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Capillary electrophoresis of homogeneous pectin fractions

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Abstract

Capillary electrophoresis (CE) has been used to characterize pectin fractions obtained from mother samples of varying degrees (and intramolecular patterns) of methylesterification (DM). These samples have been carefully produced by fractionation, (a) from the original mother samples on the grounds of molar mass, and (b) subsequently from the daughter fractions so obtained, on the grounds of charge. The results confirm theoretical predictions regarding the dependence of electrophoretic mobility on molar mass and DM and give further evidence that CE is able to give realistic information regarding the intermolecular DM of pectins.

Keywords: Pectin; Capillary electrophoresis; Degree of methylesterification; Intermolecular DM distributions

1. Introduction

Pectin is a complex carbohydrate polymer that plays an instrumental role in regulating the mechanical properties of the plant cell wall (McCann & Roberts, 1996) and has also found great utility in many diverse areas of science and technology (May, 1990). While the detailed structure of the pectin macromolecular assembly in vivo is still a matter of debate (Vincken et al., 2003) most commercially available pectin samples can be considered as a collection of polymer chains each consisting of extended regions of homogalacturonan interspersed sparsely with regions of rhamnogalacturonan I (Ralet & Thibault, 2002). Even at this level of description complexity and heterogeneity abound. In particular, the distribution of methylesterification both among chains and along individual polymer backbones is a key determinant of molecular functionality. Indeed, cell wall enzymes routinely tailor DM distributions in order to exploit structure–function relationships based on the dependence of molecular association on the pattern of methylesterification (Willats et al., 2001). The measurement of such distributions is then vital in order to understand the role that fine structure modifications play in determining the functionality of pectin both in vivo and in vitro. It is envisaged that ultimately useful pectin structure–function models will not just incorporate information on DM distributions, but will require it in order to correctly predict the properties of pectin samples.

Capillary electrophoresis has recently been shown to be a useful tool for the investigation of pectin methylester distributions (Jiang, Wu, Chang, & Chang, 2001; Ström & Williams, 2004; Williams, Buffet, & Foster, 2002a,b; Williams, Foster, & Schols, 2003; Zhong, Williams, Keenan, Goodall, & Rollin, 1997; Zhong, Williams, Goodall, & Hansen, 1998). Most simply it can be used in order to measure the average DM of a pectin sample, since there is a linear relationship between the electrophoretic mobility and the average charge per residue (Zhong et al., 1997, 1998). While many other methods perform this sample averaged DM measurement equally well (Bédouet, Courtois, & Courtois, 2003; Huisman, Oosterveld, & Schols, 2004; Lévigne, Thomas, Ralet, Quéméner, & Thibault, 2002; Maness, Ryan, & Mort, 1990; Massiot, Perron, Baron, & Drilleau, 1997; Synytsya, Copikova, Matejka, & Machovic, 2003; Rosenbohm, Lundt, Christensen, & Young, 2003), an advantage of the electrophoretic method is its inherent separation quality. For chains with lengths in excess of around 15 residues a symmetrical scaling of charge and

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hydrodynamic friction co-efficient with the degree of polymerisation (DP) is found. This means that larger polymeric chains, regardless of their DP, elute according to their average charge density and, therefore, that each CE migration time marks species with a unique DM. Peak shapes thus reflect the intermolecular methylesterification distribution (the DM distribution among chains) of the sample (Williams et al., 2003).

It is worth noting that the validity of such a methodology for obtaining intermolecular DM information from CE hinges crucially on two major assumptions. Firstly, that contributions to peak widths arising from chromatographic factors are small compared to the breadth of the intermolecular mobility (charge) distribution, and secondly that the mobility is not significantly dependent on the intramolecular distribution of charge (methylesterification), so that the migration behaviour is determined simply according to the chain-averaged charge density. The first point has been largely addressed by observing the invariance of peak widths to changes in injection time, monitoring the co-injection and subsequent resolution of discrete samples, and performing calculations of the relative contributions expected from the relevant band-broadening mechanisms (Zhong et al., 1997, 1998). The second point has been addressed by studying commercial pectin samples fractionated according to their calcium sensitivity (Zhong et al., 1998; Williams et al., 2002a) and, more recently, home-made samples molecularly engineered to have a random or block wise DM distribution by exploiting demethylesterification using chemical saponification in contrast to processive enzymes (Williams et al., 2003). It was concluded that there was no statistically significant difference between the electrophoretic mobilities of pectins of equivalent DM with differing intramolecular arrangements of methylesterification. It is interesting to note that recent work using Ion Exchange Chromotography (IEC) has also suggested that the elution can be largely governed by charge density independently of charge distribution patterns (Ralet & Thibault, 2002).

While the success of the CE work to date implies that indeed the electrophoretic mobility is independent of the molecular weight (above DP ~ 15); that is, polymeric chains of the same DM will elute together regardless of their length, here we explicitly investigate this experimentally by studying samples fractionated solely on the grounds of molar mass. Furthermore, while the co-injection and subsequent separation of pectin mixtures has provided strong evidence that the CE peak width is a reasonable reflection of the intermolecular DM distribution, collecting and re-injecting fractions from the leading and following edges of a single peak has not been possible owing to the small sample volume that can be collected from a single CE run. In this work, however, in the same spirit, we have taken pectin fractions homogenous in molar mass, and subsequently fractionated these samples again, now on the grounds of charge (using IEC). These samples, homogenous with respect to both mass and charge, have then been further studied by CE.

2. Experimental

2.1. Materials

A commercial pectin (L72) from Mexican lime peel (*Citrus aurantifolia*), with a DM of 72 was esterified in acid-methanol medium to give a pectin (E81) of a DM of 81. A series of pectins with defined DM were prepared by enzymatic treatment of E81 as fully described previously (Limberg et al., 2000). F-series samples were prepared using a fungal pectin methylesterase (f-PME) from *Aspergillus niger* purified from Pektolase™ (Danisco, Brabrand, Denmark) and P-series samples were prepared using a plant pectin methylesterase (p-PME) purified from orange peels as described by Christensen, Nielsen, Kreiberg, Rasmussen and Mikkelsen (1998). Those pectin samples (mother samples) were fully characterised in a previous study (Ralet, Dronnet, Buchholt, & Thibault, 2001). Mother samples (F69, F58, F43 and P60) were fractionated by Size Exclusion Chromatography on a column (92 × 5 cm) of Sephacryl S-500 to give daughter samples. A detailed description of chromatography conditions and physico-chemical characterisation of recovered daughter samples has been published elsewhere (Ralet & Thibault, 2002). The intermediate size fractions (pectin-S500(3)), representing the bulk of the samples, were further purified by Ion Exchange Chromatography on DEAE-Sepharose CL-6B to give granddaughter fractions as fully described elsewhere (Hellin, Ralet, Bonnin, & Thibault, in press). The isolation procedure of daughter and granddaughter samples is summarised in Fig. 1.

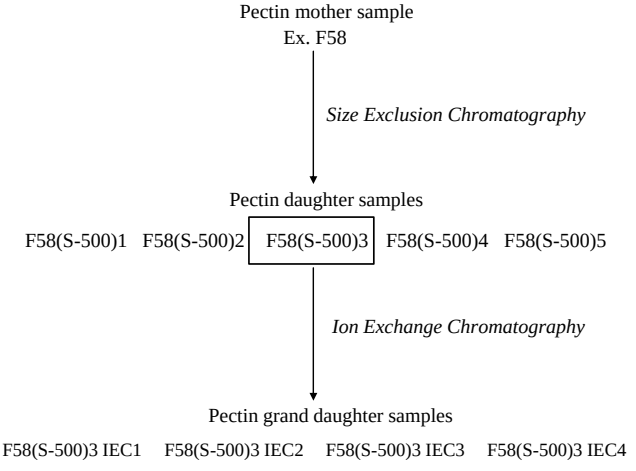

Fig. 1. Schematic diagram of the fractionation procedures employed for the production of homogeneous samples.