ELSEVIER

Carbohydrate Polymers 43 (2000) 285–289

Carbohydrate Polymers

www.elsevier.com/locate/carbpol

A comparison between MALDI-TOF mass spectrometry and HPAEC-PAD analysis of debranched starch

S. Broberg[a,*], K. Koch[b], R. Andersson[b], L. Kenne[a]

[a]*Department of Chemistry, Swedish University of Agricultural Sciences, P.O. Box 7015, SE-750 07 Uppsala, Sweden*
[b]*Department of Food Science, Swedish University of Agricultural Sciences, P.O. Box 7051, SE-750 07 Uppsala, Sweden*

Accepted 18 January 2000

Abstract

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, MALDI-TOF MS, was used in this study to determine chain length distribution of amylopectin, the main constituent of starch. The technique was compared with high-performance anion-exchange chromatography with pulsed amperometric detection, HPAEC-PAD, an established technique for this purpose. Starch from potato, wheat, and waxy maize was debranched with isoamylase and analysed using both techniques. MALDI-TOF MS is a faster and more sensitive technique and provides more detailed information on the molecular mass of the unit chains. A difference between chain length distribution for amylopectin from different sources was observed with both methods. In addition, removal of amylose was not necessary for MALDI-TOF analysis. However, the technique was less reproducible than HPAEC-PAD and overestimated the amount of longer chains.

Keywords: Starch; Quantification; MALDI-TOF MS; HPAEC-PAD; Amylopectin

1. Introduction

The ratio of amylose and amylopectin in starch and the distribution of unit chains have important effects on the physiochemical properties. Starch from various botanical sources differ in structure and consequently in functional properties when used as raw materials in, e.g. the food and paper industries (Eliasson & Gudmundsson, 1996; Hizukuri, 1996). Recently, there has been an increased interest in starch research, partly because of new knowledge of the mechanism of starch synthesis and partly due to the use of biotechnological methods in plant breeding which has increased the possibility to produce starch with modified properties (Shewmaker & Stalker, 1992). The analysis of the chain length distribution of starch is often performed by size-exclusion chromatography (SEC) of the unit chains after debranching. More details are provided by high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) since individual chains are separated with high resolution up to DP 80 (Hanashiro, Abe & Hizukuri, 1996). No correct mass distribution is directly obtained, however, since the detector response decreases for longer unit chains. Quantitative analysis of amylopectin unit chain distribution with HPAEC-PAD has recently been achieved (Koch, Andersson & Åman, 1998; Wong & Jane, 1997). Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), since its introduction by Karas, Bachmann, Bahr and Hillenkamp (1987), has been used mainly for molecular weight determination. However, recently a number of studies have shown that the method can be applied for quantification of several types of compounds including carbohydrates (Harvey, 1993; Naven & Harvey, 1996; Wang, Sporns & Low, 1999), proteins and oligonucleotides (Tang, Allman, Jones & Chen, 1993) ionized with a variety of matrices.

Here, in this study, the possibility of using MALDI-TOF MS for amylopectin characterisation was investigated. The unit chain length distributions of amylopectin from wheat, potato, and waxy maize were analysed using MALDI-TOF MS and the results compared with those from HPAEC-PAD, quantified according to Koch et al. (1998).

2. Experimental

2.1. Materials

Potato, wheat and waxy maize starch were obtained from Lyckeby Stärkelsen (Kristianstad, Sweden). Maltoheptaose

* Corresponding author.

0144-8617/00/$ - see front matter
PII: S0144-8617(00)00152-1

(Boehringer Mannheim, Mannheim, Germany) was used as a standard. Sodium hydroxide solution (analytical-reagent grade, 50%) was purchased from Baker (Deventer, Netherlands). Isoamylase (EC 3.2.1.68, crystalline, from *Pseudomonas amyloderamosa*, 71000 U/mg) was obtained from Hayashibara Biochemical Labs. (Okayama, Japan), amyloglucosidase (EC 3.2.1.3 from *Aspergillus niger*, 36 U/mg) from Megazyme (Bray, Ireland) and Merckotest Glucose (Article No. 14365) from Merck (Darmstadt, Germany). 2,4,6-Trihydroxyacetophenone and 1-hydroxyisoquinoline were purchased from Fluka (Buchs, Switzerland) and 2,5-dihydroxybenzoic acid from Aldrich (Steinheim, Germany).

2.2. Sample preparation

Starch from potato and wheat was dissolved in 1 M aqueous sodium hydroxide and the amylopectin isolated by fractionation on Sepharose CL-2B and freeze-dried as described by Lloyd, Hedley, Bull and Ring (1996). Potato and wheat starches as well as isolated amylopectin were debranched with isoamylase according to Fredriksson, Andersson, Koch and Åman (1997). Amylopectin unit chain fractions with different average degree of polymerization (DP) were obtained by debranching of waxy maize starch with subsequent fractionation on a Bio-Gel P-6 column (Koch et al., 1998).

2.3. HPAEC-PAD

The chromatography was performed on a Dionex DX 500 instrument (Sunnyvale, CA, USA) equipped with a PAD system (ED 40). Samples (20 μl) with a concentration of 0.2–1 mg debranched amylopectin per ml were injected via an autosampler (Spectra-Physics, Fremont, CA, USA) onto a CarboPac PA-100 anion-exchange column (250 × 4 mm) in combination with a CarboPac PA-100 guard column, and corrections for the different detector responses for the individual unit chains were made (Koch et al., 1998).

2.4. MALDI-TOF MS

Three different matrices were used: 2,4,6-trihydroxyacetophenone (THAP, 0.1 M in methanol) (Pieles, Zurcher, Schär & Moser, 1993), 2,5-dihydroxybenzoic acid (DHB, 0.2 M in water/acetonitrile, 1:1) (Strupat, Karas & Hillenkamp, 1991) and a mixture of DHB and 1-hydroxyisoquinoline (HIQ, 0.1 and 0.03 M, respectively, in water/acetonitrile, 1:1) (Mohr, Bornsen & Widmer, 1995). The matrix (1 μl) was deposited on the probe tip and vacuum dried to obtain a thin-film matrix layer (Vorm, Roepstorff & Mann, 1994), subsequently 1 μl of debranched waxy maize (4.6 mg/ml) was applied and vacuum dried. Alternatively, 1.5 μl of cold THAP matrix solution was mixed with an equal volume of the above solution of debranched waxy maize and 1 μl of this analyte–matrix mixture was deposited on the probe tip and dried in vacuum.

The weight concentration of the amylopectin unit chain fractions, obtained from waxy maize, ranged between 70 and 140 μg/ml. The average DP for each unit chain fraction was calculated according to Koch et al. (1998) and the concentrations were determined to 8–70 pmol/μl. The solutions were thereafter diluted with water to 6.3 pmol/μl. Each unit chain fraction was then mixed 9:1 (v/v) with maltoheptaose dissolved in water (57 pmol/μl), to gain a concentration of 5.7 pmol/μl of unit chain fraction as well as of maltoheptaose. The analyte (1.5 μl) was mixed with an equal volume of cold THAP, same conditions as above. Samples of debranched wheat and potato starch were diluted to 4.6 mg/ml and 5 μl of analyte was mixed with an equal volume of THAP.

Analyte–matrix mixture (1 μl) was deposited on a gold-plated probe tip and vacuum dried. The molecular masses of glucan unit chains were analysed in the positive mode on an LDI-1700XP (Linear, Reno, NV, USA) instrument with a nitrogen laser at 337 nm using 5–6 μJ energy for unit chain fractions and 6–8 μJ energy for debranched starch. For each acquisition, 100 laser shots were fired and the resulting spectra were averaged. For every sample 4–7 acquisitions were made, each on a different spot on the analyte-matrix surface. Calibration was done on debranched starch from waxy maize containing peaks with known *m/z* values.

All data were exported to MATLAB (The Math Works Inc., Natick, MA, USA), a general-purpose mathematical program. The peaks corresponding to the individual unit chains were integrated with background correction to obtain the areas that reflect the amount of ionized unit chains. During integration the limits were set to $[M + Na]^+ - 111.14$ mass units and $[M + Na]^+ + 51$ mass units and stepped up 162.14 mass units for every peak, corresponding to the mass increase per glucose unit.

3. Results and discussion

3.1. MALDI-TOF conditions

The matrix is of great importance for a successful ionization of the sample and several matrices are used for the analysis of carbohydrates. Therefore, three different matrices were tried with the thin-film layer method (Vorm et al., 1994) for optimal conditions. The use of THAP as matrix gave fewer extra peaks than DHB and more narrow peaks than the DHB-HIQ mixture, consequently THAP was the chosen matrix. THAP was also mixed with equal volume of analyte and 1 μl of this analyte–matrix mixture was applied on the probe tip and dried in vacuum. The latter method allowed the laser to be fired at slightly lower energy during data acquisition than the fast evaporation technique above and was consequently used in the following experiments.

Although MALDI-TOF MS is comparatively tolerant to the presence of contaminants such as buffer salts and detergents, these compounds can influence the intensity and

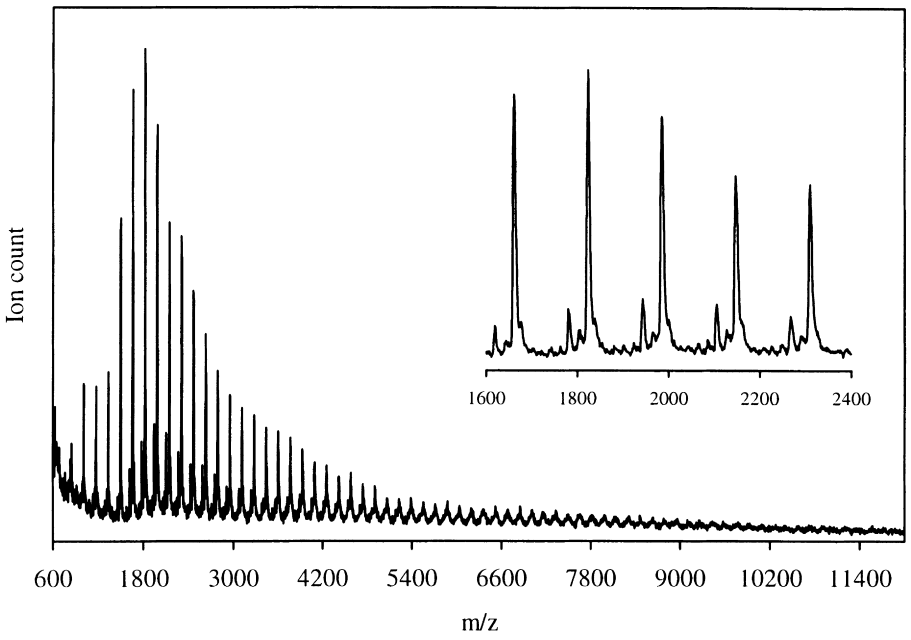

Fig. 1. MALDI-TOF mass spectrum of debranched wheat amylopectin. Inset: a fraction of the spectrum showing the adduct peaks as well as the possible fragmentation peaks.

quality of the signal (Mock, Sutton & Cottrell, 1992). The sodium acetate concentration in the examined degraded starches was through the sample preparation diluted to 0.02 M prior to MALDI-TOF MS and satisfactory mass spectra were achieved (Fig. 1). In the mass spectrum the unit chains are seen as sodium adduct peaks. Further, a small amount of potassium adducts as well as possible fragment peaks, $[M + Na]^+ - 18$ mass units and $[M + Na]^+ - 42$ mass units, are seen in the spectrum. The former is probably corresponding to the loss of one water molecule, but the latter is more difficult to find an explanation for. Due to peak broadening as *m/z* increases, it was necessary to span over 162.14 mass units per peak while integrating, thus incorporating the extra peaks.

3.2. Comparison between MALDI-TOF MS and HPAEC-PAD

The chain length distributions of debranched starch from the isolated amylopectin from potato and wheat were determined with MALDI-TOF MS (Fig. 2a and b) and compared with the corresponding distributions from HPAEC-PAD analyses (Fig. 2c). Similarities between the profiles of the two methods were observed. However, MALDI-TOF MS seemed to overestimate chains of DP over 21 when comparisons were made on a relative weight basis. Unit chain fractions from debranched waxy maize were analysed to further investigate the relationship between the MALDI-TOF detector response and DP. The results showed that the variation in response for $DP > 16$ was large. Similar results were obtained by Naven and Harvey (1996) and suggested to be a feature of the technique that is related to the inhomogeneous nature of the target. Thus, it was not possible to make reliable corrections for the fact that the signal may be overestimated for long unit chains. Furthermore, small additional peaks were detected at *m/z* that were twice as high as those for the molecules in the unit fractions. This might be a result of possible aggregation of carbohydrates and partly explain the overestimation of long unit chains. It can also be speculated that the cation, in this case mainly Na^+, has a higher probability of coordinating itself to longer unit chains due to their larger amount of hydroxyl groups compared to shorter chains.

MALDI-TOF mass spectra were obtained for isolated debranched amylopectin from potato and wheat as well as from debranched whole starch from potato and wheat, i.e. amylose and amylopectin were not separated. No significant differences between the unit chain length profiles for isolated amylopectin and whole starch samples, respectively, were found. Hence, in contrast to HPEAC-PAD the MALDI-TOF MS technique can be used for chain length distribution analysis of amylopectin without prior separation of the amylose fraction. Further, MALDI-TOF MS of a debranched starch sample is done in a few minutes compared to HPAEC-PAD analysis that takes about 1.5 h after equilibration of the system. MALDI-TOF also has the advantage of requiring much less material than HPAEC-PAD. Further studies focusing on refining the crystalline matrix-analyte surface are planned in order to improve the reproducibility of MALDI-TOF MS.

MALDI-TOF MS has in this study been used for determination of amylopectin chain length profiles, but other oligo- and polysaccharides can probably be determined by the same technique after developing an appropriate methodology. The work on fructooligosaccharides

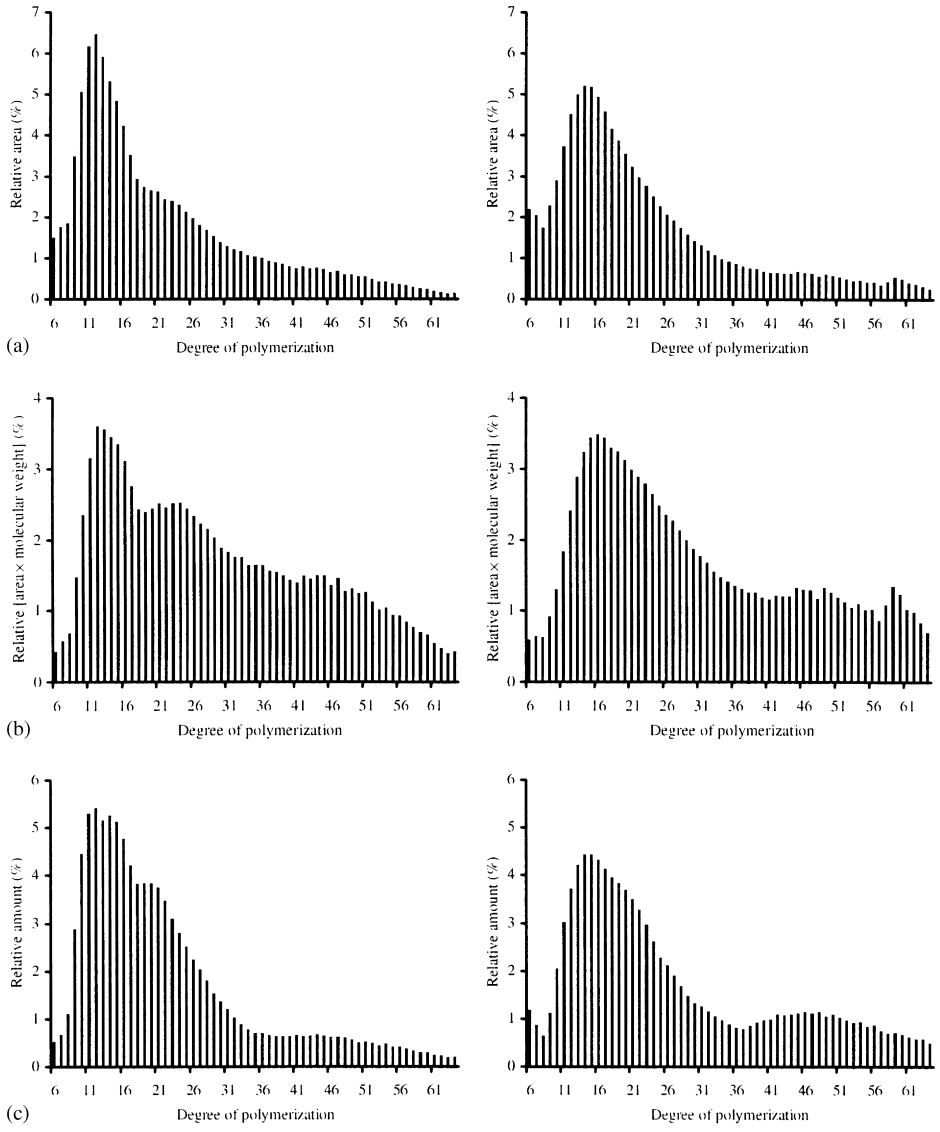

Fig. 2. Bar graphs showing the chain length distribution of wheat (left) and potato (right) amylopectin: (a) on a relative area basis obtained by MALDI-TOF MS; (b) on a relative weight basis obtained by MALDI-TOF MS. Each peak area was multiplied with the molecular weight of the corresponding chain; (c) on a relative amount basis obtained by HPAEC-PAD.

presented by Wang et al. (1999) has similarities, but the studied chain lengths were shorter (DP 3–19) and the HPAEC-PAD measurements used for comparison were not quantitative.

Acknowledgements

This work was supported by the Swedish Natural Science Research Council and the ALH-faculty at the University. The authors would like to thank Nils Nyberg for MATLAB-programming.

References

Eliasson, A.-C., & Gudmundsson, M. (1996). Starch: physicochemical and functional aspects. In A.-C. Eliasson, *Carbohydrates in food* (pp. 431–503). New York: Marcel Dekker.

Fredriksson, H., Andersson, R., Koch, K., & Åman, P. (1997). Calibration of a size-exclusion chromatography system using fractions with defined amylopectin unit chains. *Journal of Chromatography A*, *768*, 325–328.

Hanashiro, I., Abe, J.-I., & Hizukuri, S. (1996). A periodic distribution of the chain length of amylopectin as revealed by high-performance anion-exchange chromatography. *Carbohydrate Research*, *283*, 151–159.

Harvey, D. J. (1993). Quantitative aspects of the matrix-assisted laser desorption mass spectrometry of complex oligosaccharides. *Rapid Communications in Mass Spectrometry*, *7*, 614–619.

Hizukuri, S. (1996). Starch: analytical aspects. In A.-C. Eliasson, *Carbohydrates in Food* (pp. 347–429). New York: Marcel Dekker.

Karas, M., Bachmann, D., Bahr, U., & Hillenkamp, F. (1987). Matrix-assisted ultraviolet laser desorption of non-volatile compounds. *International Journal of Mass Spectrometry and Ion Processes*, *78*, 53–68.

Koch, K., Andersson, R., & Åman, P. (1998). Quantitative analysis of amylopectin unit chains by means of high-performance anion-exchange

chromatography with pulsed amperometric detection. *Journal of Chromatography A*, *800*, 199–206.

Lloyd, J. R., Hedley, C. L., Bull, V. J., & Ring, S. G. (1996). Determination of the effect of r and rb mutations on the structure of amylose and amylopectin in pea (*Pisum sativum* L.). *Carbohydr. Polym.*, *29*, 45–49.

Mock, K. K., Sutton, C. W., & Cottrell, J. S. (1992). Sample immobilization protocols for matrix-assisted laser-desorption mass spectrometry. *Rapid Communications in Mass Spectrometry*, *6*, 233–238.

Mohr, M. D., Bornsen, K. O., & Widmer, H. M. (1995). Matrix-assisted laser desorption/ionization mass spectrometry: improved matrix for oligosaccharides. *Rapid Communications in Mass Spectrometry*, *9*, 809–814.

Naven, T. J. P., & Harvey, D. J. (1996). Effect of structure on the signal strength of oligosaccharides in matrix-assisted laser desorption/ionization mass spectrometry on time-of-flight and magnetic sector instruments. *Rapid Communications in Mass Spectrometry*, *10*, 1361–1366.

Pieles, U., Zurcher, W., Schär, M., & Moser, H. E. (1993). Matrix-assisted laser desorption ionization time-of-flight mass spectrometry: a powerful tool for the mass and sequence analysis of natural and modified oligonucleotides. *Nucleic Acids Research*, *21*, 3191–3196.

Shewmaker, C. K., & Stalker, D. M. (1992). Modifying starch biosynthesis with transgenes in potatoes. *Plant Physiology*, *100*, 1083–1086.

Strupat, K., Karas, M., & Hillenkamp, F. (1991). 2,5-Dihydroxybenzoic acid: a new matrix for laser desorption-ionization mass spectrometry. *International Journal of Mass Spectrometry and Ion Processes*, *111*, 89–102.

Tang, K., Allman, S. L., Jones, R. B., & Chen, C. H. (1993). Quantitative analysis of biopolymers by matrix-assisted laser desorption. *Analytical Chemistry*, *65*, 2164–2166.

Vorm, O., Roepstorff, P., & Mann, M. (1994). Improved resolution and very high sensitivity in MALDI-TOF of matrix surfaces made by fast evaporation. *Analytical Chemistry*, *66*, 3281–3287.

Wang, J., Sporns, P., & Low, N. H. (1999). Analysis of food oligosaccharides using MALDI-MS: quantification of fructooligosaccharides. *Journal of Agricultural and Food Chemistry*, *47*, 1549–1557.

Wong, K.-S., & Jane, J. L. (1997). Effects of pushing agents on the separation and detection of debranched amylopectin by high-performance anion-exchange chromatography with pulsed amperometric detection. *Journal of Liquid Chromotography*, *18*, 63–80.