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The Potential of Urease Enzyme from Staphylococcus Aureus to Stabilize Blood Concentration Levels

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Abstract

Our current research involves investigating the potential of the urease enzyme produced by staphylococcus aureus to preserve the stability of blood concentration levels. It enables it to fulfill its function in maintaining the equilibrium of the blood concentration level, which involves preventing blood clotting within the body and completing the blood clotting process in the event of a wound; this is accomplished by producing an enzyme with a neutral acid function (PH) and a concentration that maintains the concentration and flow of blood in the circulatory system's bloodstream. The initial step in the production of the enzyme urease involves the isolation and diagnosis of bacteria under standard conditions, which is achieved through the culture of bacteria. The second step consists of extracting and purifying staphylococcus aureus through a secondary culture process. Centrifugation is employed for sedimentation and extraction, while ion exchange is used for chromatography.

Introduction.

Enzymes are essential cofactors that greatly enhance the speeds of chemical processes in living cells. Their protein structure has a substantial molecular weight, and similar to other proteins, they are composed of the fusing of several amino acids that create one or more polypeptide chains. Urea (EC 3.5.1.5) is a metalloenzyme with a three-dimensional (3D) structure. It contains nickel metal and functions as a catalyst for the hydrolysis of urea, producing ammonia (NH4) and carbamate (Carbamate). The latter compound undergoes spontaneous decomposition, forming carbonic acid and ammonia [1, 2]. Urea holds significant importance in the field of enzymology as it was the first enzyme to be purified and crystallized by scientist James Simner in 1926. This groundbreaking achievement laid the foundation for the understanding that urease and other enzymes are proteins. Urease, an enzyme, is found in most bacterial, algal, fungal, and plant species. Urea is a high-efficiency catalyst that facilitates urea decomposition at a reaction rate ranging from 10 to 14 times. The creation of urease enzyme has been observed in various organisms, including bacteria, fungi, yeasts, and algae. However, it is not synthesized within the human body. Several plants have urease [3]. The urease enzyme, principally generated by Staphylococcus aureus, is a prominent virulence factor among various bacterial taxa [4].

All known ureases possess the same structure in their active site [5, 6], where two Ni2+ ions are coordinated by lysine carbamyl, four histidines, and one aspartate [7, 8]. Assessing the enzymatic activity in the blood serum of cells is a crucial technique for medical professionals. When cells undergo destruction in diseases such as angina pectoris, hepatitis, cirrhosis of the liver, and pancreatitis, the enzymes are discharged into the bloodstream. The enzyme urease can alter the pH level, enabling bacteria to adapt to highly acidic environments (pH 9). This ability to regulate pH is one of urease's most crucial physiological functions. It can be utilized to maintain the equilibrium of blood, ensuring compatibility by reducing the concentration to prevent blood clotting while increasing the concentration to facilitate the clotting process in wounds. This function is achieved by monitoring and assessing the body's physiological state through periodic laboratory examinations. The enzyme urease plays a crucial and indispensable function in bolstering the body's immune system by facilitating biochemical interactions and chemical attraction, also known as chemotaxis. The robust immune system, along with the action of the urease enzyme acting as a vaccine, enables it to effectively suppress the essential functions of invading bacteria that cause specific diseases in the body. Hence, it functions as an enzyme with its distinct mechanism in crucial reactions and effectively controls its progression at consistent rates to uphold homeostasis in the body.

Materials and Methods.

Materials:

Hood, incubator, oven, burner, petri dish, alcohol, distilled water, loop, PH-meter, Urea Agar Based media, tubes, beaker, Tryptic Soy Broth, centrifuge, ammonium sulfate crystals, phosphate buffer solution, Phosphate buffer, EDTA, B-PEM Mercaptoethanol, ion exchangers, sodium chloride solution, sodium hydroxide, hydrochloric acid HCL, phosphate buffer (PEM), (DEAE-sepharose)**.**

Methods:

As part of the practical side of this research, a number of important and necessary steps must be taken to get the urease enzyme from *staphylococcus aureus* in the right way so that it can do its job of keeping the blood's general balance in a way that stops blood clotting by lowering it and doesn't affect the process of clotting in wounds. These standards are met by producing an enzyme whose acid function (PH) is neutral, and its concentration keeps the blood's concentration and flow in the circulatory system.

2.1. Bacterial Culturing:

A group of (20 isolates) from samples of the *staphylococcus aureus bacteria* were examined by culturing them on a Urea agar-based media (Christensen) using the planning method to see how well they would work for producing enzymes. The samples were then incubated at 37 C for 18 to 24 hours. The samples that turned pink faster are the ones that made more urease enzyme than the ones that changed color later [11].

Twenty isolates from samples of the staphylococcus aureus bacteria were prepared previously and examined by culturing them on Urea Agar-Based media (Christensen) using the planning method to see how well they would work for producing enzymes. The samples were then incubated at 37 C for 18 to 24 hours. The samples that turned pink faster produced more urease enzyme than the ones that changed color later.

2.2. Extraction and purification:

The urease enzyme is extracted from the medium (Tryptic Soy Broth) through a secondary culture of Staphylococcus aureus. The pure enzyme is then incubated in a vibrating incubator at 37 °C for 48 hours. The sediment is then separated from the sediment in a centrifuge at 10,000 revolutions per minute for 10 minutes in preparation for the sedimentation process [12]. Purification consists of two fundamental steps interspersed with a series of chemical reactions. The ultimate product is an enzyme (urease) that possesses the necessary characteristics to perform the essential function of regulating blood concentration within the body; these two steps are:

2.2.1. Sedimentation process:

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The extract (clear) obtained after centrifugation in the previous phase is subjected to precipitation by adding varying quantities of ammonium sulfate crystals. This addition occurs within specific weights with varying degrees of saturation, including (25% saturation, 50% saturation, 75% saturation, and 100% saturation). Subsequently, a centrifugation process is conducted at a rate of 10,000 cycles per minute for 15 minutes for each of these saturated concentrations that result from adding ammonium sulfate crystals to the extracted sediment. The precipitate from this centrifugation process is dissolved in a phosphate buffer solution that contains (Phosphate buffer + EDTA + B-PEM Mercaptoethanol) (20 ml. mol) to maintain a pH of approximately 7.6. The solution that is produced is capable of withstanding changes in acid function. Subsequently, a dialysis process is implemented to eliminate ammonium sulfate residues and eliminate excess fluids and wastes associated with or related to the final solution. This process yields a high-purity urease enzyme. Protein concentration and enzymatic activity were estimated in the center sediment.

2.2.2. Ion exchange chromatography:

Suspend 20 g of ion exchangers, which are insoluble granules that contain exchangeable acidic or basic radicals in their molecular structure. The exchanger is suspended in 500 ml of 0.25 M sodium chloride solution and 0.25 M sodium hydroxide after the fixed positive or negative ions in the ion radicals (placed in contact with a liquid solution) are replaced with ions with the same indication. The exchanger stagnates in distilled water, and the upper liquid is poured and washed several times until it becomes clear and filtered. The ion exchanger was subsequently separated by filtration and washing multiple times. Subsequently, it was washed with a solution of (0,25) hydrochloric acid HCL, distilled water, and equilibrated with a phosphate buffer (PEM) of 20 ml and a pH of 7.5.

The ion exchanger was then filled with (DEAE-sepharose) to a limit of 1.5 x 5 cm. After achieving equilibrium, the sample was added to the column, and the absorption was measured using a spectrophotometer. The enzyme activity was then measured using method [12] in the final step. Upon comparing these saturation concentrations, it was determined that the concentration (50% saturation) is the optimal concentration for producing a pure urease enzyme that possesses the necessary characteristics to perform the specific physiological function of the product.

Results and Discussion.

Enzymes, among the most significant biomolecules, have various applications in various fields, such as biomedicine and industrial applications. It is of the utmost importance because of its numerous advantageous characteristics. Enzymes can endure significant evolution and be synthesized from various sources, such as microbial and plant origins. Microorganisms are subject to genetic refinement and are produced with great care, resulting in economic benefits. Microbial enzymes involve a broader range of processes than enzymes used in creating plants and animals. Chemicals, biofuels, animal feed, personal care, pulp and paper, diagnostics, and treatment are among the numerous industrial applications in which enzymes are utilized [14,13].

The implementation of novel molecular methodologies, including genomics, has been made feasible by discovering new enzymes from various microorganisms. These enzymes are crucial in the refining of numerous food and beverage products, as well as the constituents that are used in them. The enzyme urease is considered a metalloenzyme because it contains a nickel ion (Nickel) in each active site (two active sites) [15], and no other type of metals such as (Zn, Cd, Fe) can replace it., Cu, Mn, etc.) to be effective and increase enzyme activity. It can be made by various bacteria, fungi, many types of yeast, and some plants [16, 17]. The urease enzyme has been well studied clinically due to its association with pathogenic bacteria [18-21]. Several studies indicated that the production of this enzyme is related to the mechanism of nitrogen regulation in addition to the presence of ammonia and the amount of nitrogen in the environment surrounding the bacteria if it is limited [22].

It is known that the purification process of the urease enzyme means separating it from the rest of the cellular components present with it to study the physical, chemical, and catalytic specifications

of this enzyme, which requires obtaining the enzyme in its pure form. The purification includes steps through which the specific activity of the enzyme increases, and the process of purifying the enzyme begins with the concentration of the enzymatic extracts from it as a first step of the purification steps to get rid of the most significant possible amount of water associated with it. Lyophilization, a process of dehydration usually used to preserve materials to avoid spoilage, occurs during the freezing of materials and the addition of enough heat to allow the frozen materials to convert from a solid to a gaseous state directly without going through the liquid state. Also, filtration makes it porous with organic solvents [12], and column chromatography is used to achieve the purification process. After these extraction steps, the enzyme will be ready to measure its efficiency. The specific activity becomes 93.13 units/mg 4.1 purification times, with an enzymatic yield of 50%. Then, the protein solution resulting from the filtration process was put into a sedimentation column (DEAE-sepharose) equilibrated by 0.25 NaCl and a pH of 7.5.

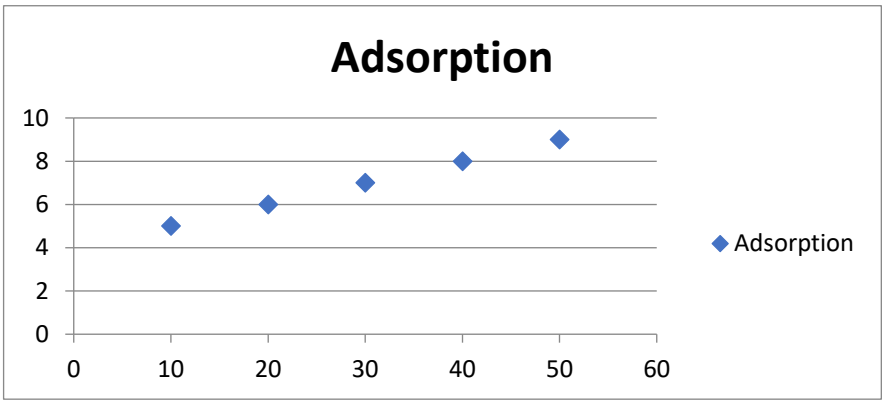

Figure (1): ***(Filtration process in a sedimentation column equilibrated by 0.25 NaCl under pH conditions of 7, 5)***

Several factors influence the activity of the urease enzyme, leading to a change in its reactive pathway in the blood, including:

- Enzyme concentration: Increasing the concentration of the enzyme leads to an increase in the speed of the reaction, so the optimum concentration of the enzyme used to maintain the balance of the blood concentration in the circulatory system is approximately 50 micrograms, which is a constant concentration of the enzyme in most experiments [23].

- pH of buffer solution: The optimal and appropriate pH for urease is 8.3, and the pH amount of 8 may be very suitable to give strong work results and effectiveness in performing its required function (23).

- Reaction time: It turns out that the time required to complete the enzymatic reaction is 30 minutes, this result is identical to [23], and at the same time, this result is close to what the researchers [24] reached, which is 25 minutes.

- Temperature: The optimum temperature for the enzyme to work is 50 degrees Celsius, which is similar to what the researcher [25] mentioned, as they mentioned that the optimum temperature for the work of the urease enzyme is 55 degrees Celsius, while stated that the appropriate temperature for the work of the urease enzyme is 45 degrees Celsius [26].

- Substrate concentration: The optimum concentration for the action of the urease enzyme is (5 milli molar), its maximum speed is 0.8 units per milliliter, and the value of the Michele's Mentin constant Kmis (6.06 milli molar), which is identical to [23]. While [27] found that the value of Km for the appropriate urease enzyme is 5.5 milli molar, and others like [28] found that 6.2 milli molar is the value of Km suitable for the enzyme's action.

- Storage conditions: The suitable storage conditions of the urease enzyme are for a period of 30 days at a temperature of 4 °C. It can be stored at room temperature but loses about 59% of its effectiveness.

- Inhibitor effect: Some inhibitors represented by compounds such as copper-chloride and methyl-urea affect the activity of urease enzyme, and it was found that this inhibition is a type of competitive inhibition, and this is consistent with previous studies [29].

The urease enzyme's function in the circulation is to preserve the platelets' normal cohesion force. The cohesion force between the platelets will decrease as the concentration of blood increases. In contrast, the urease enzyme will enhance the cohesion force of platelets in the bloodstream if the blood concentration is diminished for any reason. The urease enzyme facilitates the blood clotting process near the wound, as hemorrhaging will ensue when wounds occur. This has allowed numerous researchers and investigators to determine when the enzyme is active and performing its function accurately in the bloodstream and when it has expired from working and performing its function accurately. Additionally, we can quantify urease's quantitative and qualitative activity and analyze its kinetic behavior to determine its advantages and disadvantages, as has been done in previous studies [30].

Conclusion:

The enzyme urease can be biosynthesized within standard specifications under constant standard conditions, as demonstrated by the research presented. Conversely, the enzyme urease in the blood prevents the formation of blood clotting by maintaining the blood concentration in the body and preventing its viscosity from increasing. Conversely, it serves as the primary factor in blood coagulation during a wound, thereby preventing the continuation of hemorrhage. However, the fundamental prerequisite for its functionality and serviceability is the preservation of stable storage conditions and the periodic assessment of its suitability following the installation process.

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