Polygalacturonases from potato tubers

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Abstract: Potato tubers produce small amount of polygalacturonase. Multiple forms of this enzyme were observed with isoelectric points between 4.6 and 7.2 (major form about 6.5). Polygalacturonase isoforms were characterized by their molecular masses, pH optima, optimum of temperature, thermal stability, K_M on polymeric substrate and action pattern on polymeric and oligomeric substrate. All forms showed highly random cleavage pattern of pectate and absence of activity towards both di(D-galactosiduronic) acid and tri(D-galactosiduronic) acid, particularly the trimer. Based on these results potato polygalacturonase (endopolygalacturonase, EC 3.2.1.15) can be characterized as an enzyme with unusual degradation of oligomeric substrates yielding trimer as the end product.

Key words: pectic enzymes, polygalacturonase, multiple forms, potato tubers, action pattern.

Introduction

The production of pectolytic enzymes has been widely reported and thoroughly studied in bacteria and filamentous fungi because they play an essential role in the phytopathogenesis. Pectinase production in yeasts has received less attention and only a few species show this ability (Blanco et al., 1999). The presence of these enzymes has been reported in both sucking (Ma et al., 1990; Agblor et al., 1994) and chewing (Shen et al., 1996; Doostdar et al., 1997) insects. Plants, for instance tomato (Hobson, 1965; Tucker et al., 1980; Markovič & Slezárik, 1981; Brady et al., 1983), avocado (Awad & Young, 1979), apple (Bartley, 1978), pear (Ahmed & Labavitch, 1980), strawberry (Nogata et al., 1993), mango (Labib et al., 1995), peach (Tijskens et al., 1998), banana (Pathak et al., 2000), carrots (Stratilová et al., 1998) and many others, also produce pectolytic enzymes. Their assumed natural role in plants includes fruit maturation, growth, abscission and pollen development.

The main enzyme of pectolytic enzyme system is polygalacturonase (EC 3.2.1.15) (Rexová-Benková & Markovič, 1976). Polygalacturonases cleave α-1,4-glycosidic linkages between linked deesterified galacturonic acid units in linear part of pectin molecule by random action pattern. This is demonstrated by expressive decrease of substrate viscosity corresponding to its low degree of degradation. Total activity of polygalacturonase and exopolygalacturonase (EC 3.2.1.67) can be determined by measuring the enzyme activity by methods utilizing the increase of reducing groups of substrate during enzyme action (Somogyi, 1952). Exopolygalacturonases cleave the substrate by terminal pattern and D-galactopyranuronic acid appears in the reaction mixture as the end product. The comparison of primary structures of exopolygalacturonases and polygalacturonases showed strictly conserved active site residues, whereas the rest of the amino acid sequence may considerably vary (Markovič & Janeček, 2001). The influence of tertiary structure on the action pattern of polygalacturonases and exopolygalacturonases can be derived. So far three-dimensional structures of polygalacturonases from five microorganisms have been determined (Pickersgill et al., 1998; van Santen et al., 1999; Cho et al., 2001; Federici et al., 2001; Shimizu et al., 2002; van Pouderoyen et al., 2003). Polygalacturonases themselves differ in action pattern towards oligomeric substrates. In addition, the rate of reaction on substrates with different degree of polymerization may extensively vary between closely related polygalacturonases (Rexová-Benková & Markovič, 1976).

Concerning the potatoes, pectin methylesterase (EC 3.1.1.11) is the only pectic enzyme present in potato leaves and sprouts, which can be considered as relatively well characterized (McMillan & Pérombelon, 1995; Ebbelaar & Recourt, 1996). This study is the first report on the presence of polygalacturonase in potato tubers. Enzyme with unusual degradation of oligomeric substrates was found.

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