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Novel Application of Cucurbita maxima Peel Protease in Detergents

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ABSTRACT

The protease enzyme from Cucurbita maxima peel was isolated and purified and its application in removing blood stain was studied. The optimum pH and temperature for this newly isolated protease was found to be 7.0 and 40°C respectively. The molecular weight of isolated protease was determined by SDS PAGE and it was found to be 31 KD. This new protease enzyme was applied to blood stained cloth to evaluate the stain removing capacity of the enzyme in the presence and absence of detergents. From the results, it is confirmed that the newly isolated protease removes stain completely with detergents and even in the absence of detergents. This study confirms the application of Cucurbita maxima peel protease in removing blood stains

The thermostable crude proteolytic extract and purified protease produced by Aspergillus tamarii URM4634 were investigated at different temperatures. The activity results were used to estimate the activation energy of the hydrolysis reaction catalyzed by crude extract and purified protease ($E^* = 34.2$ and 16.2 kJ/mol) as well as the respective standard enthalpy variations of reversible enzyme unfolding ($\Delta H°u = 31.9$ and 13.9 kJ/mol). When temperature was raised from 50 to 80 °C in residual activity tests, the specific rate constant of crude proteolytic extract thermoinactivation increased from 0.0072 to 0.0378 min^{-1}, while that of purified protease from 0.0099 to 0.0235 min^{-1}. These values, corresponding to half-life decreases from 96.3 to 18.3 min and from 70.0 to 29.5 min, respectively, enabled us to estimate the activation energy ($E^*d = 49.7$ and 28.8 kJ/mol), enthalpy ($\Delta H^*d = 47.0$ and 26.1 kJ/mol), entropy ($\Delta S^*d = -141.3$ and -203.1 J/mol K) and Gibbs free energy ($92.6 \le \Delta G^*d \le 96.6$ kJ/mol and $91.8 \le \Delta G^*d \le 98.0$ kJ/mol) of thermoinactivation. Such values suggest that this protease, which proved to be highly thermostable in both forms, could be profitably exploited in industrial applications. To the best of our knowledge, this is the first comparative study on thermodynamic parameters of a serine protease produced by Aspergillus tamarii URM4634.

Proteases which are also called "enzymes of digestion" are well known biocatalysts. They are commercially. used in various industries such as detergents, food, pharma, diagnostic etc. It is reported that 60% of total enzyme market is covered by the protease and are considered as the most valuable commercial enzyme. The source of proteases are enormous and bacterial proteases are more significant as compared to plant and animal proteases because of their rapid growth and can be easily manipulated genetically. However, plant proteases which has unique substrate specificity are free from undesirable side enzyme activities which is absent in microbial or animal systems. This makes the plant based proteolytic enzyme resources as valuable source having profound applications in enzyme industry. There are minimal reports about the characterization of plant proteases.

Thus, the arduous search for new potential plant proteases continues in order to make them industrially applicable and cost effective. In the present investigation the plant Citrus decumana L. (Rutaceae family) was selected as there is no report available on the protease enzyme and its characterization. The plant selected is well documented with its medicinal uses: antispasmodic, anti-inflammatory, anti-bleeding, bronchodilator, antidiabetic, anthelminthic,

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disinfectant, etc. Therefore, the plant looked to be a promising candidate for the protease source which can be exploited for its biotechnological applications. Therefore, the aim of the present study is to characterize the protease and partially purify it from the leaves of Citrus decumana L. with a view of that these proteolytic enzymes can be commercialized as alternative source.

Alkaline protease from alkaliphilic Bacillus sp. NPST-AK15 was immobilized onto functionalized and non-functionalized rattle-type magnetic core@mesoporous shell silica (RT-MCMSS) nanoparticles by physical adsorption and covalent attachment. However, the covalent attachment approach was superior for NPST-AK15 protease immobilization onto the activated RT-MCMSS-NH_2nanoparticles and was used for further studies. In comparison to free protease, the immobilized enzyme exhibited a shift in the optimal temperature and pH from 60 to 65 °C and pH 10.5-11.0, respectively. While free protease was completely inactivated after treatment for 1 h at 60 °C, the immobilized enzyme maintained 66.5% of its initial activity at similar conditions. The immobilized protease showed higher k cat and K m , than the soluble enzyme by about 1.3-, and 1.2-fold, respectively. In addition, the results revealed significant improvement of NPST-AK15 protease stability in variety of organic solvents, surfactants, and commercial laundry detergents, upon immobilization onto activated RT-MCMSS-NH_2nanoparticles. Importantly, the immobilized protease maintained significant catalytic efficiency for ten consecutive reaction cycles, and was separated easily from the reaction mixture using an external magnetic field. To the best of our knowledge this is the first report about protease immobilization onto rattle-type magnetic core@mesoporous shell silica nanoparticles that also defied activity-stability tradeoff. The results clearly suggest that the developed immobilized enzyme system is a promising nanobiocatalyst for various bioprocess applications requiring a protease.

Keywords: Cucurubita maxima; Protease; Detergents; Enzymes; Blood stain Removal