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Improvement of thermostability and activity of pectate lyase in the presence of hydroxyapatite nanoparticles

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ABSTRACT

The activity and half-life of pectate lyase (PL) from *Bacillus megaterium* were nine- and 60-fold, respectively, higher at 90 °C in the presence of hydroxyapatite nanoparticles (NP-PLs) than in the presence of 1 mM $CaCl_2$. Thermodynamic analysis of the nanoparticle-induced stability revealed an enhanced entropy–enthalpy compensation by the NP-PLs since a reciprocal linearity of the enthalpy–entropy change to 90 °C was observed. Without nanoparticles, the linearity range was 70 °C. Such compensation reflected the maintenance of the native structure of proteins. The remarkable enhancement of activity and stability of the NP-PL system at high temperatures may be utilized commercially e.g. in the food industry or the processing of natural fibers that may require a thermotolerant enzyme.

1. Introduction

Pectin, an important constituent of the plant cell wall, is a heteropolysaccharide, which contains α-1-4 linked galacturonate chains, that are highly methyl esterified. Pectinolytic enzymes, such as pectin lyase (PL) and polygalacturonase (PG) catalyze the degradation of pectin. PG generally hydrolyzes pectic acid and PL, specific for methyl esterified substrates, catalyzes the cleavage of α-D-(1, 4) glycosidic bonds by β-elimination of the pectin substances (Pereira et al., 2002; Basu et al., 2011). These pectinolytic enzymes are produced by a variety of microbes and play an important role in biotechnological applications such as in the food and beverages industries for improving the yield and clarification of fruit juice and in the textile industries to facilitate degumming of natural fibers like ramie, jute as an alternative to conventional retting (Soriano et al., 2005; Basu et al., 2011). Thermostable enzymes are stable and active at temperatures considerably higher than their optimum temperatures (Saboto et al., 1999; Haki et al., 2003). An enzyme or protein may be termed as thermostable if a high definite unfolding (transition) temperature (T_m) or a long half-life at a preferred high temperature is observed (Turner et al., 2007). Thermostable enzymes can be used at temperatures that enhance substrate solubility and reaction rates while allowing for pretreatment of raw materials. Contamination by mesophiles can also be curtailed under these conditions (Demirijan et al., 2001; Haki et al., 2003).

Scouring of natural fibers improves water absorbency and whiteness of textiles by removing non-cellulosic substances from many natural fibers (Basu et al., 2008). Chemicals like soda-ash, oxalic acid and caustic soda, used for chemical scouring give rise to polluting effluents while weakening the strength of the finished fiber. The use of pectin-removing enzymes as an alternative has thus gained importance. However, the thermal instability of the enzyme impairs it is scouring efficiency. Nano-materials exhibit properties such as large surface-to-volume ratios, high surface reaction activity, high catalytic efficiency, and strong adsorption ability (Pendry, 1999; Hudson et al., 1997; Feldstein et al., 1997; Fukumi et al., 1994; Hagland et al., 1993). Protein adsorption on nanoparticles can lead to improved activity (Lynch and Dawson, 2008) and improvements in thermal stabilities of the enzymes were observed after adsorption (Chronopoulou et al., 2011). Enzyme stability is maximized with nano-scaled supports with possible modulation of the catalytic specificity (Konwarh et al., 2009). Hydroxyapatite (HAp), $Ca_{10}(PO4)_6(OH)2$, is a compound which can be prepared in a nano-form that is apparently non-toxic (Sheik and Kim, 2010). Although chemical and molecular biology methods have been applied to pectate lyase, thermostability concomitant with high activity were not achieved (Basu et al., 2008). In the present study, pectate lyase was treated with hydroxyapatite nanoparticles. The objective of this work was to develop a highly active thermostable pectate lyase preparation for potential application in industry.

Abbrevations: NP, nanoparticle; HAp, hydroxyapatite; NP-PL, HAp nanoparticle treated pectate lyase; PL, untreated pectate lyase; TBA, thio-barbituric acid; PGA, poly-galactouronic acid.

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