USING A CCD-BASED IMAGER TO SCAN PACE GELS PROVIDES A QUICK AND SENSITIVE METHOD OF ANALYSIS

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Analysis of polysaccharides can be problematic because of the need for complex methods and/or expensive equipment such as chromatography and mass spectrometry [1,2], NMR [3], capillary electrophoresis [4], and Fourier transform infrared spectroscopy [3,5].

These techniques are ideal for a particular polysaccharide or application, but they have limitations, such as the need for pure compounds or relatively large sample quantity.

Recently, a method known as PACE (polysaccharide analysis using carbohydrate gel electrophoresis) has been described [6,7]. This can be used in a high throughput manner and is applicable to small quantities of material.

PACE relies on derivatising reducing ends of sugars and oligosaccharides with a fluorophore label, followed by polyacrylamide gel electrophoresis. To obtain sensitive detection of fluorescent signals on PACE gels generally requires laser based scanners which can be expensive; limited to fluorescence applications; or require large amounts of bench space.

To overcome these difficulties, this article describes how CCD-based technology can be used to detect the fluorescent signal produced on a PACE gel and the sensitivity this type of system can achieve.

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MATERIALS AND METHODS

Dried polysaccharides, oligosaccharides or monosaccharides were derivatised as previously described (6,7) in the presence of either the fluorophores, ANTS (8-aminonaphthalene-1, 3, 6-trisulfonic acid) or AMAC (2-aminoacridone) both from Molecular Probes (Leiden, The Netherlands).

Derivatised samples (0.5–4 ml) were separated using different conditions, according to the type of sample being analyzed. To study uncharged oligosaccharides, samples derivatised in the presence of ANTS were loaded onto a polyacrylamide gel (20% w/v) containing bisacrylamide (0.5% w/v) with a stacking gel (2 cm) of polyacrylamide (8% w/v) and bisacrylamide (0.2% w/v). The electrophoresis buffer Tris Borate (0.1 M, pH 8.2) was used and the samples were electrophoresed at 200 V (30 minutes) and then 1000 V (90 minutes).

To detect charged sugars (monosaccharide or oligosaccharides), samples derivatised in the presence of AMAC were loaded onto a polyacrylamide gel (24% w/v) containing bisacrylamide (0.5% w/v) with a stacking gel (2 cm) of polyacrylamide (8% w/v) and bisacrylamide (0.2% w/v). Both the resolving and stacking gels were prepared in Tris–Cl, (0.1M pH 8.2). A discontinuous electrophoresis buffer system was used of Tris (0.15 M brought to pH 8.5 with 0.15 M glycine) as the cathode reservoir buffer and Tris–Cl (0.1M, pH 8.2) as the anode reservoir buffer. The samples were electrophoresed initially at 200 V (30 min) and then at 1000 V (2 h30).

The stacking gel was removed and a small amount of water was added onto each gel prior to imaging to flatten them out and reduce the wrinkling. The gels were then transferred to a G:BOX Chemi HR16 (Syngene, Cambridge, UK) for imaging. Since the emission peak for ANTS is 356 nm and for AMAC is 420 nm the gels were imaged using short and long wavelength UV, with UV and short pass emission filters and with and without neutral fielding.

RESULTS AND DISCUSSION

Using short and long wavelength UV, UV and short pass emission filters, and with and without neutral fielding applied, the G:BOX Chemi HR16 generated the same level of sensitivity for both derivatisation conditions. The system also captured PACE gel images in 2-10 seconds which allowed detection of 1pmol of ANTS and AMAC labelled sample and quantification of 5 pmol (Figures 1 -2).

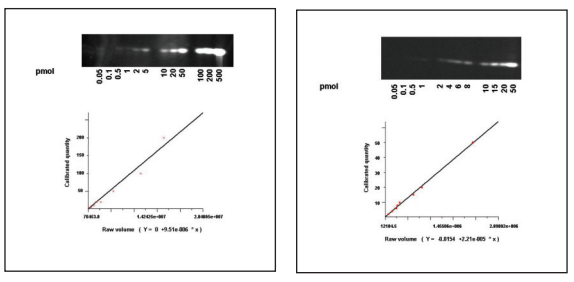

Figure 1: 1-D PACE gel labelled with ANTS and corresponding standard curve. The gel image captured using a G:BOX Chemi HR16 shows the presence of 1 pmol of uncharged oligosaccharide.

Figure 2: 1-D PACE gel labelled with AMAC and corresponding standard curve. The gel image generated with a G:BOX Chemi HR16 shows the presence of 1 pmol of charged sugar.

The ANTS labelled gels showed excess free ANTS migrates into the gel to produce a highly saturated gel image (data not shown). However, this problem can be overcome by increasing exposure time and is a benefit of using a CCD- based system integrated to image analysis software rather than a laser-based scanner.

Researchers in the Department of Biochemistry at the University of Cambridge have been testing out this system to produce a unique oligosaccharide profile of a genetically modified plant's cell wall composition.

Dr Florence Goubet, a post-doctoral scientist from Dr Paul Dupree's team in the Department of Biochemistry explained: "PACE and automated quantification can be substituted for chromatography and mass spectrometry techniques, both of which require pure or large amounts of compounds to generate results from just a few plant samples. Using our method we can process up to 100 samples a day and can detect oligosaccharides in the picomole range, which means we can rapidly identify whether we have successfully genetically engineered the plants we are modifying."

CONCLUSION

PACE is a powerful method for analyzing small quantities of carbohydrate and utilizing a CCD-based system with a high performance camera that can detect a wide range of UV excitation peaks means rapid detection in the picomole range can be achieved.

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G Box :Chemi HR16