Professorial Inaugural Lecture

Phosphates and life processes: Two Decades of Eco-toxicology and Bioinorganic Studies

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Eco-bioinorganic chemistry is that branch of chemistry, which deals with the study of metal-containing molecules within biological and eco systems. As a mix of bioinorganic and ecotoxicology, eco-bioinorganic chemistry is important in realizing the implications of xenobiotic materials in biological and life processes. Our research mainly deals with the importance of metal ions in life processes specifically in phosphate esters. Phosphate esters and anhydrides dominate the chemistry of life processes and are of great chemical, technical and biological importance. One of the reasons why Nature chose phosphate ester anions is because they undergo slow hydrolysis in the absence of enzymes, but rapid hydrolysis in the presence of enzymes. Adenosine triphosphate (ATP) is the main source of short term energy in life processes. ATP stores energy in its high energy phosphate-phosphate bonds that are tapped by the body through the process of hydrolysis. All living organisms use Adenosine triphosphate (ATP) for energy conversion. ATP is a nucleoside comprised of a central ribose sugar, a purine adenine base and a chain of three phosphate groups. It is an immediate energy source in the cell and is formed during three stages. The first stage begins by harvesting chemical energy from oxidation of a glucose molecule. This process takes place in the cytoplasm and is known as glycolysis. Since the energy within organic molecules is stored within the individual atoms, it can only be released by breaking the bonds which hold the atoms together. This requires an 'energy spend' of two ATP molecules to assist the breakdown of glucose into intermediate substrates called glyceraldehyde-3-phosphates. Further breakdown enables the coenzyme NAD+ to pick up high-energy electrons and hydrogen ions, forming two NADH molecules.

It also releases energy allowing phosphate group to bond with ADP, forming two molecules of ATP in a process called substrate level phosphorylation. Further breakdown to pyruvate generates an additional two molecules of ATP, giving glycolysis an overall energy 'profit' of two ATP. The next stage of cellular respiration also yields ATP by substrate level phosphorylation. This stage, known as the Citric Acid Cycle, completes the oxidation of glucose and takes place in the mitochondria of the cell. Pyruvate diffuses through the cell membrane and undergoes several chemical reactions to form Acetyl Co-A, producing carbon dioxide as a waste product. NADH and FADH2 also carry electrons during this stage as well. Another two molecules of ATP are produced which can be immediately used by the cell for energy.

The majority of ATP produced by our body is formed by the third and final stage of cellular respiration in a process called oxidative phosphorylation. This is known as the electron transfer stage in which NADH and FADH2 give up the electrons they gained from glycolysis and the Citric Acid Cycle, releasing energy. ATP is then generated by an enzyme called ATP synthase which uses a hydrogen ion gradient to capture the energy released from the high-energy electrons. In this way, oxidative phosphorylation yields 34 molecules of ATP for every molecule of glucose. Thus, all the chemical energy harvested from the original glucose molecule is now as ATP in the form of potential energy, ready to be used for cellular work.

Though the above reactions were known for quite some time, the involvement of divalent metal ions in the enzymes was not understood. I first embarked on the study of ATP hydrolysis and the metal ion involvement in the reactions some 25 years ago. At the time there were only six laboratories that were actively involved in similar undertakings. Three in the USA, two in Europe, and one in Australia. The intellectual environment in Prof. Milburn's laboratory, Boston University was highly conducive to my decision. The stimulating environment with the necessary critical mass of young and talented investigators with the opportunity for the free exchange of ideas with the other five laboratories is an important ingredient in the making of the scientific progress. While pursuing my PhD studies I was able to co author three of our findings in peer reviewed international journals [1-3]. After my return to Addis Ababa University, I continued with similar investigations involving pyrophosphates and triphosphates with my students. As is evident from the previous paragraph, the storage of energy is in the phosphoanhydride bonds and hence the findings arrived at from simple systems such as pyrophosphates and triphosphates can be extrapolated to those of the organic phosphates like ATP. Several important findings were published in scientific journals that emanated from the studies [4 -8]. As a result of these developments we gradually began to think of applying the knowledge gained in the laboratory to real situations in the biosphere.

Organophosphates can be classified into two groups: aryl phosphates which must be activated by liver enzymes before becoming toxic and alkyl phosphates which do not require activation for toxicity. Most organophosphate formulations are only slightly soluble in water and have high oil-to-water partition coefficients and low vapour pressures. Less harmful organophosphates are most commonly used as insecticides. They are mostly nerve poisons and may cause cumulative damage to the nervous system and liver and as well being carcinogenic. Some common organophosphate insecticides include malathion, parathion, diazinon, chlorothion and dichlorovos. More harmful chemicals in the same group include chemical warfare agents such as sarin soman tabun and VX. Nerve agents are compounds That affect the transmission of neural signals.Because the neurotransmitters that control muscle contraction are inhibited, all of the muscles in the body can’t stop moving and death results from the body breaking itself up.

Pesticides usually expire about two years after being manufactured. The expired pesticides become more harmful as they assume the role of nerve agents. Hence there is considerable concern about reducing pollution caused by organophosphate esters, which are resistant to biological degradation and can accumulate in the biosphere and in organisms. The United Nations estimates that more than 3000 tonnes of obsolete pesticides are accumulated at about 1000 sites across the African continent, threatening the health of thousands of people. Pollution is an unwelcome concentration of substances that overhelms the environments capacity for normal processing. Most approaches for handling pollution could formerly be summed up by the phrase, “dilution is the solution to pollution”. However, pollution levels have increased so much in amount and toxicity that this approach is no longer acceptable. The prevalence of these toxic organophosphates in our environment has been attributed to human activities even as they pose serious threats as environmental pollutants. These activities include (i) improper management of stockpiles of expired pesticides, (ii) improper domestic and agricultural applications of pesticides and (iii) their use as chemical warfare nerve agents in terrorist attacks or in combat situation in a war. Hence the development, production, stockpiling and use of nerve and chemical warfare agents are prohibited by the Organization for the prohibition of chemical weapons (OPCW) and other environmental agencies. Several techniques have been developed and reported to address the pollution threats caused by the presence of these toxic organophosphate esters in the environment. Such techniques like (i) gamma irradiation (ii) wet air oxidation (iii) nuclear incineration (iv) molten salt oxidation (v) plasma arc processes and (vi) hypochlorite formulations have been employed over the years. However, these known methods use compositions and formulations which have certain undesirable properties like corrosiveness, flammability and even toxicity. Hypochlorite formulations are very corrosive and toxic. Additionally, the application of hypochlorite decontaminants often requires substantial scrubbing for the removal and destruction of the organophosphate contaminant, a procedure which limits its use. In addition to the above, the technologies suffer from expensive infrastructure and high cost. No acceptable cost efficient decontamination protocol is developed. Hence formulation of decontamination solutions which are environmentally friendly fast, efficient, cheap and effective under ambient conditions is of a paramount importance. My several short term trainings in the OPCW facilities in Europe helped me understand the magnitude of the problem and upon my return from my sojourn in Europe my research direction was expanded to include decontamination effects as well. We did several investigations and reported our results to the relevant scientific journals [9-16]. Our current focus is on understanding in detail the processes of phosphorylation, dephosphorylation , pyrophosphorylation and depyrophsphorylation reactions and the requirement of metal ions in those enzyme systems. These reactions have huge implications in health issues of humans.

Phosphorus (P) loss from agricultural soils has been shown to be a major contributor to anthropogenic eutrophication of surface waters . Among the strategies which have been investigated to counter this problem are source measures which decrease the risk of P being mobilised and transported from the source. These source measures have included various soil amendments to increase the soil's capacity to retain P, mostly due to their Fe, Al or Ca content. More precise knowledge on the available phosphorous is mandatory to counteract the ill effects of excess phosphorous in the aquatic environment. Ideally a hand held device that can easily estimate the amount of phosphates in the effluents is required for quick monitoring of the phosphates. Measurement of phosphate has been of tremendous interest in recent decades. Phosphate is an essential nutrient for plants, and its measurement has been used to control fertilizers applied to maximize crop yield and quality in hydroponics and agriculture, or to control undesired growth of the algae and other aquatic vegetation to prevent the eutrophication of natural water bodies. Thus, due to the importance of phosphate and the protection of the global environment, there is a need in the development of simple and compact phosphate sensor. Such sensor is currently unavailable in the market and our research is bearing fruit along this venture. We have fabricated a new phosphate sensitive electrode and the results have been communicated to reputable journals recently [17,18].

The other aspect of our study centers on the investigation of the fate of phosphates that prevail in foods and drinks. The overloading of our body with excess phosphates has the ability to deplete important metal ions such as calcium, iron, zinc etc from our body as phosphates form strong compounds with those ions. Hence the increasing trend of osteoporosis among our old and young people is presumably related to the amount of phosphates that are consumed by individuals. Once we perfect our phosphate electrode we will embark on this aspect of our project.

In this lecture I have attempted to summarize some of my own research on the mechanism of the involvement of metal ions in organophosphate hydrolysis and condensation reactions together with some results by others which have led to our present understanding of the role of metals in enzyme systems. Looking back, I realize that I have been favored extraordinarily by external circumstances, the proper place at the proper time in terms of my PhD education under professor Milburn in the USA. My employment and short term learning opportunities afforded to me by Addis Ababa University(AAU), University of Botswana (UB), National university of Lesotho (NUL),Walter Sisulu University (WSU), and the university of South Africa (UNISA) are gratefully acknowledged. Having had incredibly brilliant co-workers and students and the cross pollination of ideas from different corners of the world has helped me shape the research ideas I currently pursue. I am indebted to the various agencies and foundations (OPCW, Matsumae International Foundation (MIF), UNESCO, and NRF) for financial assistance. In conclusion I would like to dedicate this lecture to the unsung heroes responsible for my academic career :

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Alemayehu Fikru Tafesse

And all of my students

Thank you!

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