Application of a Biomaterial (Urease) in Medicinal Field and other Industries to Remove Urea from Synthetic and Real Sample

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Urea was prepared from urine in the year of 1817 by William Prout and it is the major nitrogenous waste product generated due to protein breakdown in human liver.

Protein ⟶ Amino Acid ⟶ Ammonia

As the so-produced ammonia is toxic to our body, it is converted into harmless waste product (urea) through urea cycle.

Ammonia ⟶ Urea

This Urea is eliminated from the body through urine by the action of kidneys after glomerular filtration. And the measurement of its concentration in urine and blood is clinically important in the assessment of kidney functioning. Increased level of serum/plasma urea concentration depends on two major organsincreased production of urea by the liver and decreased elimination of urea by the kidney. The increased production of urea is also related to a high amount of protein intake, tissue breakdown during starvation to provide an energy source, ageing, etc. Excessive drinking of alcohol, having a trace level of urea, causes elevation of blood urea level.

A normal level of blood urea is 2.5-7.8 mmol/L. But when this level goes beyond this limit, many problems arise in the human body.

Keeping this in mind, researchers of an analytical section of Visva Bharati have developed a new material immobilised urease (urease immobilisation on silica gel) by which blood urea level has been detected successfully in vitro. Pure urease, due to its high cost, lack of stability,

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* Ms. Sneha Mondal, Ph.D. Scholar from Department of Chemistry, Siksha Bhavana, West Bengal, is pursuing her research on "Enzyme Immobilization." Her popular science story entitled "Application of a Biomaterial (Urease) in Medicinal Field and other industries to Remove Urea from Synthetic and Real Sample" has been selected for AWSAR Award.

limited availability, difficulties in recovery from a reaction mixture was not used. Urease enzyme is an enzyme which is derived from a leguminous plant source named Jack bean. JB Sumner first identified urease in a crystallisable form. This research group has immobilised or attached enzyme urease on an inorganic material, silica gel through covalent attachment. This newly synthesised material was applied in both human urine and blood samples. Immobilised urease hydrolyses urea (a specific substrate for urease) into ammonia and carbon-di-oxide like pure urease.

At first this research group proved the conversion of urea into ammonia by newly synthesised material in synthetic solution, i.e., they loaded measured amount of material into a glass column, then urea solution was continuously rambled through the column and the column eluate was estimated spectrophotometrically at 380 nm wavelength (absorbance value was near about 0.2 units). They also measured the spectra of pure ammonium chloride solution which also gives a peak at the same wavelength (absorbance value 0.2 units). Thus, conversion of urea into ammonia was successfully carried out and the activity of the synthesised material was surprisingly found to be 3-fold higher to native enzyme activity.

Then, they applied the above-mentioned protocol in some real samples like blood and urine. In this case, the same amount of materialas in case of column study was given to blood (serum/plasma) samples, containing the same amount of urea as was given in case of a synthetic sample and the absorbance value was found to be same. They also followed the same procedure in a human urine sample and obtained the same results. From these experiments, they found that the efficiency of newly synthesised material was 3-fold higher in comparison to pure enzyme activity.

This research group is still working to explore new technique as dialysis in the human body system utilizing this newly synthesised material. The main function of the kidney is to remove excess fluid including urea from our body. But, when the kidney fails, dialysis is the alternative treatment at the end-stage of kidney functioning. But this method is expensive and time-consuming. For this reason, they had decided to use immobilised urease in combination with zirconium phosphate which can adsorb ammonium ion. As zirconium phosphate has not enough potentiality for the complete removal of blood urea, it can't replace dialysis in patients with no renal functioning. Besides these, the use of zirconium phosphate has the following advantages: reduces the time period of dialysis, and replaces the onset of dialysis therapy for the patients having some renal functioning.

There are also various applications in which our synthesised material can work.

(1) A single domain antibody L-DOS47 was found in Jack Bean urease. When the cancer cells undergo catabolism, urea that is present within these cells also catabolises resulting in the production of ammonia and hydroxide ions. This ammonia production is responsible for the increase in pH of the medium surrounding the cancer cells. These metabolites provide a stress for the cancer cells (lung and breast cells). This stress is mainly due to the toxic effect of ammonia, increased lactic acid production and lack of removal of metabolic acids resulting in an acidic environment surrounding the cancer cells.

(2) This enzyme could be used as an antigen as it is able to stimulate a strong immunoglobulin response. H. pylori.causes ulceration diseases, possibly gastric cancer, which is inhibited by the catalytic activity of this plant enzyme, Jack Bean urease.

(3) The occurrence of Ethyl carbamate of wine imposes a harmful effect on human beings, which is derived from ethanol along with urea. This causes a carcinogenic effect on humans, mainly during the storage timings. In this case, acid urease is effectively working. So, as a precautionary measure, wine is first treated with a preparation of acid urease. This acid urease removes urea from wine, which is the potential source of ethyl carbamate.

(4) Biosensors have wide applications such as biomarkers which are used for medical diagnostics. Various pathogens and toxins are also detected by biomarkers. They have several properties such as it is highly specific and sensitive, user-friendly, low cost and compact in size. With the use of urease enzyme-based biosensor blood urea is measured.

(5) Nowadays, the use of enzymatic procedure gets high attention for the removal of phenolic pollutants from aqueous solution. Industrial fertiliser, rich in urea, is treated with immobilised urease. In this case, urease is immobilised onto polyester through chemical coupling method.

(6) In plants, urease is involved in urea metabolism. Urease within plants uses externally and internally generated urea as a nitrogen source. Urea derived nitrogen is easily available to plants only when it is hydrolysed by plants. In this way, urease plays an important role in the germination and seedlings nitrogen metabolism.

So, this research group is working hard to apply their synthesised material as an alternative to dialysis, lung and breast cancer treatment and also in the case of wine industries.

The research team includes Dr B Mandal, DrSnehaMondal, Dr. Susanta Malik, and DrMousumiChatterjee. The work is still in progress.