AN ODYSSEY INTO THE WORLD OF ENZYMES

An Inaugural Lecture Delivered at Oduduwa Hall,

Obafemi Awolowo University, Ile-Ife, Nigeria

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By

Isaac Olusanjo Adewale

Professor of Biochemistry

Inaugural Lecture Series 369

The Vice Chancellor Sir, Principal Officers of the University, royal fathers, distinguished personalities, members of the university community, visitors, ladies and gentlemen. It is my pleasure to welcome you to today's Inaugural Lecture.

It is the fifth of such from the Department of Biochemistry and Molecular Biology, Faculty of Science and the three hundred and sixty-ninth (369th) in the history of the University. The title of the lecture is 'An odyssey into the world of enzymes'. An odyssey is a journey made up of several twists and turns, full of excitement and also disappointments. I will however reserve this latter part for another time.

Enzymes are biological macromolecules capable of accelerating the rate of reactions by factors of as much as a million or more over the uncatalyzed reactions and are themselves not consumed in the process. Enzymes drive processes of life in all living things because most reactions in living things do not take place at an appreciable rate without enzymes. With some exceptions, living things are organized structures capable of life forms- movement, reproduction, nutrition, response to stimuli, growth and excretion; and all of these functions are made possible through catalytic activities of enzymes.

To drive home the points, you are able to sit down and move your body because the food you ate had been digested and converted to monomeric units which had been further broken down to generate high energy electron carriers, $FADH_2$ and NADH. The $FADH_2$ are in turn being oxidized to produce adenosine triphosphate (ATP), the universal currency of energy in living cells. Hydrolysis of ATP by adenosine triphosphatase drives your ability to see me giving this lecture and all your other activities. In terms of application, the detergent with which you used to wash your clothes contains enzymes to remove stains. Malt drinks, wine, beer, yoghurt, and bread are some of the products of enzymes in action.

My journey into Biochemistry as a discipline is probably by divine providence as I never had a permanent ambition for any course of study. My ambition oscillated between science and art-based disciplines. I must however admit that I enjoyed chemistry because it appeared magical or more adventurous- a glowing splinter could burst into flames 'magically' when dipped inside a tube. The flame of a burning splinter could be extinguished by dipping it into another tube containing gas, experiments performed by our General Science Teacher in year 1 secondary school. I eventually developed interest in Biochemistry when my brother, Dr. Ademola Adewale who had developed a keen interest in my educational development enrolled me in the then Oyo State College of Arts and Science (OSCAS), Ile-Ife, for Advanced Level program in Biology, Chemistry and Mathematics.

It was at this stage that it struck me that activities in living cells could be explained using chemistry-that the gap between chemistry, the study of structures and interactions of atoms and Molecules, and biology, the study of structures and interactions of cells and organisms could be fused. I got fascinated by the realization that since living things are composed of inanimate molecules, life, at its most basic level, is a biochemical phenomenon. I came across terms like Krebs cycle, Urea cycle, glycolysis, purines and pyrimidines, cholesterol, essential and non-essential fatty acids, etc. at this stage.

After my first degree, Obafemi Awolowo University and Professor Adeyinka Afolayan who was then the Head of Department of Biochemistry in the mow Department of Biochemistry and Molecular Biology, Faculty of Science midwifed my postgraduate training leading to a Ph.D. degree in Biochemistry in 2003. Obafemi Awolowo University provided a monthly stipend of two hundred and fifty naira (250) for the Master of Science (M. Sc.) program and Graduate Assistant, Assistant Lectureship while Professor Afolayan provided the needed mentorship.

The Chemistry of Life

The living matter contains carbon (C), hydrogen (H), oxygen (O), nitrogen (N), sulfur (S) and phosphorus (P) in bulk form in living cells and are essential for life on earth. Elements such as iron (Fe), manganese (Mm), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), sodium (Na), magnesium (Mg), potassium (K), calcium (Fa), chlorine (Cl) and iodine (I), are present in trade quantity and are also essential. Elements such as vanadium (V), chromium (Cr), molybdenum (Mo), boron (B), aluminum (Al), silicon (Si), fluorine (F), arsenic (As), selenium (Se) and bromine (Br) are also present in trace amount and may be essential for life. It is most likely that elements were selected according to their abilities to perform certain structural functions or to provide specific reactivities.

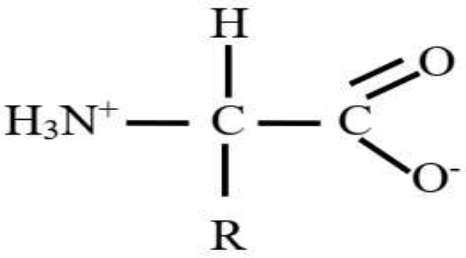
Elements found in the earth and atmosphere may have been tested by trial and error in living organisms over millions of years in the course of evolution. Those elements that most effectively performed the necessary tasks and most importantly, allowed the organism, to thrive were retained. The elements combine together to form biomolecules. This combination provides for great variety in chemical structure and reactivity and compounds representing all the three states of matter (gases, liquids and solids) are present in living cells (Alberts *et al*., 2002).

Cells contain four families of small molecules or their derivatives: amino acids, nucleotides, sugars, and fatty acids. Each of the families perform different functions and are used as building blocks of larger molecules which are polymers. such polymers include deoxyribonucleic acid (DNA), ribonucleic acid (RNA), proteins, starch, cellulose, glycogen etc. The small molecules are also involved in several complex reaction pathways and perform special functions e.g, the nucleotide adenosine triphosphate, ATP, serves as cellular reservoir of chemical energy.

Amino Acids

There are more than hundred amino acids found in nature but only about 20 amino acids are needed to synthesize proteins in most living organisms. These 20 amino acids are all L-isomer alpha amino acids. All of them except glycine contain a central chital carbon. Th3 amino acids selenocysteine and pyrolysine are considered the 21st and 22nd amino acids, respectively, and are more recently discovered amino acids which may become incorporated into protein chains during ribosomal protein synthesis (Lopeg and Mohiuddin, 2022).

The general formula for amino acids is



Each amino acid contains an amino group, protonated under physiological condition (-NH_3), a carboxyl group which is also ionized and a side chain designated as R group. Ut of the amino acids, only ten are essential i.e., they cannot be synthesized in the human body at an adequate rate from non-protein sources and need to be included from the diet. Of these ten, eight are essential at all times, whereas two (arginine and histidine) are required only during periods of rapid tissue growth characteristics of childhood or recovery from illness. The remaining eight are phenylalanine, valine, tryptophan, threonine isoleucine, methionine, lysine and leucine. The non-essential amino acids are alanine, asparagine,

aspartic acid, cysteine, glutamic acid, glutamine, glycine, proline, serine and tyrosine. All of these can be synthesized from fat and carbohydrate precursors.

Amino acids are used primarily in the synthesis of long complex polymers known as polypeptides and are joined together by peptide bond. Short polypeptides up to a length of about 50 amino acids are called peptides. Longer polypeptides are called proteins. Proteins serve many functions in nature and as such are quite complex. Some of their functions include serving as structural materials that provide protection and support e.g. collagen of our connective tissues; proteins are involved in all types of cell movement-the cytoskeleton is composed of action, tubulin and a variety of other proteins; in defense, for example, keratin, fibrinogen, antibodies, interferons are all proteins involved in defense; in regulation- many hormones e.g. insulin and glucagon are proteins; in transport- carriers of molecules e.g. hemoglobin, albumin and lipoproteins transport various molecules in vertebrates. Proteins are also involved in catalysis as enzymes, the theme of this lecture.

Enzymes

The word Enzyme was coined by German physiologist Wilhelm Kuhne in 1878 from the Greek word in leaven' or in (en) yeast (zyme) to describe alcoholic fermentation of sugars by yeast cells. In 1897, two German brothers Hans and Eduard Buchner ground a slurry of yeast cells with sand, obtained a cell-free extract by filtration and then attempted to preserve the extract with sugar syrup only to notice fermentation. He named the extract zymase thus demonstrating that yeast extract could catalyze reactions in the absence of yeast cells (Williams, 1940; Ramasarma, 2003).

It was later realized that they are present in all life forms and have remarkable properties and are capable of increasing reaction rates by factors of between one million (10^6) and one trillion (10^{12}).

They are able to perform this feat under mild conditions of pH and temperature in which cells function due to their complex structures. To illustrate the power of enzymes, consider the chemical synthesis of ammonia (NH_3) by Haber processor Haber-Bosch:

$$N_{2\,(g)} + 3H_{2\,(g)} \underset{450\text{-}500\ ^{o}C}{\overset{200\ Atm}{\rightleftharpoons}} 2NH_{3\,(g)}\ \Delta H^{o} = -91.8\ kJ/mol$$

This reaction can only take place at a temperature of 450 -500 ^{o}C, a pressure of 200 atm in the presence of reduced iron as a catalyst. This same reaction can be catalyzed by an enzyme called nitrogenase which is present in nitrogen-fixing bacteria. A particular genus, Azotobacter is present in the root nodules o leguminous plants and helps convert atmospheric N_2 to NH_3 which in turn is taken up and utilized by the plants. The reaction takes place at ambient temperature and pressure (25 ^{o}C and atm, respectively).

$$N_2 + 8H^+ + 8e^- + 16\ ATP \xrightarrow{\text{Nitrogenase}} 2NH_3 + H_2 + 16\ ADP + 16\ PO_4^-$$

An extreme example is orotidine 5'-phosphate decarboxylase. This enzyme catalyzes a reaction which would take millions of yeasts to occur in milliseconds. It must be stated at this point that not all enzymes are proteins. It is now known some RNA molecules termed ribozymes are catalytically active and play

central roles in the three kingdoms of life. Thy have been found to play roles in tRNA maturation, intron splicing, replication of RNA viruses and protein synthesis (Lescoute and Westhof, 2006). However, the vast majority of enzymes are proteins.

Properties of Enzymes

Enzymes are specific in the reactions they catalyze. (1). Enzymes are also sensitive to temperature. (2). Concentration of hydrogen ion (pH) in solution or environment where the enzyme is contained. (3) ionic strength of the solution. (4). Metals, organic or inorganic ions present in the solution which may serve as inhibitors.

Catalysis takes place at a particular site on the enzyme called the active site and catalyzes reactions by stabilizing the transition state. Enzymes are highly specific both in the reactions they catalyze and in their choice of reactants which are called substrates.

Consider the following reaction:

A and B are called substrates; C and D are called products. A chemical reaction occurs when the colliding molecules possess a minimum amount of energy called activation energy. Not all collisions result in chemical reactions because only a fraction of the molecules have sufficient energy to enter into the reaction (Mckee and Mike, 1994). The substrate molecules bind to the enzyme and they are oriented in such a manner that increases the likelihood of product formation. As products are formed, they are released from enzyme molecules which are then available for more substrates to be converted. Enzymes lower the activation energy required for a chemical reaction to proceed (Figure 1).

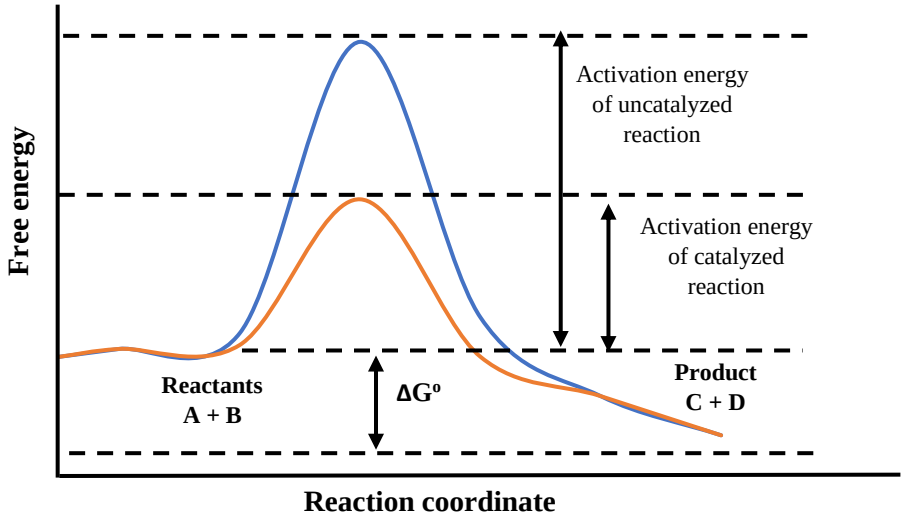


Figure 1: Activation energy of catalyzed and uncatalyzed reaction

Effect of Temperature on Enzymatic Reactions

Increase in temperature of an enzyme-catalyzed reaction results in a corresponding increase in the rate of reaction until an optimum temperature is reached beyond which further increase in temperature results into a decrease in the rate of reaction (Figure 2). At this high temperature, there is a change in the structure of the enzyme causing it to loose activity (Adewale and Ogunbiyi, 2004). The optimum temperature for a mammalian enzyme will

usually be about 40 ºC. For a 10 ºC increase in temperature between 25 ºC and 35 ºC, it is possible up to 2-fold increase in reaction rate.

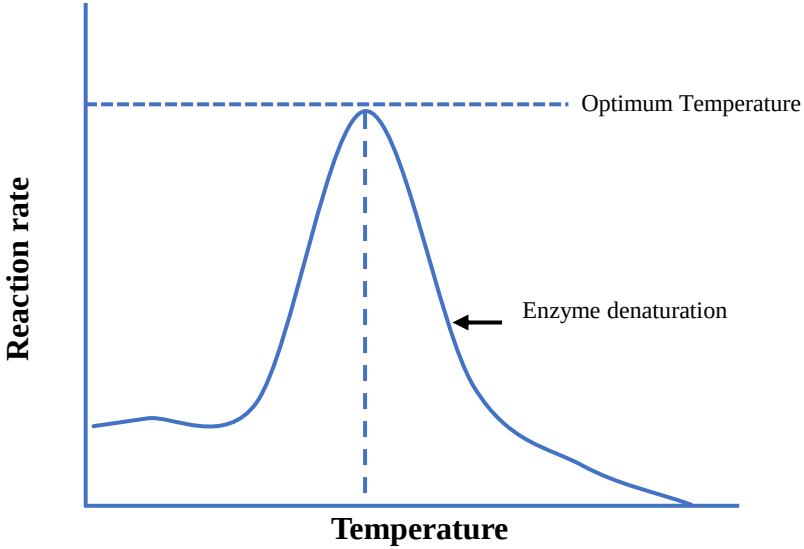


Figure 2: Effect of temperature on enzyme-catalyzed reactions

Enzymes have evolved to catalyze reactions at the temperature obtainable in the habitat of the organism where the enzyme is isolated from. A very good example is the bacteria *Thermus aquaticus*. It lives in hot springs at temperatures of between 65 - 80 ºC. Enzymes from such organism would have optimum temperature in this range. Taq polymerase (*Thermus aquaticus* DNA polymerase) is probably the most important enzyme in medical diagnosis and molecular biology, obtained from this bacterium and can withstand temperature as high as 80 ºC.

Effect of pH on Enzymes

Most enzymes are affected by the pH of their immediate environment and their activity at the highest value at the optimum pH (Figure 3). A lot of enzymes have a narrow pH range. At the pH above or below the optimum pH, there is a decrease in the activity of the enzyme. The loss in activity are also thought to be due to some subtle changes in the structure of the enzyme such as protonation or deprotonation of side group amino acid necessary for catalysis.

At extreme of pH, enzyme can be Completely denatured and precipitated out of solution. Enzymes carry electric charge which, depending on the pH of their environment can be positive, negative or zero. Proteins are least soluble when their net charge is zero.

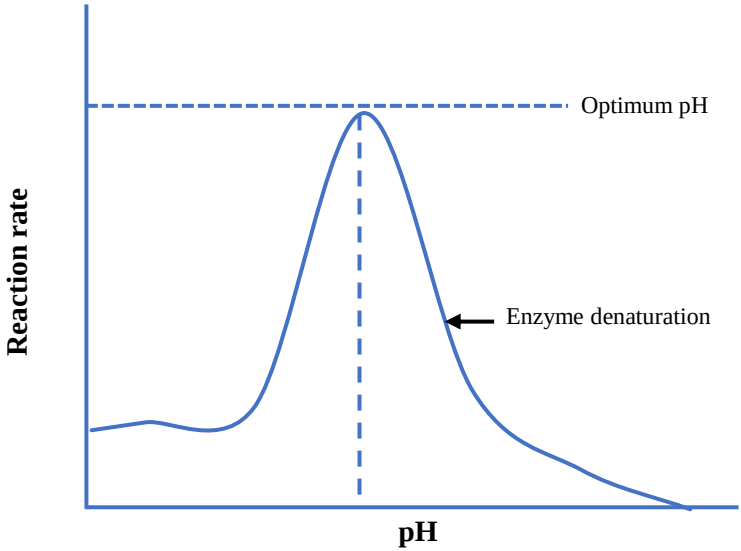


Figure 3: Effect of pH on enzyme-catalyzed reactions

Other Factors Affecting Enzymatic Activity

Other factors that affect enzyme activity include:

(1) Enzyme concentration: If the substrate concentration is sufficiently high i.e. if the rate is independent of substrate concentration, the rate of an enzymatic reaction is directly proportional to the concentration of the enzyme present. A two-fold increase in concentration will usually result in a two-fold increase in the rate of reaction if all factors are kept constant except the enzyme concentration (Robinson, 2015).

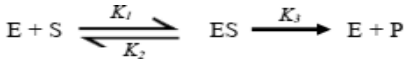
(2) Substrate concentration: At a fixed concentration of the enzyme, the rate of an enzymatic reaction will increase as the substrate concentration increases until the enzyme is completely saturated with substrate, at which time the rate is independent of substrate concentration (zero order kinetics). The enzyme is said to be operating at a maximum velocity (V_{max}). Substrates can in some cases slow down the rate of catalysis. This is called substrate inhibition.

(3) Presence of inhibitors/activator: Apart from the substrate or the enzyme itself, a variety of substances may interfere by slowing down the activity of an enzyme (termed inhibitor) or increases the activity of an enzyme. Metal ions, organic compounds, inorganic ions, may affect the activity of an enzyme. Some enzymes cannot function unless some of these compounds are present. Those that depend on metals are called metalloenzymes. Non-protein parts of the enzymes required for activity are called prosthetic groups. Cofactors are sometimes used to describe metal ions or complex organic molecules (coenzymes) which enzymes require for activity. An enzyme without essential cofactor is called an apoenzyme. Intact enzymes with their bound cofactors are called holoenzymes.

(4) End-product: The end product of an Enzymatic reaction can in some cases affect the activity of the enzyme if it accumulates, unless the product is removed as soon as it is formed. This is termed product inhibition if it slows down the enzyme activity or product activation if it increases the rate of reaction.

Enzyme Kinetics

Consider a single substrate catalyzed reaction



Where E represents the concentration of enzyme, S, the substrate concentration, ES, concentration of enzyme-substrate complex and P, the concentration of product.

The rate of this reaction is given by

$$V = \frac{Vmax\,[S]}{Km + [S]}$$

where V is the velocity (rate) of the reaction,

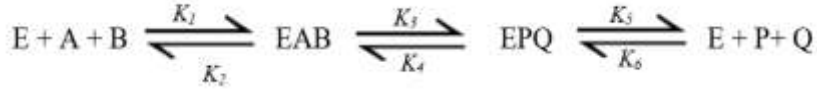
V_{max}, the maximum velocity achievable for the enzyme and K_m, the concentration of the substrate at half maximal velocity.

The equation is called Michaelis-Menten equation in honor of the scientists (Leonor Michaelis and Mand Menten) who first proposed an expression similar to this. Linearization of this equation or its integration permits the determination of the kinetic constants, Km and Vmax experimentally, in the laboratory. Nowadays, the experimental data can be fitted to several computer programs for the estimation of these parameters. It is also possible to estimate the K_{cat}, the turnover number (V_{max}/Et) i.e. the amount of product formed per second per mole of the enzyme or the amount of substrate transformed per second per mole of the enzyme.

$$K_{cat} = V_{max}/\text{Et}$$

The inverse of K_{cat} (in seconds) is the time it takes for a catalytic event. We can also estimate the catalytic efficiency, K_{cat}/K_m for the individual enzyme.

For a two substrate-enzyme catalyzed reaction which are more common:



The equation becomes:

$$V = \frac{Vmax\,[A][B]}{ki\,[A]kb + kb\,[A] + ka\,[B]}$$

The kinetic parameters can be similarly estimated.

Enzyme Inhibition

The activity of enzymes can be inhibited by molecules which bind to the enzyme either reversibly or irreversible, thus, reducing or abolishing the activity of such enzyme(s). Enzyme Inhibitors can exist naturally and assist in the regulation of metabolism. They can also be produced in the laboratory by chemical synthesis. Enzyme Inhibition is the cornerstone of drug discovery research. A lot of drugs used to treat diseases act by inhibiting enzymatic activities. Pesticides, herbicides and natural poisons also work by inhibition of enzymatic activity.

Two types of inhibition:

Reversible inhibition

Irreversible inhibition

Group-specific reagents: react with specific side chains of amino acids. Examples include diisopropylphosphofluoridate (DIPF) and iodoacetamide. DIPF and iodoacetamide can react irreversible with chymotrypsin and acetylcholinesterase.

Affinity labels or reactive substrate analogs: these are molecules that are structurally similar to the substrate for the enzyme and that covalently bind to the active site residues and are thus more specific for the enzyme active site than group-specific reagents. A very good example is Tosyl-L-phenylalanine chloromethyl ketone (TPCK) as a substrate analog of chymotrypsin.

Suicide substrates (suicide inhibitors) or mechanism-based enzyme inhibitors: this type of inhibitor binds to the enzyme at its active site and in the process of catalysis the enzyme as an intermediate bind instantaneously with the catalytic group(s) at the active site thus inactivating the enzyme. They are the most specific means to identify an enzyme's active site. The clinically available drug, (-) deprenyl is a suicide inhibitor of monoamine oxidase.

New terminologies such as catalytic antibodies (abzymes) and transition-state analogs have also come into common use in enzyme.

Enzyme classification inhibiton and properties....from PPT

My odyssey in the world of enzymes

Mr. Vice Chancellor Sir, in the last thirty years or so, my laboratory has focused its attention on the following enzymes:

1. Glutathione transferase
2. Aldehyde dehydrogenase
3. Amylase
4. Invertase
5. Cellulase
6. Tyrosinase/ peroxidase
7. Proteases
8. Glucose-6-phosphate dehydrogenase

Glutathione Transferase

Glutathione transferases, EC. 2.5.1.18, (GSTs) are a group of phase II detoxification enzymes that are found in eukaryotic and prokaryotic organisms. They are grouped into different classes such as alpha, mu, pi, omega, delta, zeta, beta, etc. on the basis of amino acid sequence, substrate utilization and inhibition characteristics. They conjugate compounds with sufficiently electrophilic centre to an endogenous tripeptide called glutathione (ɤ-glutamylcysteinylglycine).

The electrophilic compounds are usually xenobiotics– drugs, pesticides, herbicides, environmental contaminants and conjugation with glutathione makes the xenobiotics more soluble and can then be eliminated from the body via the mercapturic acid pathway (Adewale and Afolayan, 2004). As a result of their activities, they have been implicated in resistance to drugs in humans and resistance to herbicides and pesticides in plants and insects respectively.

They are also overexpressed in organism living in polluted environment, thus conferring on them adaptive strategies. A good example to illustrate this is African River prawn (crayfish) that are

abundant in Osun River at Asejire dam, Ikire. We have studied GST in this organism to provide information if there is anything unique about this enzyme in the organism's adaptation.

We purified GST from the organs of this organism to homogeneity and noted that the concentration of the enzyme was highest in the hepatopancreas. Sodium dodecyl polyacrylamide gel electrophoresis (SDS-PAGE) showed the subunit molecular weight to be 24.1 ± 0.35 kDa and native molecular weight to be 46 ± 1.4 kDa. The GST has two isoenzymes with the same molecular weight. The mechanism of model substrate (CDNB) addition is by steady-state random kinetic mechanism (Figure 4) and could unfold via a three-state unfolding process.

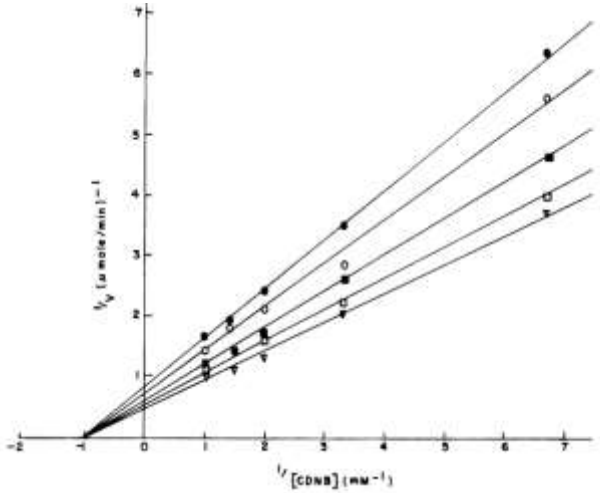


FIGURE 4: Initial-velocity pattern for the crayfish GST. The varied-concentration substrate was CDNB, and the fixed-concentration substrate was GSH

The enzyme uses different mechanisms for different electrophilic substrates that the organism may encounter in its habitat. It was concluded in the study that the high K_m of Asejire crayfish GST for its physiological substrate may be a special physiological adaptation for effective xenobiotic detoxification (Adewale and Afolayan, 2004, 2005). This information is useful in monitoring aquatic contamination. In a similar study, the ability of grasshopper to feed on different plan foods containing different allelochemicals (Figure 5) was attributed to the unique properties of GST which was also partly responsible for the hardiness of African catfish (Adewale and Afolayan, 2006, Famutimi and Adewale, 2021).

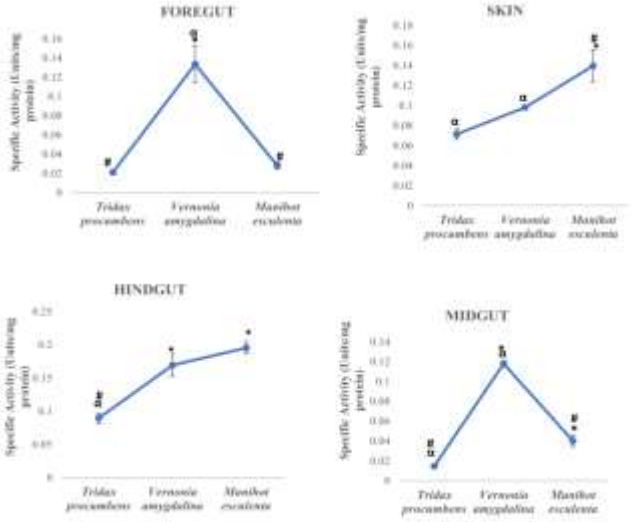


Figure 5: Specific activity of crude GST in tissues of *Z. variegatus* fed on different food plants.

Our work on glutathione transferase from *Bulinus globosus*, the intermediate host of the disease-causing *Schistosoma haematobium* concluded that different isoenzymes of GST are expressed in the same class of molluscs even when they belong to the same genus or species and that the expression may depend on whether the snails are on aestivation or not. During aestivation, the organism is under stress and multiple GSTs belonging to the alpha class with elevated activities were found in the organs of the snail contributing to its drought tolerance (Ojopagogo and Adewale, 2010; Ayinuola and Adewale, 2019). The information provided on these organisms are necessary for the design of effective pesticides/ molluscides. Furthermore, our work on GST has been applied in quality control to measure freshness assessment of stored fish or meat samples (Adewale, 2010).

Aldehyde Dehydrogenase

Aldehyde dehydrogenases, EC. 1.2.1.3, are a group of enzymes that catalyzes the conversion of aldehydes to their corresponding carboxylic acids, they usually use NAD or NNP as a coenzyme.

They are also ubiquitous, being present in plants and animals. Our interest in plant aldehyde dehydrogenase (ALDH) arose because of their role in stress tolerance in plants. Plants have evolved a variety of definite strategies to remove reactive oxygen species (ROS). ROS accumulation to an excessive amount could cause disruption to cellular machinery and homeostasis. The harmful effect of excessive amount of aldehyde is well established and they are directly involved in various illicit reactions such as membrane lipids, nucleic acids, proteins to form adducts which

alter their functionality and manifest phenotypically in form of cell/ tissue death, organ failure, tumour formation.

Family 3 aldehyde dehydrogenase is an important determinant of resistance to toxicity mediated by intermediate chain-length aldehydes that are produced during lipid peroxidation. Overexpression of class 3 ALDH isoenzymes in *Arabidopsis thaliana* caused more than 30% increase in tolerance to drought and high NaCl concentration in the laboratory of one of my German collaborators- Prof. Dr. Dorothea Bartels of the Institute of Molecular Biology and Biotechnology of Plants (IMBIO) at the University of Bonn, Germany. In order to construct drought-tolerant transgenic plants especially food crops such as maize, rice, wheat, etc., overexpression of family 3,5 and 7 of this enzyme had been suggested, but they must be fully characterized biochemically to achieve this feat. They are however difficult to characterize because they are quite unstable. In 2007, I was brought in as a Guest Scientist with a generous grant from European Molecular Biology Laboratory (EMBO) to solve the stability problem, with research experience gained at Enzyme Research Laboratory at Ife. The isoenzymes (ALDH 3H1 and ALDH 3I1) were overexpressed in *Escherichia coli*, stabilized with some biomolecules and characterized. The work was published in a prestigious foremost Biochemical Journal (Stiti *et al.*, 2011).

Amylases

Amylases are a group of enzymes that catalyzes the hydrolysis of starch producing oligosaccharides notably maltose, glucose, maltotriose. They hydrolyze α-1,4 and α-1,6 glycosidic bonds in starch and are classified into three different groups namely: α-amylase, β-amylase and ɤ-amylase.

α-Amylase (EC. 3.2.1.1) also called 1,4-α-D-glucan glucanohydrolase are widespread in nature, being found to be present in most organisms from bacteria, fungi to man. They are metalloenzymes, as most require Ca^{2+} for activity and hydrolyze starch from non-reducing end producing smaller fragments and can thus reduce the viscosity of starch slurry i.e. liquefy gelatinized starch. They are thus valuable industrial enzymes and are extensively used in food industries, breweries, bakeries and confectioneries. They are also used in laundries.

β-Amylase (EC. 3.2.1.2) also called 1,4-α-D-glucanmaltohydrolase is present in bacteria, fungi and plants. It hydrolyzes starch or oligosaccharides produced by the action of α-amylase to produce maltose starting from the non-reducing end. The presence of α-amylase and β-amylase in a suspension of gelatinized starch at the right pH and temperature may lead into the hydrolysis of such starch producing sweetened sugary solution together with limited dextrins. It is therefore also used extensively in many applications like α-amylase.

ɤ-Amylase (EC. 3.2.1.3) also called glucan 1,4-glucosidase, amyloglucosidase, exo-1,4-α-glucosidase can hydrolyze α-1,4-glycosidic bonds at the non-reducing end of starch comprising amylose and amylopectin as well as α-1,6-glycosidic bonds present in amylopectin yielding glucose.

Grain Amylases

Industrial uses of amylases in Nigeria are widespread in the breweries to break down cereal (maize/sorghum) starch into fermentable sugars (glucose/maltose); in beverage manufacture (production of high glucose/maltose syrup); in laundry, to remove starch; and also as additives in detergents, to remove stains. Increasingly, they are also used in biofuel production to degrade

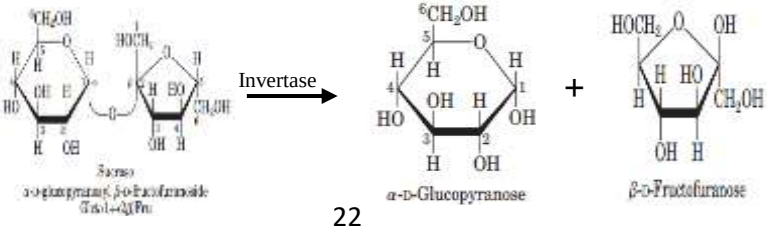
cassava starch into fermentable sugar, which is fermented to give ethanol.

Over the years, my laboratory has carried out extensive work on substitution of bacterial/fungal amylases with amylases obtained from local grains particularly, sorghum and millet (Adewale *et al*., 2006; Adewale and Oladejo, 2009; Adefila *et al*., 2012). We have malted the grains, essentially using locally-fabricated materials, kilned the grains, extracted and purified amylases from the grains using conventional purification procedures and shown that the sorghum amylases can be used as substitutes for imported bacterial/fungal amylases. We compared malted kilned and unkilned grain enzymes and noted that kilned malted sorghum can be stored for several months with the amylases still intact for further application.

Mr. Vice Chancellor, I introduced the word 'unkilned' into the dictionary of science in the process. We have optimized the condition for maximal amylase production: when sorghum grains are steeped in water adjusted to pH 6.5 at 20 ^{o}C, 8-fold higher amount of amylolytic enzymes are produced (Adefila *et al*., 2012).

Invertases

Invertases (EC. 3.2.1.26) also known as β-fructosidase or β-fructofuranosidase are found in yeast, bacteria, fungi and other organisms such as bees and also present in higher plants. Invertase catalyzes the hydrolysis of sucrose (table sugar) into fructose and glucose by cleaving the α-1,2-glycosidic bond.



By hydrolysing sucrose to glucose and fructose, the resulting product fructose is sweeter than sucrose and so less of it is required to sweeten our drinks. It is therefore used in large quantity in food industries, ethanol production, drugs and cosmetics. Invertase is mainly sourced from yeast.

Our interest also stems from the fact that locally-sourced yeast from our environment can replace the imported enzyme. We have screened palm wine from several sources in Southwestern Nigeria for yeast that can produce invertase in large quantities. To my surprise, yeast from Abagboro village around this area produced the highest amount of invertase and the highest concentration of ethanol compared to brewers yeast (obtained from an international brewery in Nigeria) and other local yeast within the Southwest and commercially available yeast. Invertase from Abagboro yeast has been characterized in my laboratory.

Mr. Vice Chancellor Sir, to fully demonstrate the applicability and domesticate the technology, we got cassava tuber from local farmers, mashed it into slurries, gelatinize the starch and used sorghum grain amylolytic enzymes to liquefy and saccharify the starch to obtain a sugary, fermentable solution. Using locally-sourced soyabean as a source of nitrogen for the sugar solution, we fermented the work to obtain 24.2% ethanol compared to 16.7% for yeast from an international brewery in Nigeria. This work has now been patented and the patent certificate issued by the Federal Republic of Nigeria on 23rd December, 2021. Further distillation of our fermented product will result in fuel-grade ethanol which can power automobiles providing alternative to petrol as a non-renewable supply.

Tyosinases

Tyrosinases (EC. 1.14.18.1) also called phenolase, monophenol monooxygenase, monophenol oxidase is a copper-containing enzyme which is quite ubiquitous in nature. It catalyzes the oxidation of various phenolic substances such as tyrosine and 3,4-dihydroxyphenylethylamine (dopamine). These reactions have been a subject of intense investigations in the last two decades or so because of their applications in several biotechnological processes.

Some of the processes include production of 3,4-dihydroxy-L-phenylalanine (L-DOPA), a drug widely used to treat Parkinson's disease, production of cross-linked protein networks for use as novel food additives. It is also an oxidase that is directly involved in the production of melanin, which is responsible for the colour of our skin. Inhibitors of tyrosinase are used in various cosmetics as skin-whitening agents. In plants, tyrosinase is responsible for browning reactions, observed in apple, yam, etc. because of the oxidation of phenolics found in such fruits. The enzyme has traditionally been sourced from mushroom *Agaricus bisporus.*

My interest in tyrosinase research stems from the fact that different species of yam could serve as a cost-effective source of tyrosinase for various industrial uses. With a grant from the University Research Council (URC) of the senate of OAU, we devised a novel method of purification of tyrosinase from four species of yam, namely, *Dioscorea praehensilis, Dioscorea alata, Dioscorea rotundata, Colocasia esculenta* (cocoyam) which were found to contain highest tyrosinase protein rather than other tubers such as *Xanthosoma mafaffa, Ipomea batatas* and *Dioscorea dumetorum.*

Tyrosinase from the four species was purified to homogeneity (Figure 6) as measured by sodium dodecyl polyacrylamide gel electrophoresis.

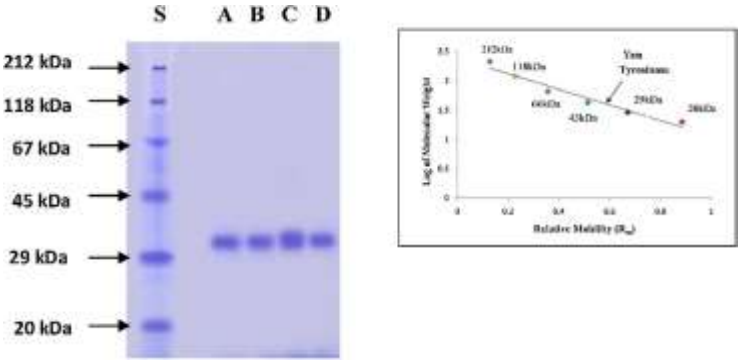


Figure 6: **SDS-PAGE of purified tyrosinase from *D. praehensilis, D. alata, D. rotundata* and *C. esculenta*.**

We showed that all the purified enzymes were activated in 40% ether by between 120 and 170% and maintained 100% residual activity at up to 65% ether for 17 hours. Optimum pH and temperature were 6.5 and 50 ^{o}C, respectively. B-mercaptoethanol and glutathione were inhibitors of the enzyme. We showed that different colored products (Figure 7) could be formed in aqueous environment in the presence of substrates or additives including catechol, pyruvic acid and ammonia (Ilesanmi *et al*., 2014; Ilesanmi *et al*., 2021).

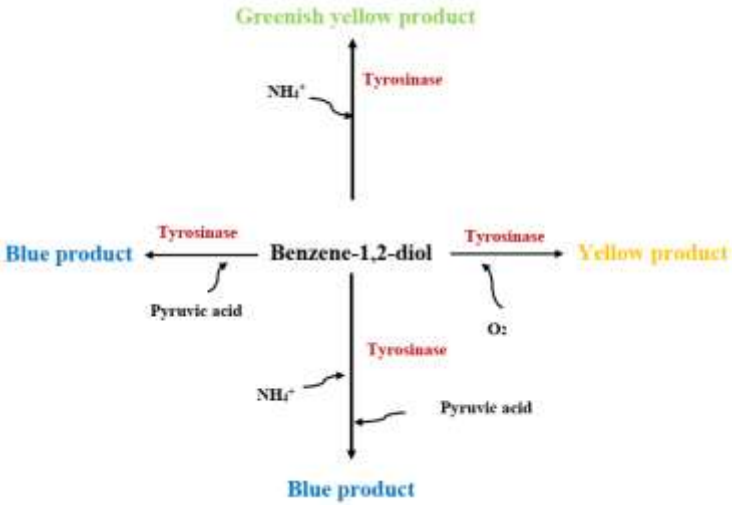


Figure 7: Colored products formed in aqueous environment in the presence of substrates

Mr. Vice Chancellor Sir, this piece of information is valuable for organic and pharmaceutical chemists for the green synthesis of drugs and novel food and pharmaceutical compounds. We have extended this study to *Moringa oleifera* leaves as a source of this enzyme (Agunbiade and Adewale, 2022). Interestingly, there was a spike in the international demand for Nigerian yams when we first published this work in 2014.

In a study quite similar to tyrosinase work, we also showed that white and red cultivars of kolanut, *Cola* nitida could be a source of peroxidases which are also used in various diagnostic reagents as reporter enzymes (Adewale and Adekunle, 2018). The fund for this work was provided by Tetfund Institution-Based Research.

Serine Proteases: Furin and Transmembrane Serine Protease 2 (TMPRSS2)

Serine proteases are found in many living cells as part of ‘molecular scissors’ responsible for cutting unwanted proteins; in food digestion, to break down the polymeric proteins into peptides and amino acids to facilitate absorption; or in posttranslational modification of proteins to generate new functionality for roles in cell proliferation, immunity and inflammation. Aberrant activity (hyperactivity) or inactivity of some of the proteases have therefore been implicated in many diseases including cancer, cardiovascular disorders, autoimmune diseases and viral infections. We have focused our attention on two proteases which have been implicated in several human pathologies including COVID-19: furin and TMPRSS2.

Furin is a member of the evolutionary ancient family of proprotein convertase and has been termed ‘master switch’ of tumor growth and progression at its aberrant expression or activation can promote the formation and progression of various malignancies (Figure 8), and are involved in the infectivity of severe acute coronavirus 2. Several studies have shown that the spike proteins are cleaved by host cell furin at the S1/2 site thus allowing the virus to transfer its genome into the host cell (Braun and Sauter, 2019).

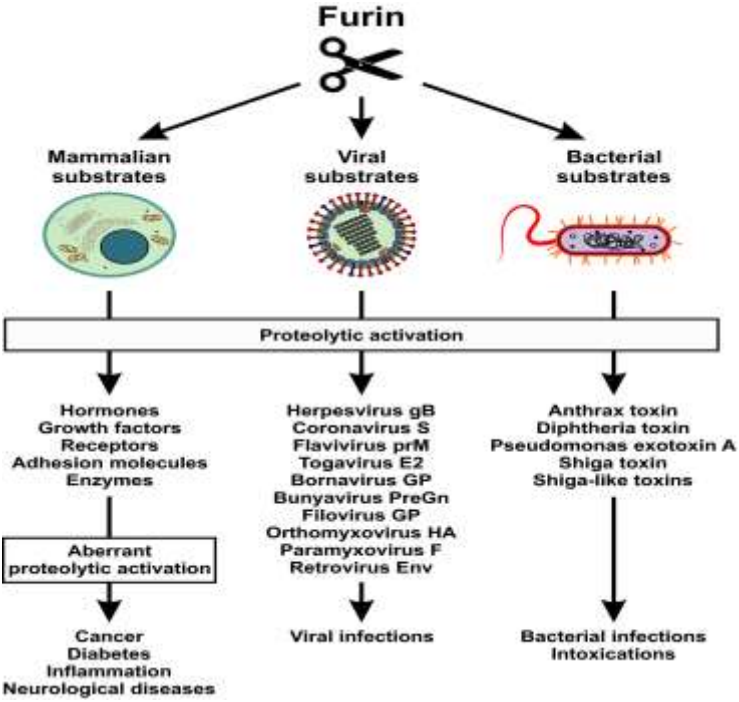


Figure 8: Some roles of furin in normal and disease states (Adapted from Braun and Sauter, 2019)

TMPRSS2, on the other hand, has been an important target in the treatment of seasonal influenza infections and contributes to prostate carcinogenesis and metastasis (Pászti-Gere *et al*., 2016). In order for SARS-Cov-2 to enter cells, TMPRSS2 has also been implicated as being responsible for cleaving the spike protein at the S2' site. The studies suggest that inhibitors of these proteases have high therapeutic potential for treatment of these diseases.

Based on a report by Majumdar *et al*. (2010), many medicinal plants which had hitherto thought, to exhibit their medicinal properties by virtue of high polyphenols and flavonoids present in

them, actually contain inhibitors of the serine proteases; proprotein convertases. With international funding support from seven funding agencies namely Science Granting Councils Initiative in Sub-Saharan Africa (SGCI) and administered by South Africa's National Research Foundation (NRF) in collaboration with Canada's International Development Research Centre (IDRC), the Swedish International Development Cooperation Agency (Sida), South Africa's Department of Science and Innovation (DSI), the Fonds de Recherche du Quebec (FRQ), the United Kingdom's Canada's International Development Research Centre (IDRC), the Swedish International Development Cooperation Agency (Sida), South Africa's Department of Science and Innovation (DSI), the Fonds de Recherche du Quebec (FRQ), the United Kingdom's Department of International Development (DFID), United Kingdom Research and Innovation (UKRI) through the Newton Fund, and the SGCI participating councils under the COVID-19 Africa Rapid Grant Fund, I as the Principal Investigator in collaboration with Prof. Dr. Daniel Sauter of the University Hospital Tubingen, Germany and other partners from University of Johannesburg, South Africa and OAU Ife are screening four medicinal plants- *Momordica charantia, Lawsonia inermis, Xylopia aethiopica* and *Hymenocardia acida* for inhibitors capable of inhibiting the proteases. Each of the inhibitors could be further developed to manage COVID-19. These plants have traditionally been used to manage at least four viral diseases. Preliminary results suggest that the plants have phytochemicals that are protease inhibitors (Adewale *et al*., 2023). We are currently carrying out whether the purified extracts would block the replication of HIV and SAR-CoV-2.

Mr. Vice Chancellor Sir, I am also happy to announce to you that my collaboration with the nano laboratory of Prof. S.O.

Oluwafemi, University of Johannesburg, South Africa, an authority in nanoscience and technology is yielding positive results as we have observed for the first time, that quantum dots could serve as inhibitors of serine proteases.

Recommendations

Mr. Vice Chancellor Sir, the study of enzymes as catalysts within living cells and *in vitro* (outside) is a cornerstone of development in medicine, agriculture, biotechnology and even in green chemistry. In medicine, it was stated earlier that enzymes which may be derived from bacteria, fungi, plants or animals; or produced by recombinant DNA technology, could be taken directly as drugs for some diseases lacking the enzyme for a particular reaction or to breakdown a particular substance in the body e.g. streptokinase (SK) which breaks down blood clots in some cases of heart attack (myocardial infarction) and embolism; glucocerebrosidase (Cerezyme, Velaglucerase, Taliglucerase) to manage Gaucher's disease or recombinant deoxyribonuclease (Pulmozyme) to manage cystic fibrosis.

Except for industrial enzymes, which don't necessarily have to be highly homogenous, most of the enzymes in chemical and other biotechnological applications are supposed to be used in pure form, otherwise, unwanted side reactions may occur. Enzyme isolation, purification and characterization is an expensive venture that requires high level of skills. No wonder, biochemists in the field of enzymology have moved to other less financially-demanding areas thus the school authority should consider this area of research for funding as it was in the beginning when Ife was the foremost institution with facilities for enzyme/ protein chemistry research.

Our Molecular Biology programme in the Department of Biochemistry and Molecular Biology, probably the first in the country has not taken off since council approval about eight years ago as a result of no funding. I recommend that the University should consider funding our Molecular Biology programme a priority, in the process, enzymology research will benefit for they are closely integrated. To borrow a leaf from other countries who have recognized enzymology between 1974 and to late eighties, our degree programme in Biochemistry was so well equipped that our graduates who chose to pursue further studies in Europe or America has a seamless transition, because they had been exposed to all necessary tools similar to what obtained in those climes. There was no University webometric ranking then, but we were getting commendation letters from Ivy League schools in Europe and America. Biochemistry or Molecular Biology is the language of Life Sciences. Allocating resources to biochemical research will stimulate high-quality, cutting-edge research in life sciences such as the creation of high-yielding transgenic crops able to withstand drought or salt stress, diseases and pests; production of novel foods and nutraceuticals; novel drugs for old and emerging diseases; novel catalysts for biotechnological applications etc. These will lead to high-quality publications in high-impact journals, improving the image of the University in addition to solving problems in our immediate environment.

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limited degree of freedom. These two extremes moulded my character. I appreciate all my brothers particularly Dr. Ademola Adewale who took an unusual interest in me since I was about ten years old and guided me even up till this moment. I gained knowledge and wisdom from him and his wife- Mrs. Tinuade Adewale; Late Hon (Dr.) Elijah Adewale and Bishop Mike Adewale. I acknowledge the contributions of my former teacher and supervisor- Professor Adeyinka Afolayan, he was responsible for the sponsorship of my Ph.D. programme in Biochemistry and gave me a cash donation of six thousand naira (₦6,000.00) for the payment of my first article in Journal of Nigerian Society for Biochemistry and Molecular Biology. He recommended me for the award of Obafemi Awolowo University Postgraduate Fellowship Award which enabled me to enroll and complete my M.Sc. degree in Biochemistry. I appreciate my other teachers in the Department of Biochemistry and Molecular Biology and Department of Microbiology particularly, Prof. F. D. Onajobi, Prof. A. Aboderin, Late Prof. (Oba) Folayan, Late Prof. Shonekan, for they showed love to me. I appreciate all my other lecturers in the Faculty through which I have gained knowledge. I appreciate all past Deans in the Faculty who have been assigning responsibilities to me, which enabled me to acquire requisite experience which I now found to be very useful: Prof M. Badejo, Prof. V. Olarenwaju, Prof. W. Muse, Prof. A. O. Ogunfowokan, Prof. A. Adedeji and Prof. M.A Eleruja. I always enjoyed their company and the company of my good friends- Gbenga Alebiowu and Akinropo Akinfala.

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I want to appreciate my international collaborators: Professor Samuel Oluwatobi Oluwafemi has been wonderful, hard-working and God-sent. Through him, the University and the country stand to gain tremendously for he is the leading expert in nanoscience in the world. I appreciate Prof. Dr. Sauter (University of Tubingen, Germany) and Prof. Dr. Dorothea Bartels of the University of Bonn, Germany. I appreciate all my present and former students and all my other collaborators in OAU- Prof. Mrs. Adedeji, Prof. A.E. Folorunso, Prof. M.A. Aderogba and Prof. B. Omafuvbe.

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References

Adefila, O. A., Bakare, M. K., & Adewale, I. O. (2012). Characterization of an α-amylase from sorghum (Sorghum bicolor) obtained under optimized conditions. *Journal of the Institute of Brewing*, *118*(1), 63–69.

Adewale, I. (2010). Freshness assessment of stored meat by specific activity of glutathione transferase. *Emirates Journal of Food and Agriculture*, *22*(2), 140. https://doi.org/10.9755/ejfa.v22i2.4901

Adewale, I. O., & Afolayan, A. (2004). Purification and catalytic properties of glutathione transferase from the hepatopancreas of crayfish macrobrachium vollenhovenii (herklots). *Journal of Biochemical and Molecular Toxicology*, *18*(6), 332–344. https://doi.org/10.1002/jbt.20044

Adewale, I. O., & Afolayan, A. (2005). Organ distribution and kinetics of Glutathione transferase from African river prawn, Macrobrachium vollenhovenii (Herklots). *Aquatic toxicology (Amsterdam, Netherlands)*, *71*(2), 193–202. https://doi.org/10.1016/j.aquatox.2004.11.005

Adewale, I. O., & Afolayan, A. (2006). Studies on glutathione transferase from grasshopper (Zonocerus variegatus). *Pesticide Biochemistry and Physiology*, *85*(1), 52–59. https://doi.org/10.1016/j.pestbp.2005.10.004

Adewale, I. O., & Oladejo, A. (2009). Properties of the isoforms of α-amylase from kilned and unkilned malted sorghum (Sorghum bicolor). Carbohydrate Polymers, 77(1), 105–109. https://doi.org/10.1016/j.carbpol.2008.12.011

Adewale, I. O., Adebiyi, V. G., Famutimi, O. G., & Dada, O. V. (2023). Kinetics of trypsin inhibition by methanolic and solvent-partitioned fractions of two medicinal plants – Momordica charantia and Xylopia aethiopica. *South African Journal of Botany*, *152*, 174–181. https://doi.org/10.1016/j.sajb.2022.11.037

Adewale, I. O., Agumanu, E. N., & Otih-Okoronkwo, F. I. (2006). Comparative studies on α-amylases from malted maize (Zea mays), millet (Eleusine coracana) and Sorghum (Sorghum bicolor). *Carbohydrate Polymers*, *66*(1), 71–74. https://doi.org/10.1016/j.carbpol.2006.02.022

Adewale, I., & Adekunle, A. (2018). Biochemical properties of peroxidase from white and red cultivars of kolanut (Cola nitida). *Biocatalysis and Agricultural Biotechnology*, *14*, 1–9. https://doi.org/10.1016/j.bcab.2018.01.013

Agunbiade, O. J., & Adewale, I. O. (2022). Studies on latent and soluble polyphenol oxidase from Moringa oleifera Lam. Leaves. *Biocatalysis and Agricultural Biotechnology*, *45*, 102515. https://doi.org/10.1016/j.bcab.2022.102515

Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K., & Walter, P. (2002). The Chemical Components of a Cell. *Molecular Biology of the Cell. 4th Edition*. https://www.ncbi.nlm.nih.gov/books/NBK26883/

Ayinuola, Y. A., & Adewale, I. O. (2019). Proteomic identification of an alpha class glutathione S-transferase in freshwater snails (Bulinus globosus). *Animal Biology*, *69*(3), 377–390. https://doi.org/10.1163/15707563-00001045

Braun, E., & Sauter, D. (2019). Furin-mediated protein processing in infectious diseases and cancer. *Clinical & Translational Immunology*, *8*(8), e1073. https://doi.org/10.1002/cti2.1073

Famutimi, O. G., & Adewale, I. O. (2021). Induction and catalytic properties of grasshopper (Zonocerus variegatus) glutathione transferase fed on different food plants. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, *241*, 108970. https://doi.org/10.1016/j.cbpc.2020.108970

Ilesanmi, O. S., Adedugbe, O. F., & Adewale, I. O. (2021). Potentials of purified tyrosinase from yam (Dioscorea spp) as a biocatalyst in the synthesis of cross-linked protein networks. *Heliyon*, *7*(8), e07831. https://doi.org/10.1016/j.heliyon.2021.e07831

Ilesanmi, O., Ayinuola, Y., & Adewale, I. (2014). Kinetic characteristics of purified tyrosinase from different species of Dioscorea (YAM) in aqueous and non-aqueous systems. *Journal of Molecular Catalysis B: Enzymatic*, *108*, 111–117. https://doi.org/10.1016/j.molcatb.2014.07.009

Lescoute, A., & Westhof, E. (2006). The interaction network of structured RNAs. *Nucleic Acids Research*, *34*, 6587–6604. https://doi.org/10.1093/nar/gkl963

Lopez, M. J., & Mohiuddin, S. S. (2022). Biochemistry, Essential Amino Acids. In *StatPearls*. StatPearls Publishing. http://www.ncbi.nlm.nih.gov/books/NBK557845/

Majumdar, S., Mohanta, B. C., Chowdhury, D. R., Banik, R., Dinda, B., & Basak, A. (2010). Proprotein convertase inhibitory activities of flavonoids isolated from Oroxylum indicum. *Current Medicinal Chemistry*, *17*(19), 2049–2058. https://doi.org/10.2174/092986710791233643

Ojopagogo, Y. A., & Adewale, I. O. (2010). Alteration in the status of glutathione transferase of the water snail, Bulinus globosus, during aestivation and recovery. *Animal Biology*, *60*(2), 145–155. https://doi.org/10.1163/157075610X491680

Pászti-Gere, E., Czimmermann, E., Ujhelyi, G., Balla, E., Maiwald, A. and Steinmetzer, T. (2016). *In vitro* characterization of TMPRSS2 inhibition in IPEC-J2 cells. *Journal of Enzyme Inhibition and Medicinal Chemistry* 31(S2): 123-129**.**

Robinson P. K. (2015). Enzymes: principles and biotechnological applications. *Essays in biochemistry*, *59*, 1–41. https://doi.org/10.1042/bse0590001

Stiti, N., Adewale, I. O., Petersen, J., Bartels, D., & Kirch, H. H. (2011). Engineering the nucleotide coenzyme specificity and sulfhydryl redox sensitivity of two stress-responsive aldehyde dehydrogenase isoenzymes of Arabidopsis thaliana. *The Biochemical journal*, *434*(3), 459–471. https://doi.org/10.1042/BJ20101337