ENZYMOLOGY AND RADIATION BIOLOGY IN THE UNDERSTANDING OF BIOCHEMISTRY

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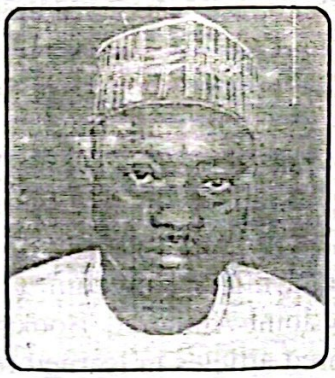

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University of Stanford, United Kingdom	1991-1994
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Professor of Biochemistry, Bayero University, Kano	2005 to Date
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Graduate Assistant, Bayero University, Kano	1990-1993

Professor Muhammad Sani Sule has won several awards and prizes, including Northco Holding Prize for the best student Graduating Student in B.Sc. Biochemistry, Usmanu Danfodio University, Sokoto (1989); Zamfara Textiles Prize for the Best Graduating Student in the Faculty of Science, Usmanu Danfodio University, Sokoto (1989); Vice Chancellor's Prize for the overall Graduating Student in Academic Performance, Usmanu Danfodio University, Sokoto (1989); and Mountbatten Award Winner, University of Stanford, United Kingdom (1994). Professor Sule has published widely: Two Joint-Authored Books; and Thirteen Single and Thirty Nine Joint-Authored articles in learned reputable academic journals. Professor Sule has attended several Conferences and Seminars and presented scholarly papers. Professor Sule was the National Assistant Secretary of Nigerian Society of Biochemistry and Molecular Biology from 1995 to 2003. He is a Member, Nutritional Society of Nigeria; Member, Nigerian Society for Experimental Biology; and Member, Nigerian Institute of Food Science and Technology.

PROFESSORIAL INAUGURAL LECTURE

ENZYMOLOGY AND RADIATION BIOLOGY IN THE UNDERSTANDING OF BIOCHEMISTRY

1.0 INTRODUCTION

Biochemistry is simply the chemistry of life. It deals with study of the molecules of life, which are carbohydrates, lipids, proteins, nucleic acids and water.

Proteins, which are polymers of amino acids, are essential molecules of life. They carry out many functions in living organisms – from structural, transport to enzymatic.

Enzymes are special types of proteins with catalytic function. They catalyse thermodynamically feasible reaction at a rate compatible with biochemical processes essential for the maintenance of the cell and the whole organism. Even though most of them are proteins, a few of them have been shown to be ribonucleic acids (RNA). Some enzymes are made up of only amino acids or proteins part and are therefore simple proteins e.g. guanase, ribonuclease. Others require additional non-protein part, called cofactor, for activity. These are conjugated proteins. The cofactor can simply be a metal ion, in metalloproteins such as superoxide dismutase, xanthine oxidase, or a coenzyme such as lipoic acid, thiamine pyrophosphate or pyridoxal phosphate.

Some enzymes consist of a single polypeptide chain i.e. they are monomeric proteins e.g. lysozyme, fructose 1,6 bisphosphate aldolase. Others consist of 2 or more polypeptide (pp) chains and are, therefore, polymeric proteins. Some examples are phosphorylase a (2 pp chains), chymotrypsin (3 pp chains), glyceraldelyde 3-phosphate dehydrogenase (4 pp chains), *E. coli* RNA polymerase (5 pp chains) and glutamine synthetase (12 pp chains).

Enzymes are also diverse in their relative molecular mass and amino acid (aa) contents. They range from 13,700 Daltons (D) and 124 aas for ribonuclease, 13,930 D and 129 aas for lysozyme, 21,600 D and 241 aas for chymotrypsin to as high as 619,000 D

and 5,628 aas for glutamine synthetase and 1,604,000 D and >10,000 aas for acetyl coA carboxylase.

2.0 NITRIC OXIDE SYNTHASE

Nitric oxide was previously considered to be an unstable gaseous chemical produced either in automobile engines or in industry. It was later discovered to be an essential biomolecule with beneficial homeostatic and regulatory functions in the cardiovascular and nervous system of animals. Some of the reported functions of NO include (Sule, 2000a): Relaxing the smooth muscles of blood vessels and corpus cavenosum, cytotoxic effects of macrophages and neutrophils, inhibition of platelet aggregation, regulation of capillary pressure in the kidney etc.

After the confirmation of NO as biomolecule, its source in the biological system was discovered. It was shown to be formed from arginine in various tissues in the body of mammals, such as endothelial cells, macrophages, neutrophils and brain (Tayer and Marletta, 1989; Bredt and Snyder, 1990). It was demonstrated that the NO-synthesizing enzyme stoichiometrically converts arginine to citrulline and NO (Bredt and Snyder, 1990).

The L- arginine undergoes two sequential monoxygenation reactions, the first leading to the formation of N-hydroxyarginine and the second leading to the formation of NO and citrulline.

Bredt and Snyder (1990) purified the enzyme, Nitric oxide synthase (E.C. 1.14.23.1), for the first time from rat cerebellum. The enzyme is an NADPH- dependent dioxygenase, which also requires calcium and calmodulin for its activity (Mayer *et al.*, 1990). It exists in two isoforms (Forstermann *et al.*, 1990), viz: the one that is constitutively expressed in tissues like brain, adrenal gland and lungs and is termed constitutive NOS; and the other that is only expressed when induced by cytokines, such as tumour necrosis factor ∝, interleukin-1 and interferon Ý and is called inducible NOS. The inducible NOS is formed in tissues such as macrophages, liver and human myocardium.

NO was later implicated in several pathological states, including septic shock, vascular leak syndrome associated with cytokine

therapy, uraemia, diabetes and neurological diseases. The constitutive NOS was observed to be linked with all the beneficial effects of NO and the inducible NOS was implicated in all the harmful effects of NO (Pfeilschifter and Vosbeck, 1991). This led to the suggestion that therapeutic inhibition of the inducible NOS could be beneficial if it is sufficiently specific not to interfere with the constitutive NOS.

2.1 Contribution to Knowledge

The development of selective inhibitor of inducible NOS, which would have no effect on constitutive NOS, could only be possible if the mechanisms of the reactions catalyzed by both isoforms and their other properties are elucidated. The first step in the achievement of this elucidation is the purification and characterization of the isoforms. Ion-exchange chromatography on DEAE cellulose column and affinity chromatography on 2,5 – ADP agarose were utilized to purify the constitutive NOS from porcine cerebellum (Sule, 1997a). The purification enzyme was 3200 – fold with 47% yield. The purified enzyme was found to be homogenous, migrating as a single 150 KDa band on sodium dodecylsulphate – polyacrylamide gel electrophoresis. The Km of the enzyme for L-arginine was found to be 1.30μM and Vmax to be 0.91 μmol min $^{-1}$ mg $^{-1}$ protein.

2.2 Further Developments

In another development, it was reported that NO may have neuroprotective or neurotoxic effects depending on its oxidation – reduction state (Lipton *et al.,* 1993). The workers found that all the neuroprotective effects were caused by oxidized NO i.e. nitrosonium ion, NO^+) while all the neurotoxic effects were caused by reduced NO (i.e. nitric oxide, $NO^{\cdot}$). NO^+ reacts with thiol group of proteins, in a process called S-nitrosylation, which might bring about a physiological regulation function. On the other hand, $NO^{\cdot}$ reacts with peroxide ion, O_2^- to form peroxynitrate, $ONOO^-$, which is the final neurotoxic agent.

As a result of that finding, it was clear that a different therapeutic approach was needed. Snyder (1993) suggested that the best therapeutic agent in any of the neurotoxic conditions of NO should either prevent the formation of $NO^{\cdot}$ whilst enhancing the formation of NO^{+}, or be able to be converted to NO, but only the NO^{+} form.

The mechanism by which NO relaxes smooth muscles was later elucidated in the corpus cavernosum. It was found that NO^{+} activates guanylate cyclase through S-nitrosylation. The enzyme catalyses the formation of cGMP from GTP. The cGMP activates cGMP – dependent protein kinase which phosphorylates some muscle protein leading to smooth muscle relaxation (Figure 1).

The cGMP is normally removed by phosphodiesterase V, which converts it to GMP. In some individuals or under certain conditions e.g. old age, fatigue and diseases like diabetes; the activity of phosphodiesterase V becomes enhanced, leading to erectile dysfunction (Boolell *et al.*, 1996).

The workers developed an inhibitor of phosphodiesterase V i.e. sildenafil, which maintains cGMP concentration necessary to achieve and maintain erection when sexual stimulation exists. Sildenafil, in form of sildenafil citrate (or Viagra) has been found to manage erectile dysfunction caused by spinal cord injury, depression, hypertension or diabetes (Steers, 1998).

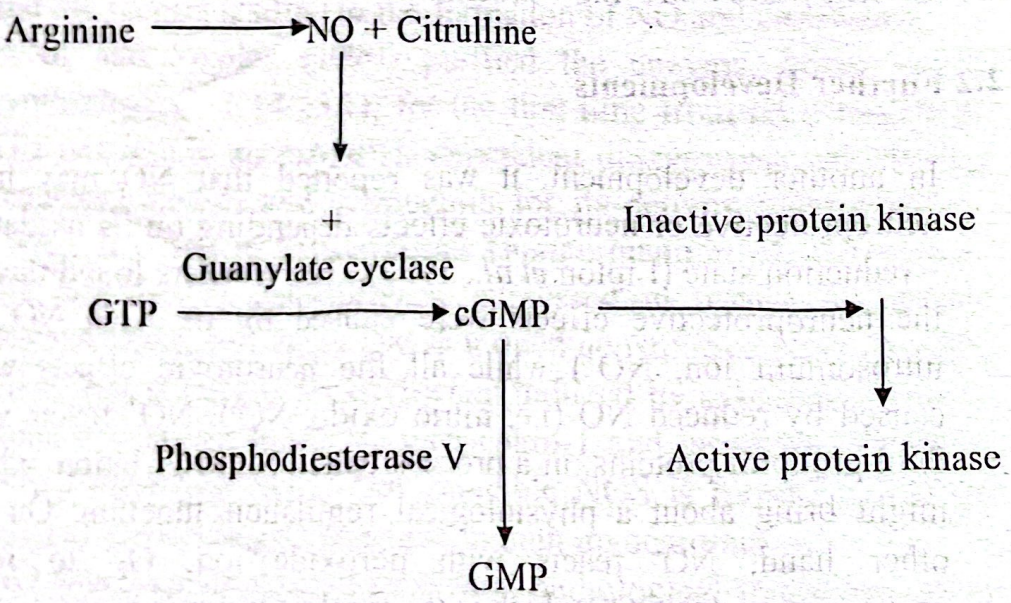

Figure 1: The formation of NO and its mechanism of activation of protein kinase

3.0 RADIATION BIOLOGY STUDIES

Radiation biology is the study of effects produced on living organism or biomolecules as a result of exposure to ionizing radiation. Ionizing radiations are those radiations capable of causing ionization and excitation of molecules along their track. They include electromagnetic radiations (X and gamma rays), particle from radioactive decay (α and β- particles), neutrons, high-energy electrons and fission fragments.

Interaction of ionizing radiations with biomolecules or living organisms leads to the production of free radicals. Free radicals are species (atoms or molecules) with unpaired electrons, making them very reactive. Some examples are $H^{\cdot}$, $OH^{\cdot}$, $NO^{\cdot}$, $HO_2^{\cdot}$, $N_3^{\cdot}$ etc.

In addition to exposure to ionizing radiation, free radicals can be produced in living cells by normal metabolic processes, such as incomplete reduction of oxygen in electron transport chain, purine catabolism, degradation of oxygenated hemoglobin and cyt P_{450} metabolism. With the exception of $NO^{\cdot}$, most free radicals formed in living tissues are toxic and are implicated in various pathological states. Living cells, therefore, have mechanism by which free radicals are detoxified, such as the use of enzymes (like catalase, superoxide dismutase and glutathione peroxidase) and antioxidants (like ascorbic acid, alpha-tocopherol, uric acid and bilirubin)

My main areas of interest in radiation biology are in food irradiation and enzymology.

3.1 FOOD IRRADIATION

According to a world health organization report, the world has ample food to feed everybody and that the problem lay in the fact that large quantities of food are lost after harvest. In Africa, the losses are as high as 30 – 40% of the total production (Chinsman, 1987). In addition to being not very effective, certain insecticides and fumigants, like ethylene dibromide, have been found to be carcinogenic. One of the most effective and viable alternative is food irradiation (Sule, 1997b). Food irradiation refers to the

process of subjecting food to a dose of ionizing radiation in order to kill off bacteria and fungi, inactivate food degrading enzymes and halt the food's natural decaying process.

It was reported that irradiation of foodstuffs up to an overall dose of 10 kGy did not make the foodstuffs to become radioactive, did not produce special radiolytic products, presented no toxicological hazard or microbiological problems and did not introduce special nutritional problems (Wynne, 1989; Loaharanu, 1990). It was further reported that even though the individual food components were sensitive to irradiation, they were observed to be relatively resistant to it when present in a complex matrix of a food (Loaharanu, 1990).

The first food irradiation was carried out in the United States in 1943 when hamburger taken by its troops was irradiated to increase its shelf-life. In 1958, irradiation of potatoes was permitted in the then USSR. The irradiation of some specific foodstuffs is permitted in about 50 countries, such as Argentina, Brazil, South Africa, USA, France, Germany, Russia, Japan and Thailand (Table 1, IAEA, 1989). The introduction of food irradiation in African countries could curb the menace of post-harvest food losses, provided that the accompanying safeguards are strictly adhered to. The safeguards include labeling of irradiated foods, food inspection (or policing) to verify the irradiation dose and consumer education programme (IAEA, 1989).

Table 1. Some of the countries currently irradiating food and the types of food irradiated

	S.Africa	Argentina	Brazil	Germany	France	Israel	Thailand	China
Strawberries	+	+	+			+	+	
Beans			+					
Onions	++	+	+	++	+	+	++	+
Potatoes	++	+	+		+	+	++	++
Wheat			+				+	+
Poultry	+		+			+	+	+
Spices	+	++	++	+	++	++	+	

Key: + Permitted

++ Currently irradiated

3.2 ENZYME STUDIES

Ionizing radiations lead to the production of free radicals especially in aqueous solutions. The free radicals produced cause inactivation of enzymes. The free radical – induced inactivation of enzymes has been used to study the mechanism of enzyme action. It is a type of chemical modification, which is one of the steps involved in elucidating enzyme reaction mechanism.

There are 2 types of free radicals employed in radiation biological studies on enzymes, viz: primary radicals of water radiolysis and secondary (or inorganic) radicals (Bisby *et al.*, 1984).

The primary radicals of water radiolysis (PRWR) are generated when aqueous solution of enzyme is irradiated. The water molecules absorb most of the radiation energy, leading to their ionization and electronic excitation. They eventually form PRWR i.e. hydroxyl radical ($OH^{\cdot}$), hydrogen atom ($H^{\cdot}$) and hydrated election (e^{-}_{aq}) as depicted in reactions 1 – 4.

$$2H_2O \xrightarrow{\sim\sim} HO_2^{+} + e^{-};\ H_2O^{*} \quad \ldots\ldots\ldots\ldots\ldots (1)$$

$$H_2O^{*} \longrightarrow H^{\cdot} + OH^{\cdot} \quad \ldots\ldots\ldots\ldots (2)$$

$$e^{-} \xrightarrow{H_2O} e^{-}_{aq} \quad \ldots\ldots\ldots\ldots (3)$$

$$H_2O^{+} + H_2O \longrightarrow H_3O^{+} + OH^{\cdot} \quad \ldots\ldots\ldots\ldots (4)$$

The secondary radicals are formed by irradiating aqueous solution of enzyme containing sodium or potassium salt of the radical to be produced. The radical Br_2^{-}, for example, is formed by adding sodium or potassium bromide, so that $OH^{\cdot}$ from water radiolysis reacts with Br^{-} to form $Br^{\cdot}$ which complexes with Br^{-} to form Br_2^{-} (reactions 5 and 6)

$$Br^{-} + OH^{\cdot} \longrightarrow Br^{\cdot} + OH^{-} \quad \ldots\ldots\ldots\ldots\ldots\ldots (5)$$

$$Br^{-} + Br^{\cdot} \rightleftharpoons Br_2^{-} \quad \ldots\ldots\ldots\ldots\ldots\ldots (6)$$

Cl_2^{-}, I_2^{-} and $(SCN)_2^{-}$ can similarly be formed (Adams *et al.*, 1972). Another inorganic radical employed is azide radical,

which is formed by the reaction of azide ion with hydroxyl radical.

$$N_3^- + OH^\cdot \longrightarrow N_3^\cdot + OH^- \quad \text{.....(7)}$$

Azide radical has two advantages over the other radial anions. Firstly it is uncharged and, therefore, may be capable of reacting in hydrophobic environments of proteins. Secondly, it has only a very weak absorption in the ultraviolet and visible regions of the spectrum and, therefore, would not interfere with the absorption of the transient species produced following its reaction with enzymes (Hayon and Simic, 1970).

The study of mechanism of enzyme action using primary radicals did not give promising results due to lack of sufficient reaction specificity of the radicals. However such studies using inorganic radicals revealed significant results, and in many cases the amino acids responsible for the catalytic activity of enzymes have been identified (Baverstock *et al.*, 1974).

The technique involves both steady-state radiolysis and pulse radiolysis. In steady-state radiolysis, the aqueous solution of the enzyme is irradiated in conditions that lead to the generation of a specific free radical at certain pH, and the activity of the enzyme is then tested. Since different secondary radicals react with certain amino acid residue/s in proteins at specific pH, it is possible to determine the amino acid/s which is/are the target of the radicals. In pulse radiolysis the reaction of the enzyme with the free radical, which led to its inactivation, is studied. From the absorption spectrum of the transient species produced within 100 μs after the reaction, it is possible to confirm the involvement of the amino acid suspected in the steady-state radiolysis. This is because amino acids in free form or in proteins react with inorganic radicals to produce species with characteristic absorption spectrum in the ultraviolet or/and visible regions of the spectrum.

Many enzymes have been studied using the techniques and the crucial amino acids have been identified (Table 2).

Table 2: The amino acid residues crucial to the activity of some enzymes determined
by free- radical induced inactivation.

Enzyme	Crucial amino acid residues	References
Ribonuclease	Histidine	Aldrich *et al.*, 1969
Chymotrypsin	Histidine	Baverstock *et al.*, 1974
Papain	Tryptophan and Cysteine	Adams and Redpath, 1974
Carboxypeptidase	Tyrosine	Roberts, 1973
Bovine Carbonic anhydrase	Typtophan, Tyrosine and Histidine	Redpath *et al.*, 1975
Superoxide dismutase	Histidine	Roberts *et al.*, 1974
Fructose 1,6-bisphosphate aldolase	Tyrosine and Histidine	Felicioli *et al.*, 1975
Pepsin	Tryptophan	Adams *et al.*, 1979
Guanase	Histidine and Cysteine	Sule, 1997d

3.2.1 GUANASE

Guanase (EC 3.5.4.3), also known as guanine aminohydrolase or guanine deaminase, catalyses the hydrolytic deamination of guanine to xanthine. The reaction is the first step in the pathway for the catabolism of guanine to an end product, which might be allantoin, uric acid or urea depending on the animal species.
The enzyme is a simple protein found mainly in the liver and brain of animals (Lewis and Glantz, 1974). The enzyme from rabbit liver was found to have a molecular weight of 55 KDa and pH optimum of 8.0 (Lewis and Glantz, 1974).

Contribution to Knowledge

The effect of gamma radiation generated by CO^{60} source on the Michaelis constant (Km) of rabbit liver guanase was studied. The Km of the enzyme, using guanine as substrate, was found to increase from 6.1 μM to 8.33μM and 9.30 μM following a dose of 48 and 96 Gray respectively. Steady state gamma radiolysis and pulse radiolysis were also used to study the radiation inactivation of guanase, specifically the reactions of the enzyme

with primary radicals of water radiolysis. The efficiency of the PRWR in the inactivation of guanase was found to increase in the order $O_2^{\cdot-}$ < $OH^{\cdot}$ < $H^{\cdot}$, with efficiency of 1.2%, 2.86% and 18.18% respectively. The reaction of the enzyme with hydrated electron was diffusion controlled, but it was found to be ineffective in causing inactivation of the enzyme (Sule, 1997c).

In addition the efficiency of inorganic radicals in the inactivation of guanase was determined. The efficiency of the radicals was found to increase in the order $(SCN)_2^{\cdot-}$ < $I_2^{\cdot-}$ <$Br_2^{\cdot-}$ < $N_3^{\cdot}$. The rate of reaction of 5 $\mu moldm^{-3}$ guanase with $N_3^{\cdot}$ radicals increased as the pH was increased. The second order rate constant increased from 8.64 x 10^8 $dm^3mol^{-1}s^{-1}$ at pH 5.66 to 7.88 x 10^9 $dm^3mol^{-1}s^{-1}$ at pH 10.9. The enzyme also showed considerable reactivity with $(SCN)_2^{\cdot-}$ at pH 11.2. On the basis of data obtained, it was concluded that histidine and cysteine residues are essential in the activity of guanase (Sule, 1997d).

Chemical modification study using reagents was carried to confirm the involvement of histidine and cysteine residues in the catalytic activity of guanase. The enzyme, in which the cysteine and histidine residues were chemically modified, was completely inactivated, thereby confirming the involvement of the amino acids in catalysis by the enzyme (Sule, 1999)

3.2.2 GLYCERALDEHYDE 3-PHOSPHATE DEHYDROGENASE

Glyceraldehyde 3-phosphate Dehydrogenase (GAPDH) catalyses the oxidative phosphorylaton of D-glyceraldehyde 3-phosphate to form 1,3 – bisphosphoglycerate and NADH. The enzyme is abundant especially in the muscles of various animal species.

The most common isoform in animals, i.e. the NAD^+- dependent phosphorylating enzyme, is made up of 330 amino acids per subunit. The active enzyme was shown to be a tetramer with a molecular weight of 146,000 (Harrington and Karr, 1965). The amino acid responsible for its catalytic activity has been found to be cysteine – 149 (Harris *et al.*, 1963).

Contribution to Knowledge

Pulse radiolysis was used to study the reaction of porcine muscle GAPDH with $N_3^{\cdot}$ radicals at pH 7.4 and 11.7. There was marked increase in the reactivity of the enzyme with the radicals as the pH was increased from 7.4 to 11.7. The k_2 for the reaction between GAPDH and $N_3^{\cdot}$ radicals at pH 7.4 and 11.7 was found to be 5.48×10^8 and 5.88×10^{10} $dm^3mol^{-1}s^{-1}$ receptivity. The transient spectrum obtained following the reaction of GAPDH with $N_3^{\cdot}$ Radicals showed that the radicals attacked accessible tyrosine and histidine residues in GAPDH at 7.4. However, the attack of the radicals was mainly on tyrosine residues at pH 11.7 (Sule, 1998).

The reaction between GAPDH and thiocyanate radical anion was also studied at pH 11.68. The radical anion was also shown to attack tyrosine residues at that pH with k_2 of $4.72 \times 10^{10} dm^3mol^{-1}s^{-1}$ (Sule, 2000b)

3.2.3 FRUCTOSE 1,6 – BISPHOSPHATE ALDOLASE (EC 4.1.2.13)

Aldolase catalyses the reversible cleavage of fructose 1,6 - bisphosphate to dihydroxyacetone phosphate and glyceraldehyde 3-phosphate.

It is an intracellular enzyme usually found in the cytosol of many tissues of animals except in the brain, where it was found to occur in the mitochondrial fraction (Brumgrabber and Abood, 1960). It was first purified from rabbit muscle in 1940, crystallized in 1943 and its molecular weight was reported to be 150 kD (Velick and Ronzoni, 1948). Four amino acids, Lys 107, 227, His and Tyr, were shown to be responsible for the activity of the enzyme, out of a total of 1,444 amino acids residues (Lai and Horecker, 1972).

The enzyme was reported to be inactivated by X-rays, and the inactivation efficiency of PRWR and some inorganic radicals were also determined (Felicioli *et al.*, 1975).

Contribution to Knowledge

Pulse radiolysis was used to study the reaction of rabbit muscle aldolase with $N_3^{\cdot}$ radicals at pH 10.9. The k_2 for the reaction of aldolase with $N_3^{\cdot}$ radicals at that pH was found to be 4.39×10^{10} $dm^3mol^{-1}s^{-1}$. The transient spectrum obtained following the reaction of aldolase with the $N_3^{\cdot}$ radicals showed that the radicals attacked accessible tyrosine residues in aldolase at pH 10.9 (Sule, 1998).

3.2.4 β- LACTOGLOBULIN

β -Lactoglobulin is one of the major whey proteins of milk. It accounts for about 7% of milk proteins and contributes to the cooked flavour of milk through its donation of thiol groups during heat treatment of the milk (Ganguli, 1989). It is rich in tyrosine and trytophan residues.

The pulse radiolysis of β-lactoglobulin was studied in order to investigate the phenomenon of charge transfer which was earlier reported to exist between some amino acid residues in proteins (Butler *et al.*,1982)

The most reactive amino acids in proteins include the aromatic and sulphur-containing amino acids. The reactions of tyrosine and tryptophan with inorganic radicals have been shown to be by electron transfer in which an electron is removed from the phenol and indole groups respectively (Butler *et al.*, 1982).

$$TyrOH + N_3^{\cdot} \longrightarrow TyrO^{\cdot} + N_3^{-} + H^{+} \quad \text{.....(8)}$$

$$TrpH + N_3^{\cdot} \longrightarrow Trp^{\cdot} + N_3^{-} + H^{+} \quad \text{.....(9)}$$

The products of the reaction between free radicals and some amino acids in protein are capable of transferring their electrons to other amino acids within the protein. For instance the oxidation products of trytophan and methionine have been observed to transfer their electrons to tyrosine (Prutz *et al.*, 1982).

This has shown that care has to be taken in using free radical inactivation in the study of mechanism of enzyme action.

Contribution to Knowledge

Pulse radiolysis was used to study the reactions of azide radicals with tyrosine, tryptophan and β-lactogbulin. The transient absorption spectra obtained following the reactions showed that tyrosinyl and trytophyl radicals have wavelength of maximum absorption of 400 nm and 520nm respectively.

The transient spectra also showed that azide radicals attacked accessible tyrosine and tryptophan residues in β-lactoglobulin. When the transient spectra obtained 100 and 200μs after the pulse radiolysis were compared, there was firm evidence to conclude that electron transfer took place from typtophan to tyrosine residues in the protein (Sule and Butler, 1999).

4.0 CLINICAL SIGNIFICANCE OF ENZYMES

Enzymes have the following clinical applications:

4.1 DETERMINATION OF ENZYME ACTIVITIES FOR CLINICAL DIAGNOSIS

The principle of the use of measurement of enzyme levels in the serum as an aid to diagnosis involves the use of non-plasma specific enzymes (NPSEs). NPSEs are enzymes with no known function in the plasma, and their substrates and cofactors are lacking in the plasma. They may only be present in the serum at low levels due to turnover of cells which cause release of their enzyme content. If a tissue is broken down or becomes necrotic, there is an elevation of activity of enzymes peculiar to that tissue in the serum.

For the measurement of an enzyme to be useful in clinical diagnosis the enzyme must be present in a readily available tissue fluid, blood or urine; be easy to assay; be sufficiently stable and there should be significant difference in its activity in the normal and diseased subjects (Price and Stevens, 2003)

Some of the enzymes currently in use in diagnosis of diseases include aminotransferases i.e alanine and aspartate

aminotransferases (liver diseases), alkaline phosphatase (obstructive jaundice), creatine kinase and lactate dehydrogenase (myocardial infarction), acid phosphatase (prostatic carcinoma), lipase and amylase (acute pancreatitis) etc.

4.1.1 SERUM GUANASE ACTIVITY

The determination of serum guanase activity in various pathological conditions showed that it increased only in patients with liver diseases (McLeods, 1967). Other investigators later showed that its elevation in the serum was an indicator of viral hepatitis (Mandel *et al.*, 1970). The determination of serum guanase activity was found to be a reliable indicator of liver cell damage to the extent some workers suggested it to be more sensitive than serum alanine aminotransferase, aspartate aminotrasferase or bilirubin determination (Shiota *et al.*, 1989).

Contribution to Knowledge

Serum guanase activity was determined in healthy adults and in patients suffering from liver diseases in Kano. The enzyme levels in the normal adults and patients suffering from liver diseases were found to be 0.32±0.13 U/L and 1.45±0.41 U/L respectively. The significant difference in the serum guanase levels in the 2 categories of subjects indicates that it could be used in the diagnosis of liver disease in Kano (Sule *et al.*, 2002)

The enzyme was found to be significantly higher in patients suffering from hepatitis B than in other liver diseases (Sule and Dangalan, 2005).

In another study, the enzyme activity was determined in diabetic normotensive and diabetic hypertensive patients attending Murtala Mohammed Specialist Hospital. The study found significant decrease in the serum levels of the enzyme in both conditions compared to normal, and hypertension had no effect on serum guanase activity (Sule and Falgore, 2004)

4.2 CLINICAL CONDITIONS DUE TO ENZYME DEFICIENCIES

Enzymes, being proteins, are synthesized from DNA through mRNA. Once the portion of DNA coding for certain enzyme (known as gene) undergoes mutation, a defective mRNA would be produced. This would be translated to a defective protein – one with replaced, added or removed amino acid. If the replaced or removed amino acid is crucial to the activity of the enzyme or to its structural integrity, the enzyme produced may totally be inactive or may have a fraction of its normal activity. This could lead to clinical condition or in-born error of metabolism.

There are over 400 different genetic defects as a result of enzyme deficiencies. Most are referred to as in-born errors of metabolism (IBEM) when the deficient enzyme participates in a particular metabolic pathway. Some examples are albinism (deficiency of tyrosinase), alkaptonuria (homogentisate 1,2-dioxygenase), galactosemia (galactose 1-phosphate uridyl transferase), Gaucher's disease (glucocerebrosidase) and phenylketonuria (phenylalanine hydroxylase). Phenylketonuria is one of the most common IBEM with a frequency of 1:12000 in western Europe and USA (Price and Stevens, 2003). It leads to hyperphenylalanaemia, mental retardation and early death. It is currently managed only through dietary therapy i.e. by giving diet with proteins with low phenylalanine content up to the age of 9 years.

As a result new born babies are screened for phenylketonuria in many countries in Europe and in USA.

Contribution to Knowledge

Incidence of phenylketonuria was studied in a mentally retarded children home (Terry-Home) in Kano, and three out of the total of 32 patients (constituting 3.4%) were found to be phenylketonurics (Sule and Alhassan, 1998). It was recommended that the screening of new-born babies for phenylketonuria should be carried out here in Nigeria also so that

dietary management could start at the appropriate time in order to prevent clinical symptoms of the disease.

4.3 USE OF ENZYUSE OF ENZYME INHIBITION IN DRUG DEVELOPMENT

Enzyme inhibitors are being used as drugs in certain clinical conditions, where the activities of the enzymes are not desirable. Some examples include the use of allopurinol (an inhibitor of xanthine oxidase) in condition of gout or hyperuricaemia, statins (mevinolin, compactin and monacol K, inhibitors of hydroxymethylglutaryl coΛ reductase) in familial hypercholesterolaemia, sildenafil (inhibitor of phosphodiesterase V) in erectile dysfunction.

Many antibiotics work by inhibition of certain enzymes in the pathogenic organism (in some cases without major consequence in the host or patient). Examples are penicillin inhibits transpeptidases involved in peptidoglycans synthesis in bacteria, chloramphenicol inhibits peptidyl transferase in bacteria and ciprofloxacin inhibits DNA gyrase in bacterial protein synthesis etc.

4.4 USE OF ENZYMES TO DETERMINE THE CONCENTRATIONS OF METABOLITES OF CLINICAL IMPORTANCE

The advantages of high specificity and operations under mild conditions have made the use of enzymes in the determination of concentrations of metabolites of clinical importance increasingly popular in clinical laboratories. Some of the metabolites determined using enzymes are blood glucose (using glucose oxidase and peroxidase), serum urea (using urease), serum uric acid (using uric acid oxidase), serum cholesterol (using cholesterol esterase, cholesterol oxidase and peroxidase) etc.

4.5 ENZYME THERAPY

Some enzymes are administered intravenously for therapeutic purposes. Some examples are urease in cases of kidney failure, bilirubin oxidase in jaundice, fibrinolysin to remove fibrin from blood clots (in thrombosis), beta-glucosidase in Gaucher's disease; lipases, amylase and proteases in pancreatic insufficiency and asparaginase in some acute leukaemias.

5.0 MEDICINAL PLANTS

From 2002, my areas of interest expanded to include medicinal and toxicological effects of medicinal plants. This arose as a result of widespread use of medicinal plants for treatment of various diseases, often without regard to proper dosage, sometimes leading to toxicity.

A medicinal plant is any plant which one or more of its organs contains substances that can be used for therapeutic purpose or which is a precursor for the synthesis of drugs (Lewis, 1981). They are medicinal because they contain bioactive chemicals (or phytochemicals) and some essential mineral elements. These phytochemicals include alkaloids, tannins, flavonoids and phenolic compounds. It was estimated that there are between 200,000 and 700,000 species of tropical plants that have medicinal properties (Atata *et al.*, 2003).

In China, traditional herbal preparations account for between 30 to 50% of the total medicinal consumption (WHO, 2003). According to the same report, the first line of treatment for 60% of children with high fever resulting from malaria in Ghana, Mali, Nigeria and Zambia is the use of herbal medicines at home.

In spite of the expansion of my research interest to include assessing the claim of some medicinal plants and animals (eg. white grubs) used as medicines and determining the safe dose limit, my interests in enzymology and free radical studies remained. This is due to the strong relation between them, as follows:

Firstly, in assessing the medicinal values of the plants, experimental models of diseases have to be produced in animals,

usually rats, mice or guinea pigs, in the laboratory. This usually uses the generation of free radicals. For example, in order to assess the effect of a medicinal plant on liver disease, a model liver disease has to be produced in experimental animals. This usually uses the subcutaneous administration of 100 mg/kg carbon tetrachloride (CCl_4) (Alhassan *et al.*, 2009). The CCl_4 produces trichloromethyl radical by reductive dechlorination. The trichloromethyl radical abstracts a hydrogen atom from polyunsaturated fatty acids to form chloroform and a lipid radical, which reacts with molecular oxygen to initiate lipid peroxidation, which ultimately causes liver cytotoxic response (Brattin *et al.*, 1985).

Secondly, the mechanism by which most plants exert their medicinal effect is through the antioxidant properties of their phytochemicals and mineral elements (such as manganese, copper, zinc and iron).

Thirdly, enzymes are used in biochemical analyses in the cause of the study of the medicinal effect. For example, in order to find out if liver disease is produced in the animals or if a medicinal plant is potent against the disease, serum liver enzymes are assayed; or if diabetes is imposed, blood glucose is analysed using enzymes.

Some of the medicinal plants studied include *Guiera senegalensis (sabara), Psidium guajava (gwaba), Cassia occidentalis (rai dorai), Khaya senegalensis (madaci), Securidata longipedunculata (uwar magunguna), Balanites aegyptiaca (aduwa), Ricinus communis (zurman), Datura stramonium (zakami) and* white grubs *(gwazarma).*

6.0 CONCLUDING REMARKS

The molecules of life are not inert. They undergo various transformations by which some of them are broken down to generate energy for life processes to take place; some of these transformations lead to biosynthesis of macromolecules, which are components of cell structures. Most of these transformations require the use of enzymes. Any living organism is alive when there are functional enzymes in it and is dead when there are no

functional enzymes in it. Hence, Biochemistry may be defined as the study of action of enzymes, their substrates and products.

Ionizing radiations are now increasingly being used for various purposes: medical (in radiotherapy and photographs), food irradiation, energy generation and in making of weapons. Due to these reasons, the study of the effects of their exposure by living organism is an important aspect of biochemistry.

Some medicinal plants have scientifically been proven to be effective in the treatment of certain diseases. However, recommended dosages should not be exceeded as this has been found to cause toxicity in some plants.

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