding and unfolding involve conformational entropy change (ΔS_{conf}). Loss of conformational entropy leads to greater rigidity/stability of protein in general [2,3]. This is further corroborated with the suggestion that entropy is seen to be related directly to the number of different conformations a particular state can adopt; a reduction in the number of rotamers (different conformations/ conformers) leads to a significant loss of entropy opposed to protein folding [4]. Related to increase/decrease in the ΔS_{conf} is the hydrophobic effect that primarily results from the release of ordered waters when nonpolar groups are removed from solvent which leads to increase in translational entropy (TE) of the solvent molecules, described as the maximum driving force promoting folding [5]. Support in part to this position is the conclusion that the unfolding rate constants in water are related to the amount of buried hydrophobic residues in the tertiary structure of protein [6]. Thus partial exposure of the hydrophobic groups leads to a partial unfolding expressed as an increase in the conformational entropy or dynamics. Forces opposed to TE as well as what has been called major factors such as hydrophobic factor – burial of non-polar surface area – and van der Waal interactions together with the peptide H-bond, and peptide solvation, and auxiliary factors such as salt bridges, side-chain H-bonds, disulphide bridges, and propensities to form α-helices and β-helices can result in an increase in the conformational entropy.

Measurement of backbone conformational entropy of unfolded β-sheet polyproteins has been carried out yielding entropy loss whose value is equal to 19 (± 2) J/(mol.K residue) [7]. It seems the entropy decreased by an amount equal to 19 (± 2) J/(mol.K residue). Other methods which gave estimates of the backbone ΔS_{conf} of the unfolded state ranging from 12 J/(mol.K residue) to 30 J/(mol.K residue) while majority cluster around the median value of ~ 20 J/(mol.K residue) have also been reported. Using different methods, Filter [8] reported ΔS_{conf} values ranging from 10.85-14.45 J/(mol.K per residue) corresponding to temperature range of 30-70℃.

There are different kinds of conformational entropy. They are protein backbone and side chain conformational entropies. But according to Baxa et al. [3], accurate determination of the quantity is very challenging. Several methods/models exist for the determination of ΔS_{conf}. Use of Boltzmann equation, $\Delta S_{conf} = -R\ln W$, where W is the number of different conformations adopted in the unfolded state [4] is an example. Also, $\Delta S_{conf} = -k_B \Sigma p_i \ln p_i$ where p_i is the fractional population of each rotamer state i in the unfolded state [2-4]. Some approaches include (but not limited to) molecular dynamic simulations combined with nuclear magnetic resonance spectroscopy [3,9]. Other approaches used to probe conformational dynamics in proteins over nanosecond to millisecond time scales as observed by Narayanan et al. [9] are X-ray diffraction, single molecule FRET *etc*. In the same paper is the observation that NMR Carr-Purcell-Meiboom-Gill (CPMG) is one of the most commonly used experiments to characterize conformational events experienced by enzymes on the time scale of their catalytic rate k_{cat} – typically occurring on the order of

millisecond [9]. However, a model proposed and used by Fitter [8] appears to be simple. This entails determination of ΔS_{conf} for unfolding with a radius of confinement for motions in the folded state r_f and in the unfolded state r_u given as $\Delta S_{conf} = 3R\ln r_u/r_f$ [8]. This presupposes confined or restricted diffusive motion within certain conformational space [10].

Research has shown that human salivary alpha amylase is known to have been targeted for design of therapeutic agents against type II diabetics, obesity, hyperlipidemia and caries, and to be involved in anti-inflammatory reactions; it is also implicated in the symptoms of its deficiency such as psoriasis, eczema, hives, insect bites, allergic bee and bug stings, atopic dermatitis, and all types of herpes *etc* [11]. Therefore, the study of its conformational changes has become a worthwhile exercise. Since the dynamical behavior of α-amylase has been seen to be a function of temperature it is therefore, indicative of importance of conformational entropy change during unfolding of protein in general [8]. Therefore, the objectives in this research are: 1) To formulate models based on defined principle for the application of Fitter's model and 2) ultimately show that there are changes in the radius of an enzyme in solution and consequently conformational entropy change with temperature before and during catalytic activity of an enzyme.

1.1 Theoretical Background

It is well established fact that enzymes are classified in terms of their capacity to function in different environment. The environment in question is purely thermal in nature. Hence there are low temperature adapted, moderate temperature adapted, and high temperature adapted enzymes. The low temperature adapted enzymes exist with high conformational flexibility while the higher temperature adapted enzymes have rigid conformational state. Hence there are psychrophiles, mesophiles, and thermophiles [12]. The main purpose of higher temperature as applicable to mesophiles and thermophiles is to enable the enzymes achieve greater conformational flexibility needed for function. Therefore, if a dry sample of higher temperature dependent enzyme is placed in water at temperature ≥ room temperature, the radius of such enzyme may not be equal to the radius at temperature < room temperature. The usual practice is to determine the radius of the enzyme using Einstein-Stokes equation, $6\pi\eta r_P = k_B T/D$ where, η, r_P, k_B, T, and D are viscousity constant, radius of the protein, Boltzmann constant, absolute temperature, and translational diffusion constant respectively. However, D must be experimentally determined as often the case [13]. Within tolerable biological temperature limits, the values of r_P may not differ widely. However, the value of r_P for human salivary alpha amylase (HSαA) is taken as 2.9998 nm as the minimum for HSαA at temperature as low as room temperature (293.15K). This value of r_P is calculated by substituting relevant parameters into $k_B T/6\pi\eta D$. The value of D is as reported by Sky-Peck [14].

The mathematical aspect of this proposition is based, in part, on the time tested observation that density of a liquid water and its viscousity decreases with increasing temperature. If the H-bond in water is vulnerable to increasing thermal energy, then without losing scientific relevance, virtually all weak interaction in globular protein cannot be an exception. This proposition is similar to the following deduction: "The flexibility of the protein is thus sufficient for water or a base catalyst to access the exchanging amide groups, suggesting that conformational fluctuations of the magnitude of the hydrogen bond opening occur throughout the protein" [15]. Placing a soluble protein in water at temperature ≥ than room temperature will compel the protein to assume a radius proportional to the given thermal energy quantified in terms of absolute temperature. The experimental model for the testing of model is the determination of enzyme activity. This is anchored on the premise that enzyme activity is the most sensitive probe to study the changes in enzyme conformation during various treatment because it reflects subtle readjustments at the active site, enabling very small conformational variations of an enzyme structure to be detected; enzyme activity measurements also indicate the integrity of the tertiary structure of the enzyme against denaturants [11].

There is need to approach the determination of $r_{p\infty}$ by exploring the rate of hydrolysis of starch by HSαA. It is postulated that the length of the radius of the 3-dimensional (3-D) structure of an enzyme during catalytic activity is directly proportional to the absolute temperature.

$$r_{p\infty} = \beta T \quad (1)$$

where β is the proportionality constant. Also, increasing hydrolytic activity of the enzyme with increasing temperature is coupled to increasing

conformational flexibility of the enzyme. This implies that the radius of the enzyme must increase with increase in the temperature. In this case, it is postulated that the rate of the hydrolytic activity is directly proportional to the increase (Δr) in length of the radius of the enzyme which affects the size of the active site.

$$\Delta r = \beta T - r_{p0} \quad (2)$$

If there is decreasing activity with increasing temperature, the velocity of hydrolysis (v) is postulated to be inversely proportional to Δr.

$$v = \mu / \Delta r \quad (3)$$

where μ is the proportionality constant. Then substituting Eq. (2) into Eq. (3) gives:

$$v = \mu / (\beta T - r_{p0}) \quad (4)$$

Expansion of Eq. (4) leads to:

$$\mu = v\beta T - v r_{p0} \quad (5)$$

Dividing Eq. (5) through by $v\mu$ gives:

$$1/v = \beta T/\mu - r_{p0}/\mu \quad (6)$$

A plot of $1/v$ versus T gives intercept r_{p0}/μ from which μ can be determined and its substitution into the slope, β/μ can enable one to calculate β.

On the other hand, there may be increasing hydrolytic activity with increasing temperature, but not indefinitely within the short duration of assay at temperatures that are tolerable to the enzyme.

Thus,

$$v = \omega \Delta r \quad (7)$$

Substituting Eq. (2) into Eq. (7) gives:

$$v = \omega (\beta T - r_{p0}) \quad (8)$$

Expansion of Eq. (8) yields:

$$v = \omega \beta T - \omega r_{p0} \quad (9)$$

A plot of v versus T gives intercept, ωr_{p0} from which the constant ω can be calculated and substituted into the slope so as to calculate the second constant, β for a reaction increasing with increasing temperature. With the values of the constant established, the value of $r_{p\infty}$ can be calculated using Eq. (1) or as $\Delta r + r_{p0}$ since Δr can be calculated using either Eq. (3) or Eq. (7) as the case may be.

2. MATERIALS AND METHODS

2.1 Chemicals

Hydrochloric acid, sodium hydroxide, and sodium chloride were purchased from BDH Chemical Ltd, Poole England, and potato starch was purchased from Sigma, USA. Tris was purchased from Kiran Light Laboratories, USA, 3, 5-dinitrosalicylic acid and maltose were purchased from Kem light laboratories, India. Sodium potassium tartrate tetrahydrate was purchased from Kermel, China while calcium chloride was purchased from Lab Tech Chemicals, India. Distilled water was purchased from local market. Crude extract of human salivary amylase (E. C. 3, 2.1.1 1, 4 - α - D – glucanohydrolase) was used.

2.2 Equipment

Electronic weighing machine was purchased from Wenser weighing Scale Limited and 721/722 visible spectrophotometer was purchased from Spectrum Instruments China. *PH* meter was purchased from Hanna Instruments, Italy.

2.3 Method

A stock solution of a potato starch was prepared by mixing 1 g in 100 mL of distilled water, subjected to heat treatment at 100℃ for three minutes, cooled to room temperature, and final volume was made by topping the volume with buffer (TrisHCl, *p*H = 6.9), to 100 mL to give 1.0 g/100 mL. A volume = 20 mL of saliva sample was obtained few hours after meal by spitting into a 50 mL beaker. Then the 20 mL of saliva was mixed with calcium salt (5 mM), buffer (TrisHCl, *p*H = 6.9), sodium chloride, and distilled water to give 1:2 diluted saliva. This was subjected to 3 minutes of centrifugation at 3000 rpm (or at 1343 *g*) using ordinary laboratory centrifuge (model 800D). Centrifugation can raise the temperature of saliva and exposure to ambient condition for a longer time before assay at different temperatures may lead to a partial loss of catalytic power preventable by the presence of the calcium salt. This was finally diluted with the buffer to give 1:20 dilution of saliva as stock. Two different concentrations of

HSαA, 1:100 and 1:1000 dilutions of stock saliva, as working stock, were prepared. The buffer, TrisHCl, shows decreasing *p*H values with the increase in temperature. Therefore, the pH was adjusted by adding few drops of the base to the stock solution of the buffer until the desired pH is restored at each incubation temperature for assay. The assay of the enzyme was carried out according to Bernfeld method [16]. This method entails the termination of activity of the enzyme by adding 1 mL of Bernfeld reagent (3, 5 - dinitrosalicylic acid) to the reaction mixture at exactly 3 minutes. Spectrophotometer readings for the determination of the amount of maltose yielded were taken at 540 nm and the extinction coefficient was 181.1/M.cm. Both test in duplicate and blank readings were taking. One unit of enzyme activity is expressed as the amount of the enzyme which catalyzes the production of 1μmol maltose per minute.

In order to determine conformational entropy change for an enzyme undergoing hydrolytic activity at different temperatures, according to the method employed by Fitter [8], the reciprocal of velocity of hydrolysis of starch is plotted versus the temperature if there was decreasing velocity of hydrolysis with temperature (Eq. 6); where there is increasing velocity of hydrolysis with the increasing temperature, the velocity of hydrolysis is plotted versus the temperature (Eq. 9). This is with a view to determine various proportionality constants (as shown in theoretical section) that can be used to calculate the radius $r_{p\infty}$, at higher temperature, and increase in radius, Δr, of the enzyme due to the combined effect of the presence of the substrate and the increase in temperature.

2.4 Statistical Analysis

Assay of the enzyme at each temperature was carried out in duplicate. Mean values of the parameters are reported.

3. RESULTS AND DISCUSSION

The assay of HSαA was carried out to determine the effect of increasing temperature on global structural aspect (the change in the radius) of the enzyme in the presence of the substrate, in raw or native form and in gelatinized form. This was carried out with and without the presence of extra calcium salt apart from initial salt added to the saliva at the preparatory stage. In line with report by Singh et al. [11] the assay is based on the assumption that, "enzyme activity can be regarded as the most sensitive probe to study the changes in enzyme conformation during various treatments because it reflects subtle readjustments at the active site, enabling very small conformational variations of an enzyme structure to be detected". As Fig. 1 shows, the enzyme (1:1000 dilution of the stock solution of the enzyme) showed higher hydrolytic activity at lower temperatures above room temperature. At temperatures approximately between 310.15K and 323.15K, there was gradual decrease in activity, while rapid decrease occurred at higher temperature in the absence of extra calcium salt. This may be as a result of the partially destabilized active site portraying native like activity within defined temperature limit outside which activity → zero. There was increasing activity (Fig. 2) of the enzyme (subjected to 100-fold dilution) in the presence of the salt with increasing temperature up to approximately 323.15K followed by decreasing trend at higher temperatures as observed in previous research [17-21]. Increasing activity with increasing temperature can be described as native activity with conformational stability as against native-like activity, that is activity less than what should have been the case, if the enzyme was to be stable at higher temperature given the fact that, research report by Baldwin and Rose [22] shows that, there is the existence of "'wet' molten globule (WMG), a highly dynamic state with native-like secondary structure and a protein core that admits solvent and lacks close-packing". This is in addition to the dry molten globule (DMG) described as "an expanded form of the native protein in which close-packing has been released at the start of unfolding, but native-like conformation persists and water does not penetrate the core" [22]. It seem therefore, that the native-like attributes may enable the enzyme retain less than full native capacity for its function.

The assumption of the occurrence of DMG, seem to point to structural issue in terms of an increase in perhaps, the hydrodynamic radius implied in the expanded form of the native enzyme. What is important is that there will always be at least an infinitesimal expansion, not just as a result of hydration but due to the thermally induced vibrations that can partially compromise the active site configuration [15]. Thus plots of v versus T and $1/v$ versus T were carried out with respect to the observed trends, increasing v and decreasing v respectively in order to determine the proportionality constants for the calculation of $r_{p\infty}$ and Δr as the case may be. The plots are

shown in Fig. 3 where the salt-treated enzyme showed increasing trend in activity with gelatinized starch and Fig. 4, where the same salt-treated enzyme showed decreasing trend in activity with higher temperature. Fig. 5 is the case for the salt-free enzyme showing decreasing activity. On the other hand Fig. 6 is the case for the salt treated enzyme showing increasing activity at all temperatures investigated with raw starch as substrate.

The structure-function paradigm which explains the mechanism of enzyme function under different adaptations in particular has been followed by new theories which include now-accepted induced fit, conformational selection, and transition-state stabilization models, which explain the behaviour of a protein-ligand recognition and catalysis in the molecular function of receptor/enzyme [9]. Therefore, the increase in the radius of the enzyme (Tables 1-5) may be accounted for in terms of thermal perturbation and emerging theories. The conformational selection model may be similar to the population-shift model because “selection” implies one out of many alternatives available among population of different conformations. With the induced-fit model, substrate binding is believed to induce conformational alterations in the active site to cause a new conformation for the entire enzyme [15]. No conclusion can be reached on the best theory to adopt. In the population shift model, the enzyme is believed to adopt a conformational equilibrium among many native states and the substrates selectively bind to suitable native conformation, shifting equilibrium towards the binding conformation [23]. It can be suggested that temporary existence of subpopulation of proteins with different conformation may be a possibility when a solution of the protein in a test tube is transferred to a water bath at higher non-destabilizing temperature before thermal equilibration in the test tube content. Structural studies on several glycosyltransferases have shown that flexible loops at the substrate binding site of the enzymes undergo a marked conformational change from an open to a closed conformation on binding the donor substrate [24]. Perhaps, this may represent a mechanism of catalysis at a given temperature without precluding the possibility that, there should always be conformational change with the increase in temperature.

Since every particle has at least infinitesimal degree of any kind of motion except perhaps at absolute zero, it is therefore, inconceivable to feel that when a given mass of an enzyme is dissolved in aqueous buffer or pure water, at or above room temperature, there cannot be any form of intra-molecular motion. Thus conformational dynamics of all kinds, be it side chain, backbone etc, can altogether lead to global conformational change before the presence of substrate can induce additional conformational expansion similar to what seem to have been observed elsewhere [25]. Thus conformational change can occur by means of thermal effect and either the presence of substrate for the enzyme or a ligand for the receptor (a transport protein, for instance). The presence of the substrates under ambient temperature (thermal energy) may precipitate what has been described as the conformational motions of 2 kinds namely in line with different time scale, the fast, local picoseconds-nanosecond (ps-ns) fluctuations of individual residues to the slower microsecond-millisecond-second (μs-ms-s) global conformational exchange [9]. Thus the increasing activity (Figs 3 and 6) and increasing radius of the enzyme, with the increasing temperature (Tables 1, 4 and 5), should be the case for a mesophile which require higher temperature than the psychrophilic enzymes [12] to achieve needed conformational flexibility/entropy (Tables 1, 4 and 5) for catalytic function. There was also decreasing activity and increasing radius with increasing temperature in the presence and absence of 5 mM calcium chloride (Figs. 4 and 5 respectively) using gelatinized starch as substrate accounting for the fact that the enzyme may have lost its conformational stability by exceeding its optimum radius and consequently its physiological global conformational entropy (Tables 2 and 3).

It can be observed that in the presence of calcium chloride with gelatinized starch, the values of Δr at higher temperature were larger than values at lower temperature as shown below Tables 1 and 2. This is a clear evidence of the failure of calcium ion to stabilize the enzyme at higher temperature similar to report elsewhere [21]. This is further buttressed by the assertion that the loss of rate acceleration is ascribable to the thermally induced vibrations compromising the active site catalytic configuration thereby giving the impression that excess rigidity of the active site residue is obviously not a limiting factor in enzyme catalysis [15]. This claim by Karshikoff et al. [15] is not in agreement with research elsewhere which shows that at room temperature hyperthermophilic enzymes are often barely active, but are as active as their

mesophilic counterparts (HSαA) at their corresponding physiological temperature [26]. The same author [26] also reported that the reduced activity at ambient temperature is due to the high molecular rigidity of the enzyme, which is then relieved at the elevated in vivo temperature**.** This is very applicable to the trend observed in this research as depicted in Figs 3 and 6. The increase in global conformational entropy (Tables 1, 4 and 5) is just what is needed for catalytic function (or increasing velocity of hydrolysis with increasing temperature) of the enzyme with partial decrease in the number of interactions between amino acid residues [27]. Another factor is the relative abundance of buried hydrophobic residues which, explains slow unfolding of hyperthermophilic enzymes [6]. This means that any enzyme with much less proportion of hydrophobic residues in the interior may exhibit greater conformational flexibility as should be the case with mesophiles like HSαA in this study**.** Increasing temperature below T_m may therefore, partially weaken the hydrophobic binding interaction as to achieve local and ultimately with time, global conformational entropy increase for catalytic function.

Table 1. Physicochemical parameters for an enzyme showing increasing hydrolytic activity on the gelatinized potato starch in the presence of calcium chloride

Temperature/K	Radius/nm		Conformational entropy/J/K	
	a	b	a	b
290.15	3.13	3.13	1.07	1.07
310.15	3.34	3.31	2.66	2.50
313.15	3.38	3.40	2.91	3.15

'a' and 'b' stand for data resulting from the use of $r_{p\infty} = \beta T$ and $\Delta r + r_{p0}$ respectively. Values are as extrapolated from Fig. 3. A plot of v versus T gives intercept, ωr_{p0} from which ω is determined; from the slope ($\omega\beta$), β is determined (see Eq. 9). The radius of HSαA is ≈ 2.9998 nm. The values of Δr, range from 1.30Å-4.00Å and correspond to a temperature range of 290.15 K-313.15 K; the proportionality constants, ω and β are ≅ 7.72 exp (6) mU/m and 10.78 exp (-12) m/K respectively

The nondestabilizing conformational entropy has been accounted for in terms of the local conformational entropy below melting point (T_m) [28] occurring on ps-ns time scale [9] that is not strongly opposed to overall 3-D structure of the enzyme. The imbalance between the conformational entropy and the energy of interaction between residues skewed to the positive conformational entropy change (Tables 2 and 3) [27], that may be above normal physiological limit, resulting from excessive thermally induced vibration that can substantially or totally weaken the buried hydrophobic interaction [6], may lead to the decrease in catalytic activity or velocity of hydrolysis of the substrate (Figs. 4 and 5). What should be clearly observed is that higher global conformational entropies (Tables 2 and 3, where gelatinized starch was the substrate) were applicable to the decreasing activity of the enzyme while lower entropies (Table 1 where gelatinized starch was the substrate) were applicable to the increasing activity of the enzyme. This is a clear demonstration of the fact that when the physiological conformational entropy is exceeded, unfolding and decrease in catalytic activity become the outcome.

Table 2. Physicochemical parameters for an enzyme showing decreasing hydrolytic activity on the gelatinized potato starch in the presence of calcium chloride

Temperature/K	Radius/nm		Conformational entropy/J/K	
	a	b	a	b
318.15	3.49	3.39	3.78	3.05
323.15	3.55	3.40	4.20	3.12
328.15	3.60	3.44	4.55	3.15
333.15	3.66	3.48	4.96	3.70

'a' and 'b' stand for data resulting from the use of $r_{p\infty} = \beta T$ and $\Delta r + r_{p0}$ respectively. Values are as extrapolated from Fig. 4. A plot of 1/v versus T gives intercept r_{p0}/μ from which μ can be determined and its substitution into the slope, β/μ can enable one to calculate β (see Eq. 6). The radius of HSαA is ≈ 2.9998 nm. The values of Δr, range from 3.71Å-4.80Å and correspond to a temperature range of 318.15 K-333.15 K; the proportionality constants, μ and β are ≅ 1.49 exp (-12) mU.m and 10.98exp (-12) m/K respectively

Table 3. Physicochemical parameters for an enzyme showing decreasing hydrolytic activity on the gelatinized potato starch in the absence of calcium chloride

Temperature/K	Radius/nm		Conformational entropy/J/K	
	a	b	a	b
293.15	3.45	3.45	3.45	3.49
308.15	3.62	3.64	4.69	4.82
310.15	3.65	3.64	4.86	4.82
313.15	3.68	3.65	5.09	4.87
318.15	3.74	3.69	5.49	5.15
323.15	3.80	3.85	5.88	6.19

'a' and 'b' stand for data resulting from the use of $r_{p\infty} = \beta T$ and $\Delta r + r_{p0}$ respectively. Values are as extrapolated from Fig. 5. See Table 2 foot note for further detail. The radius of HSαA is ≈ 2.9998 nm. The values of Δr, range from 4.50Å-8.45Å and corresponds to a temperature range of 293.15 K-323.15 K; the proportionality constants, μ and β are ≅ 3.92exp (-13) mU.m and 11.75 exp (-12) m/K respectively

The outcome of the "tradeoff between increasing conformational flexibility and the need for

stability" is vital for effective catalytic function. Thus as reported in literature [29], human serum albumin (HSA) binds its ligand via hydrophobic interaction, while bovine serum albumin (BSA) does so electrostatically. The same authors observed that HSA interacts with C-7 position of the xanthone ring of mangiferin via H-bonding with its Gly-196. Dipole-dipole interaction ascribed to BSA [29] does not fall short of Coulomb law (in terms of electrostatics) and H-bonding cannot be an exception. However, there are conformational changes in the protein structure accompanying the binding of the ligand, depending on the concentration of the ligand [29]. This issue is substantiated (so long as ligand must not always be the case) in this research when cognizance is taken of the fact that gelatinized starch in solution has higher concentration of polysaccharide, starch, than the raw counterpart. Thus as shown below Tables 1-5, the increase in radius, Δr, with increasing temperature with raw starch is < the values obtained with gelatinized starch with increasing temperature.

If hydrophobic interaction binds weakly, dipole-dipole interaction is either attractive or repulsive; but if binding is the case, it may be attractive like hydrogen bonding so as to stabilize the ligand-receptor complex and by extension, enzyme-substrate complex. This is without prejudice to the expectation of conformational changes in the vicinity of the binding site, which suggests substrate-induced adjustments in the macromolecular structure of the receptor/enzyme [29]. Thus this issue of stabilization is in line with the age-long function of the enzyme, the lowering of kinetic barrier (activation energy) that cannot be different from transition-state stabilization models [9]. The role of calcium chloride, the calcium ion component in particular, is clearly shown in this research whereby its presence lead to lower Δr than values obtained in its absence with raw starch (Tables 4 and 5) and with gelatinized starch (Tables 1 and 2). Since calcium ion and chloride ion have opposing effect, it seems the cation effect may have overcome the effect of the anion. Research in the past [17-21], points to similar direction.

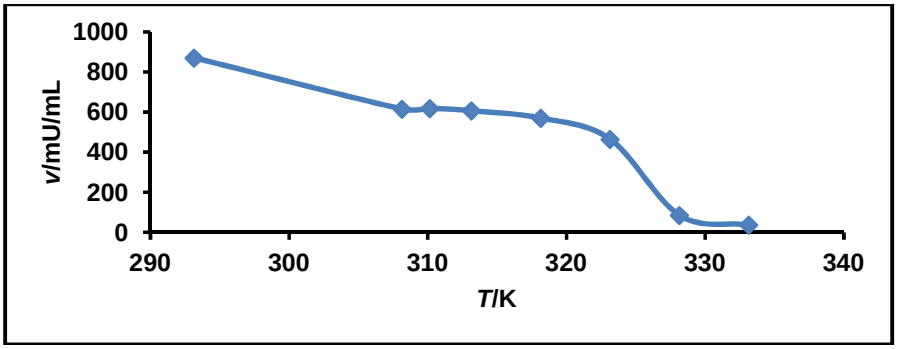

Fig. 1. A plot showing the trend of velocity (*v*) of hydrolysis of gelatinized potato starch with absolute temperature without additional calcium chloride (5 mM/L) using 1:1000 diluted crude HSαA

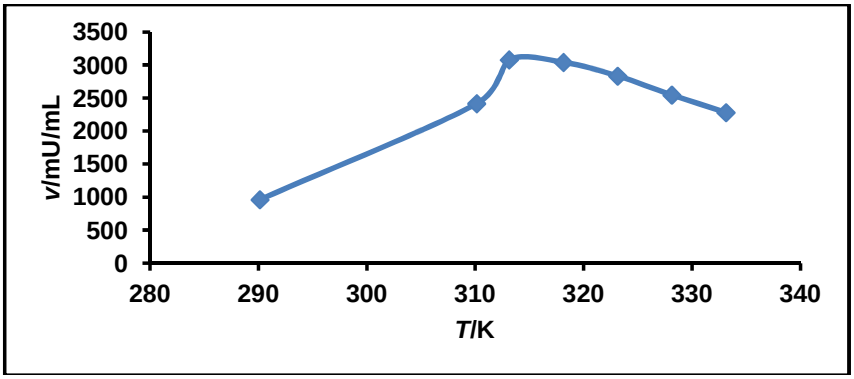

Fig. 2. A plot showing the trend of velocity of hydrolysis of gelatinized potato starch with absolute temperature with additional calcium chloride (5 mM/L) using 1:100 diluted crude HSαA

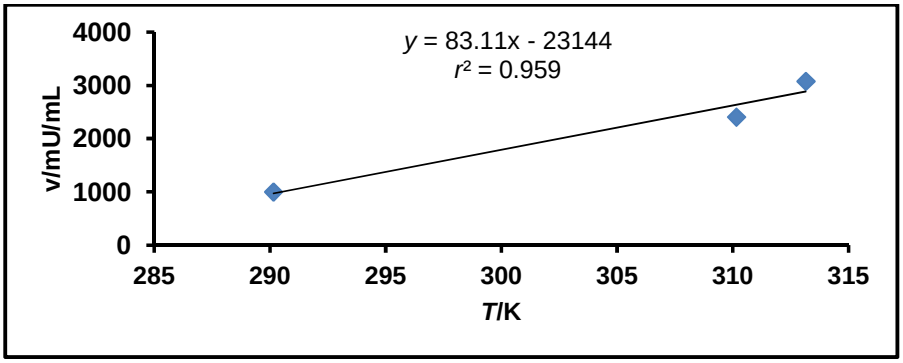

Fig. 3. Plot of velocity of hydrolysis of gelatinized starch versus absolute temperature ranging from 290.15-313.15K for the determination changes in the radius of the 1:100 diluted, $CaCl_2$ treated crude HSαA showing increasing activity

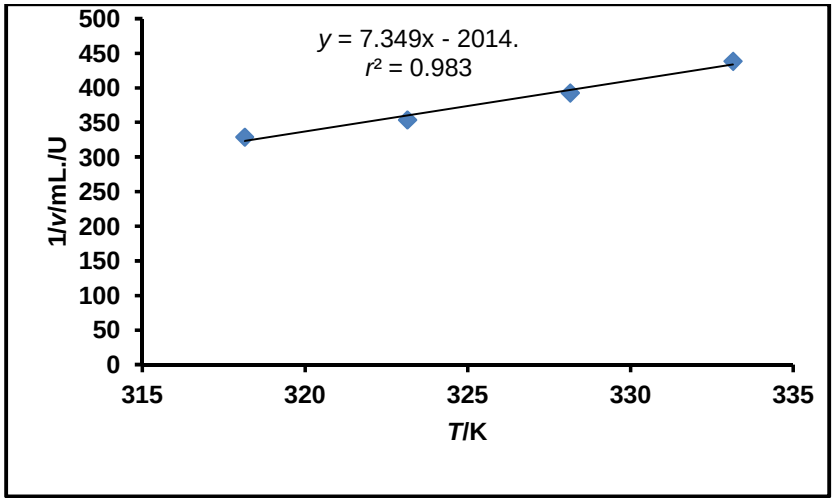

Fig. 4. Plot of velocity of hydrolysis of gelatinized starch versus absolute temperature ranging from 313.15-333.15K for the determination changes in the radius of the 1:100 diluted, $CaCl_2$ treated crude HSαA showing decreasing activity

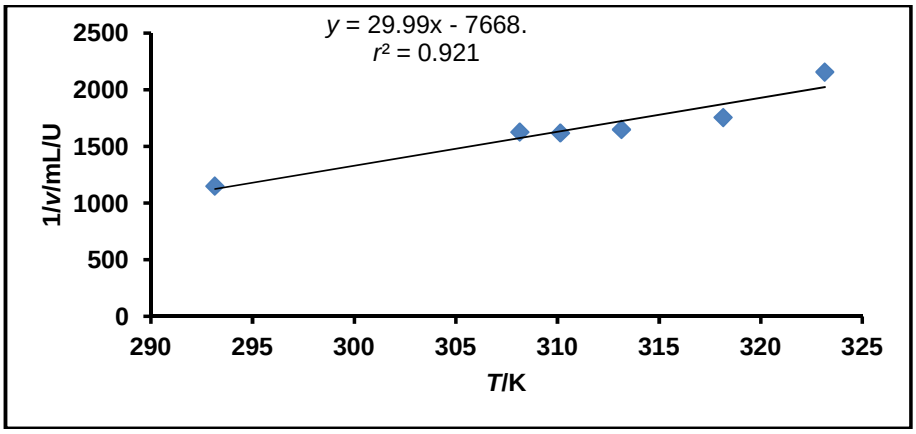

Fig. 5. A plot of the reciprocal of the velocity of hydrolysis of gelatinized potato starch without calcium chloride versus temperature for the determination of changes in the radius of 1:1000 diluted HSαA

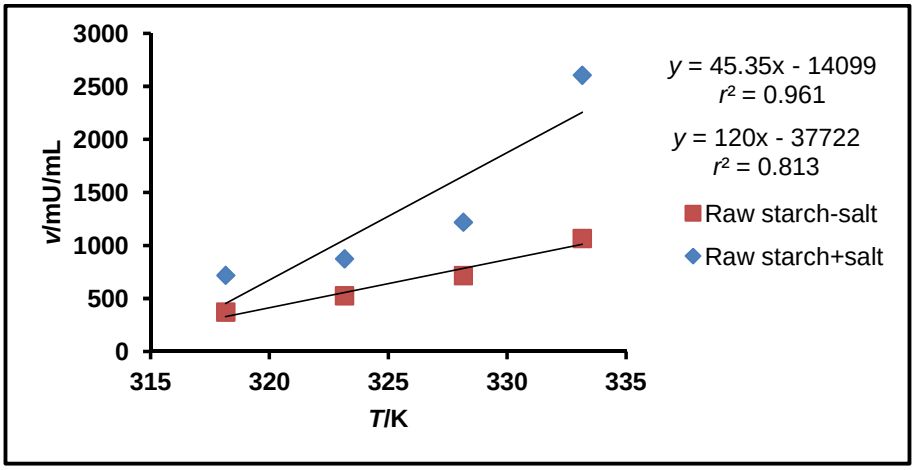

Fig. 6. The plot of the velocity of hydrolysis of raw potato starch versus temperature for the determination of changes in the radius of 1:4 diluted crude HSαA. (■) and (◆) stand for assays with calcium chloride and without the salt respectively

Table 4. Physicochemical parameters for an enzyme showing increasing hydrolytic activity on the raw potato starch in the presence of calcium chloride

Temperature/K	Radius/nm		Conformational entropy/J/K	
	a	b	a	b
318.15	3.04	3.06	0.33	0.46
323.15	3.09	3.06	0.72	0.53
328.15	3.13	3.13	1.09	1.09
333.15	3.18	3.19	1.47	1.54

'a' and 'b' stand for data resulting from the use of $r_{p\infty} = \beta T$ and $\Delta r + r_{p0}$ respectively. Values are as extrapolated from Fig. 6. See Table 1 foot note for further detail. The radius of HSαA is ≈2.9998 nm. The values of Δr, range from 0.55Å-1.91Å and correspond to a temperature range of 318.15 K-333.15 K; the proportionality constants, ω and β are ≅ 1.37exp (7) mU.m and 9.55 exp (-12) m/K respectively

Table 5. Physicochemical parameters for an enzyme showing increasing hydrolytic activity on the raw potato starch in the absence of calcium chloride

Temperature /K	Radius /nm		Conformational entropy /J/K	
	a	b	a	b
323.15	3.12	3.11	0.98	0.93
328.15	3.17	3.15	1.09	1.25
333.15	3.22	3.23	1.74	1.84

'a' and 'b' stand for data resulting from the use of $r_{p\infty} = \beta T$ and $\Delta r + r_{p0}$ respectively. Values are as extrapolated from Fig. 6. See Table 1 foot note for further detail. The radius of HSαA is ≈2.9998 nm. The values of Δr, range from 0.81Å-2.30Å and correspond to a temperature range of 318.15 K-333.15 K; the proportionality constants, ω and β are ≅ 4.70 exp(6) mU.m and 9.65 exp (-12) m/K respectively

4. CONCLUSION

The assay of the crude HSαA for the purpose of testing the model reaffirmed the proposition that enzyme activity measurements indicate the integrity of the tertiary structure of the enzyme against not just chemical denaturants but heat. The derived models were theoretically/ conceptually and experimentally compatible and usable given that the hydrodynamic radius, r_{p0} (or positive Δr) increased with increase in the temperature whether or not the enzyme showed increasing activity. The activity of the enzyme decreases when its physiological limit of the conformational entropy change, ΔS_{conf}, is exceeded. The values of Δr were higher at higher temperatures in the presence and absence of calcium chloride. Therefore, the models can be used to ascertain the conformational integrity of any enzyme in both healthy and pathological state. Further research on other enzyme or homologue subjected to either chemical denaturant or higher temperatures, or mutants may be carried out in the feature.

ACKNOWLEDGEMENTS

I wish to thank Madam R. Udema for her financial assistance in my most trying moment which enabled the continuation of my research. I appreciate the effort of Mrs E. Ugboma who helped in the procurement of hard currency.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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