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Investigation of enzymatic activity, stability and structure changes of pectinase treated in supercritical carbon dioxide

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ABSTRACT

The activity and stability of enzymes in supercritical carbon dioxide fluid are the crucial points and basis for developing and applying green, environmentally friendly processes and/or reactions in this water free media in different industries, which has attracted increasing interesting recently. The objective of the present work is to investigate the activity and stability of pectinase in supercritical carbon dioxide media, as well as for its structure and conformation changes. The results show that the activity and stability of pectinase were significantly improved under appropriate conditions. Significant increases in activity and stability of treated pectinase could be available with pressure lower than 15.0 MPa, whereas, temperature tends to reduce enzymatic activity and stability. An excellent stability of pectinase with improved activity was observed with duration from 0.5 h to 4.0 h. Fourier transfer infrared spectra, ultraviolet spectra, fluorescence spectra and scanning electron microscopy analyses indicate that alterations in the secondary and tertiary structures, and morphology of treated pectinase, were occurred without conspicuous changes in its primary structure. An exposure of the residual side aromatic groups of tryptophan on polypeptide to outer surface of the enzyme in solution was also detected. Therefore, all the investigations further demonstrate that the supercritical treatment is an efficient method to improve the activity and stability of the enzyme due to conformational changes, and there is also a feasible to perform cleaner and sustainable production processes or reactions with pectinase in supercritical carbon dioxide media.

1. Introduction

As a biological catalyst, enzyme has received a growing demand in the last decades as an alternative to most of conventional chemical catalysts in different industries, due to its high catalytic efficiency, specificity and selectivity, minimum side reactions, mild reaction conditions and readily available, as well as industrially economically feasible and more environmentally friendly, etc (Wohlgemuth, 2010; Ciftci and Saldana, 2012; Mukhopadhyay et al., 2013). Up to date, there are many available reports about various enzymes and their applications in various industries, such as α-amylase, cellulase, β-galactosidase, protease, lipases, pectinase, Amyloglucosidasee pullanase, glucose oxidase, xylanase, which frequently employed in chemical engineering, organic synthesis, food engineering, textiles, environmental treatments, etc (Choi et al., 2015; Kalantzi et al., 2008; Chand et al., 2012). Among all the employed enzymes, pectinase or pectinolytic enzyme refers to all the species that can catalyze the hydrolysis of pectin substances, and can be divided into protopectinase, pectin esterase and depolymerizing enzymes according to their reaction mechanisms, methods, action patterns, as well as substrates of hydrolysis (Kalantzi et al., 2008; Mukhopadhyay et al., 2013). Pectinase is widely utilized in food industry for extraction and clarification of fruit and vegetable juice, fermentation of red wine and tea, extraction of oil (Kashyap et al., 2001), as well as for scouring of cotton fabric and degumming of ramie fiber in textile industry, etc (Mukhopadhyay et al., 2013).

However, all the traditional enzymatic processes for biocatalysis reactions in aqueous bath involve a large amount of water and energy consumptions, and readily cause some concerns of environment pollution by discharging wastewater with high biologic oxygen demand (BOD) and high chemical oxygen demand (COD) values, such as in textile industry. In fact, the conventional enzymatic processes employed for textile treatments usually still require a large bath ratio and long cultivation duration for a

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satisfactory efficiency of enzymatic reactions and commercial production, especially for the biodesizing, biodegumming and bioscouring of natural fiber based substrates by employing various enzymes. Thus, a lot of water resource, energy and effluents with high concentration of pollutants still can't be avoided in the conventional enzymatic processes, although they possess more advantages than traditionally harsh chemical processes. Therefore, the effective, useful and Ecofriendly methodologies or processes with non-aqueous media or in which water is not the predominant content for enzyme applications, are very popular in textile and/or other industries. Fortunately, supercritical carbon dioxide fluid, a green and hydrophobic medium, has received increasing attentions in recent years as an alternative to conventional water and organic solvents in industries, due to its numerous advantages such as non-toxic, environmentally friendly, high diffusion rates, less side reactions, readily downstream treatments, absence of gas–liquid mass transfer limitations (Gremos et al., 2012; Long et al., 2011; Long et al., 2015). Consequently, it is very meaningful and necessary to investigate the activity and stability of enzymes in supercritical carbon dioxide media in order to develop different biocatalytical processes by integrating or combining enzymes with non- or less content aqueous media of supercritical carbon dioxide fluid for cleaner and sustainable production in textile and other industries, with more advantages than conventional enzymatic processes in elimination and/or reduction of the consumption of water resource and the discharge of effluents inherent as well as decreasing total production costs, etc.

Theoretically, the activity and stability of enzymes in supercritical carbon dioxide media are the crucial points and basis prior to a development and/or an application of any bio-catalytical methodologies or processes in this water free media. Up to date, part of research for some enzymes is available in literature. Melgosa et al. (2015) investigated the effect of supercritical carbon dioxide treatment on the activity and conformational, morphology of four commercial lipases, and revealed that all the employed enzymes remained activities in the supercritical medium and the activity of some enzymes could be improved, whereas some other enzymes reduced their activity by means of conformational changes and structural alterations according to the treatment conditions. Leitgeb et al. (2013) investigated the activity of cellulase and α-amylase from *Hortaea werneckii* after cell treatment with supercritical carbon dioxide, and found that sufficient residual activity of both the examined enzymes were detected and could be still used as biocatalysts in this medium. Natalia et al. (2012) explored the stability, activity, and selectivity of benzaldehyde lyase in supercritical fluids including carbon dioxide, and revealed that supercritical fluids could be an alternative media to organic solvents. Senyay-Oncel and Yesil-Celiktas (2013) treated immobilized α-amylase in supercritical carbon dioxide in order to enhance enzyme stability and activity, and found that the activity of immobilized α-amylase after consecutive enzymatic reactions could be enhanced by the retreatment with supercritical carbon dioxide. In addition, an enhancement in activity and stability for free α-amylase was also achieved after being treated in sub- and supercritical carbon dioxide by Senyay-Oncel and Yesil-Celiktas (2011). Moreover, Senyay-Oncel et al. (2014) investigated the activity and stability of protease in sub- and supercritical carbon dioxide, and demonstrated that enhanced activity and stability were observed with the raise of pressure, and the potential mechanism was also explored. Andrade et al. (2008) assessed the influence of compressed CO_2 and propane treatment on the specific activity of partially purified D-hydantoinase from adzuki bean (*Vigna angularis*), then found that good stability of this enzyme in the two solvents was observed although some activity losses were occurred for resolubilized enzyme extract with compressed CO_2. Manera et al. (2011) explored the influences of pressure, exposure time and depressurization rate on the β-galactosidase activity of permeabilized cells of *Kluyveromyces marxianus* CCT 7082 submitted to treatment with compressed carbon dioxide, propane and *n*-butane, and showed that the activities of this biocatalyst were always higher than those of the non-treated ones. Furthermore, Rezaei et al. (2007) reviewed effects of pressure and temperature on enzymatic reactions in supercritical fluids including carbon dioxide, and demonstrated that most of the enzymes were active in supercritical fluids, and their activity could be improved with temperature within limits.

However, some negative effects from treatments with supercritical carbon dioxide on the activity and stability of some enzymes were also reported. Santos et al. (2016) investigated the activity of immobilized lipase from *Candida antarctica* (Lipozyme 435) and its performance on the esterification of oleic acid in supercritical carbon dioxide, and showed that the activity of Lipozyme 435 decreased with the increase of pressure, temperature, exposure time and the number of pressurization/depressurization cycles. Oliveira et al. (2006) also investigated the influence of temperature, pressure, exposure time and depressurization rate on the activity of an immobilized lipase from *Yarrowia lipolytica* in compressed carbon dioxide, propane and n-butane, and showed that significant activity losses were obtained in carbon dioxide in comparison with other organic solvents. Moreover, the effects of treatment parameters on enzyme activity depended on its nature and source, as well as its forms. However, Dhake et al. (2011) investigated the activity and stability of *Rhizopus oryzae* lipase via immobilization for citronellol ester synthesis in supercritical carbon dioxide, and revealed that the immobilization method by using the blended polymer of hydroxylpropyl methyl cellulose and polyvinyl alcohol could greatly overcome the drawback by enhancing the catalytic activity of *R. oryzae* lipase for synthesis in supercritical carbon dioxide.

It is obvious that most of the reported research works about the activity and stability of enzymes in supercritical carbon dioxide are mainly concentrated on α-amylase, cellulase, β-galactosidase, protease, free and/or immobilized lipases, etc. Very few works have been reported about the activity and stability of pectinase in supercritical carbon dioxide; especially, it is very lack of investigations about the alterations of pectinase in primary, secondary and tertiary structures, as well as its conformational and morphology changes induced by the supercritical media, although it is very important as a crucial basis for developing or performing green and environmentally friendly processes, reactions by combination of the two ecological technologies in different industries.

The objective of the present work is to investigate the activity and stability of pectinase in supercritical carbon dioxide media, and also to explore some information about its structural, conformational and morphology alterations during treatment. The effects of system pressure, temperature and treatment duration on the activity and stability of pectinase were evaluated. Moreover, Fourier transfer infrared spectra (FT-IR), ultraviolet (UV) spectra, fluorescence spectra and scanning electron microscopy (SEM) analyses were performed for investigating the structural, conformational and morphology information of treated pectinase.

2. Material and methods

2.1. Materials

A pectinase (from aspergillus Niger) in an analytical pure grade was purchased from Beijing Solarbio Technology Co., Ltd. (Beijing of China), and was used without further purification. A pectin (Galacturonic acid > 74.0%, dried basis) from citrus peel was obtained from Sigma–Aldrich Co., Ltd. (Shanghai of China), and used as a