Research Article

Assessment of urease activity in *Pisum sativum* seeds

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ABSTRACT

Introduction: Urease is an enzyme which catalyzes urea into ammonia and carbon dioxide. Urease is abundantly found in seeds of *Leguminosae* family. It is an important enzyme for plants as it converts atmospheric nitrogen into absorbable form of nitrogen. It is even found in some bacteria and fungi and sometimes acts as defense mechanism. This research aims at extracting urease from *Pisum sativum* seeds. **Materials and Methods:** The dry seed sample was bought from a local departmental store. The extraction of the enzyme was done in three steps. They are precipitation, dialysis, and electrophoresis. The activity of the protein extracted was checked under changing pH and temperature. The molecular weight of the enzyme was found using sodium dodecyl sulfate-polyacrylamide gel electrophoresis technique. **Results:** The optimum pH was found to be 7.5, and the optimum temperature was found to be 40°C. The molecular weight of the enzyme was found to be 90kD. **Conclusion:** Urease was extracted from *P. sativum*. The molecular weight was estimated. The enzyme activity was checked in changing pH and temperature. Urease can be used to regulate the level of urea in diabetic patients.

KEY WORDS: Urease, Pisum sativum, Optimum pH, Optimum temperature

INTRODUCTION

Nitrogen is the most important plant nutrient for plant growth and development.[2] It has been proposed that plant urease functions in the assimilation of urea normally formed in plants as a result of the hydrolysis of arginine to ornithine catalyzed by the enzyme arginase.[6] In plant cells, urea is an important source of nitrogen that must be converted to ammonia for nitrogen assimilation.[1-3] In nature, two major biochemical processes lead to urea production: (1) Arginase-catalyzed production of urea from arginine and (2) purine degradation to glyoxylate and urea. In addition to the nitrogen assimilatory function of urease, plant ureases appear to have defensive roles against herbivore and fungal attack.[4-6] Urea can be hydrolyzed by two different enzymes: Urease and an ATP (and biotin)-dependent urea carboxylase/allophanate hydrolase. The latter, found in some fungi, algae, and at least one bacterium,[7,8] has never been reported in plants. Rather, all plants appear to have a urease.[17]

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Website: jprsolutions.info | ISSN: 0975-7619

A number of eukaryotic and prokaryotic organisms are reported to produce proteolytic enzymes.[19] The plant and fungal ureases are homo-oligomeric with identical proteins repetition. On the other hand, the urease that is found in bacteria is composed of repetitions of two or three subunits of different sizes.[9] The structure of the protein crystal plays an important role in the activity of the enzyme. Even environmental factors such as temperature, pH, and substrate concentration can affect the enzyme activity.[10,11]

Urease is also important in human bodies because many urinary tract and gastroduodenal diseases, including cancer, are related in some ways to this enzyme. Some of urease applications include treatment of industrial waste, the industry of alcoholic beverages, use in hemodialysis, and its potential use in space missions as life supporter.[12-15]

The aim of this experiment was to extract urease from *Pisum sativum* seeds and to check its activity in changing pH and changing temperature. This was done to provide an insight on the various aspects of the enzyme.

MATERIALS AND METHODS

Sample Preparation

Dry seeds of *P. sativum* were purchased from the local department store. They were grinded into a fine

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Received on: 25-02-2018; Revised on: 27-04-2018; Accepted on: 30-05-2018

powder by a mechanical grinder. 10 g of this powder was then mixed with equal amount of water to convert it into a fine paste.

Ammonium Sulfate Precipitation

The extracted protein was precipitated using ammonium sulfate. This was done to further purify the protein to obtain the pure enzyme. Ammonium sulfate is the most commonly used salt for salting out proteins because of its large solubility in water and its relative freedom from temperature effects, and it has no harmful effects on most of the proteins. The salt was added to a solution of macromolecules to a concentration just below the precipitation point of the protein. After centrifugation, the unwanted precipitated proteins were discarded, and more salt was added to the supernatant to a concentration sufficient to salt out the desired protein. After a second centrifugation, the protein was recovered as a precipitate.

Dialysis

Dialysis is a useful step in adjusting a protein sample from one buffer to another and in correcting the metal and salt ion concentration. A dialysis tube was used in this method. A tubing with a cutoff of 30kD was used.

Assay of Protein

The urease assay was performed as described by Sharma *et al*. method. The sample was added to 10 ml of urea solution. 2ml of the previous solution was added to each test tube containing 5 ml of Nessler's reagent and incubated at 40°C for 5 min. They were followed by the addition of 1.0 M HCl to terminate the reaction after a specific time. Absorbance measurements were taken for the resulting solutions at 405 nm. The estimation of urease was carried out using the standard curve of ammonium sulfate.[3]

Estimation of Protein

Estimation of protein was done according to Lowry *et al*. using bovine serum albumin (BSA) as standard material. The results of the BSA were used to obtain a standard curve. This standard curve was used to determine the protein concentration in the assay and estimated for the original sample.

Effect of Temperature on Enzyme Activity

5 ml of buffer substrate was taken in a series of test tubes, and 0.2 ml NaCl is added to it. The reaction was started by adding 0.3 ml of the sample and incubated at different temperatures. The reaction was started by adding 1 ml of DNS reagent. The contents were mixed well, and the tubes were heated in a boiling water bath for 10 min. The test tubes were cooled and 10 ml of distilled water was added. The color developed was read at 520 nm calorimetrically.

Effect of pH on Enzyme Activity

Four test tubes were prepared. 1.0 ml of tris-HCl buffer and glycine NaOH buffers of pH 5.0, 7.0, 8.5, and 10.0 were pipetted out into respective tubes. The sample was added to one tube at a time and was viewed under a spectrometer. The readings were recorded every 15 s. The rate of the reaction was calculated.

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

The molecular weight of the enzyme was determined using SDS-PAGE. This technique was also used to check enzyme purification. Electrophoresis is the process of migration of charged molecules present in the solution in response to an electric field. They have been developed to separate macromolecules on the basis of their molecular weight. The glass plate was sealed with the spacer, and the separating gel was prepared, which was poured to the three-fourth of the glass plate. The stacking gel was then prepared and poured up to the rim of the notched plate. The combs were placed immediately. After polymerization, the combs and bottom space were removed. The glass plate was fixed in the electrophoresis apparatus. The sample was prepared by adding it in 10µl of sample solubilizing buffer. The sample was loaded and electrophoresis was done till the dye reached the bottom. The gel was removed from the glass plate. The gel was stained by placing it in the staining solution for 30 min. The gel was destained by placing it in a destaining solution overnight. The protein bands were observed on the gel. The molecular weight of each sample was compared with protein molecular weight marker.

RESULT

Optimal pH for the enzyme urease is 7.5, and the optimal temperature was found to be 40°C. The molecular weight of the enzyme was found to be 90 kD [Figures 1-3].

DISCUSSION

The enzyme was extracted, purified, and its activity was checked at changing pH and temperature. The molecular weight of the enzyme was found to be 90 kD.

The optimum temperature for the enzyme activity was found to be 40°C. A similar test done by Srivastava *et al.* showed the optimum temperature to be 47°C.[13] Increase in temperature increases the kinetic energy of the molecules. When the temperature was further increased, the hydrogen bonds that held the enzyme together started breaking.[12]

The optimum pH for enzyme activity was found out to be 7.5. This was comparable to results in experiments done by *Aspergillus niger* and *Coccidioides immitis*.[14,15]

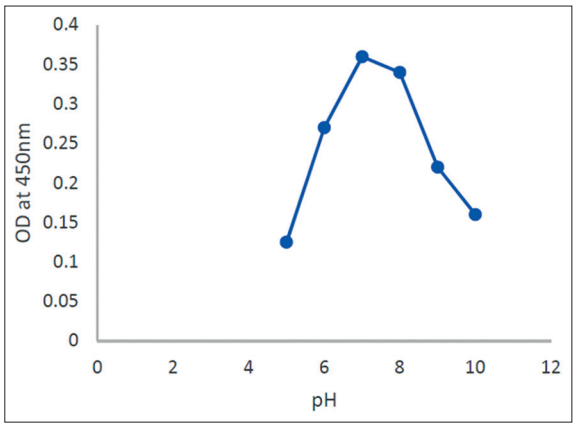

Figure 1: Effect of pH on urease activity

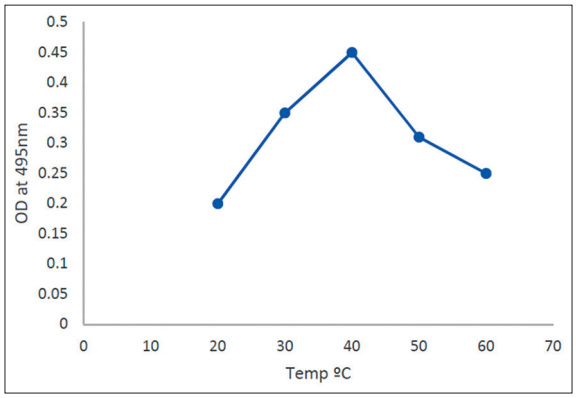

Figure 2: Effect of temperature on urease activity

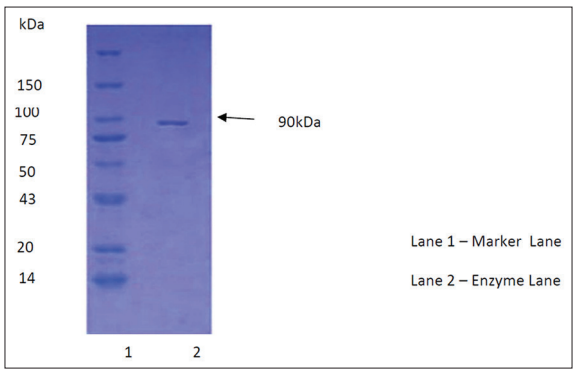

Figure 3: Sodium dodecyl sulfate gel electrophoresis

Many other studies reported basic pH as an optimum value for the extracted urease. This could also be seen by the other experiments. Acidic pH has an inhibitory effect on the enzyme, thus reducing its activity. Furthermore, the existence of the active sites in amino acids will be influenced by the change in pH which may alter the ionization of these amino acids.[18]

There were three factors which affected the rate of dialysis. The first was the difference in the concentration of the molecules between the internal and the external solution, which is the driving force for the movement of the molecules. The second was mixing on opposite sides of the dialysis membrane which will increase the rate of movement of particles. The third was dialyzable particle size versus pore size of the membrane, and substances that are only very much smaller than the pore size will reach equilibrium faster than the substances that are only slightly smaller than the pores[16-19].

Carda *et al.* showed that there are increased levels of urea and decreased level of amylase in the saliva of diabetic patients.[20] *Dioscorea villosa* Tubers have been indicated to be antidiabetic as they inhibit α-glucosidase and α-amylase enzymes responsible for the metabolism of carbohydrates.[21] Since the level of urea increases in diabetic patients, urease can be used to regulate urea level in the oral cavity of diabetic patients.

CONCLUSION

Urease was extracted from *P. sativum* seeds. The molecular weight was estimated. The enzyme activity was checked in varying pH and temperature. This enzyme can be further purified and analyzed for its clinical applications.

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Source of support: Nil; Conflict of interest: None Declared