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Qualitative and quantitative analysis of branches in dextran using high-performance anion exchange chromatography coupled to quadrupole time-of-flight mass spectrometry

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ABSTRACT

Dextran, a family of natural polysaccharides, consists of an α (1→6) linked-glucose main (backbone) chain having a number of branches. The determination of the types and the quantities of branches in dextran is important in understanding its various biological roles. In this study, a hyphenated method using high-performance anion exchange chromatography (HPAEC) in parallel with pulsed amperometric detection (PAD) and mass spectrometry (MS) was applied to qualitative and quantitative analysis of dextran branches. A rotary cation-exchange cartridge array desalter was used for removal of salt from the HPAEC eluent making it MS compatible. MS and MS/MS were used to provide structural information on the enzymatically prepared dextran oligosaccharides. PAD provides quantitative data on the ratio of enzyme-resistant, branched dextran oligosaccharides. Both the types and degree of branching found in a variety of dextrans could be simultaneously determined online using this method.

1. Introduction

Dextran, a family of natural polysaccharides consisting of an α (1→6) linked-glucose main (backbone) chain and a number of branches, is often produced as extracellular polysaccharide by bacteria, such as *Streptococcus*, *Lactobacillus*, *Leuconostoc mesenteroides*, and *Weissella* [1–4]. Dextran is one of the most important polysaccharides used widely in industrial and medical applications [5–10]. The average molecular weight (Mw) of a dextran typically determines its applications [11,12]. In addition, the types and the quantity of its branches are important for dextran to play its different roles [13–16].

Gas chromatography coupled with mass spectrometry (GC-MS), and nuclear magnetic resonance (NMR) spectroscopy are two prominent strategies to investigate the types and number of branches in dextran [15–18]. However, multiple steps of chemical derivatization, such as methylation, hydrolysis, acetylation, etc., are generally required before GC-MS analysis [19–23]. Structural information is often lost during these chemical processes [24]. Furthermore, the GC-MS method provides only linkage information rather than an accurate determination of the composition of branches due to differences in the ionization efficiency of polysaccharide hydrolyzates in MS detection [25,26]. NMR is an effective method to determine the structure and branching degree of dextran [1,10,16,17,19]. However, NMR requires a large amount of sample, and the NMR measurements and interpretation can be very demanding and time consuming [27]. In our previous study, a new method relying on an ultra-high performance hydrophilic interaction chromatography coupled with quadrupole time-of-flight MS/MS (UP - HILIC - Q/TOF - MS/MS) was applied to analyze enzymatically digested dextran oligosaccharides, which has linkage information of branches [28]. In that work, optimized UP-HILIC afforded an effective separation of digested oligosaccharides based on both size and linkage. The subsequent application of MS/MS was used to sequence each oligosaccharide product, including those branching domains. The enzyme-resistant oligosaccharides with the linkages of 1–3 and 1–4 were recognized as the branching points of original dextran with this method. Unfortunately, the resolution of the isomers having the same dp but with different linkages still left large room for improvement, and a quantitative analysis of dextran branches was still remaining.

Anion exchange chromatography (HPAEC), also known as ion chromatography (IC), with pulsed amperometric detection (PAD) offers an effective platform to separate and quantitate

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carbohydrates with high selectivity, sensitivity and accuracy [29–31]. However, the high concentration of nonvolatile salt used in HPAEC-PAD prevents its direct application with MS, and PAD also only provides limited structural information. A desalter is required between HPAEC and MS to facilitate continuous online desalting, converting the highly salted HPAEC eluent into an MS compatible solvent. In 2005, an ion suppresser with the electrolysis-driven cation-exchange membrane had been used between HPAEC and MS for carbohydrate analysis [32,33]. However, in those studies, the analytes were generally all monosaccharides [32] and the chromatographic resolution and the quality of the MS data for oligosaccharides were insufficient to justify the use of HPAEC–MS [33].

In this paper, the HPAEC was coupled in parallel with PAD and electrospray ion (ESI) - Q/TOF-MS using a rotary cation-exchange cartridge array desalter. This online system, HPAEC coupled with PAD, MS and MS/MS, provides high resolution, quantitative data and structural information of digested dextran oligosaccharides. Based on the qualitative and quantitative analysis of dextran-digested products, the types and numbers of the branches in dextran were determined.

2. Experimental

2.1. Materials

Dextran T-10, Dextranase from *Penicillium sp.* and sodium acetate were purchased from Sigma–Aldrich (St. Louis, MO). Dextran A, B and C were kindly provided by Bright-Gene Pharmaceutical Corp. (Suzhou, China). Sodium hydroxide solution 50% was purchased from Merck (Darmstadt, Germany). High-purity water (resistivity $\geq$18.2 MΩ × cm, 25 °C) was used throughout the study. All other chemicals and reagents were of HPLC grade.

2.2. Dextran digestion

Dextran T-10 (5 mg) was dissolved in 0.5 mL water and incubated with 0.5 U dextranase at 37 °C. Aliquots were taken at 1, 3, 12 and 24 h. Another 0.5 U dextranase was added to the forth aliquot and it was incubated for an additional 12 h. The final digestion products of dextran A, B, and C (1 mg) were obtained by incubation with 0.1 U dextranase for 24 h, respectively. Boiling each aliquot for 20 min terminated the action of the enzyme. The denatured enzyme was removed by centrifugation (1,5000 rpm) for 10 min.

2.3. HPAEC

Samples were analyzed on a Metrohm 850 Professional system with a 919 IC auto-sampler plus, dual pumps and a PAD (Herisau, Switzerland). Each aliquot of 20 μL was injected on a CarboPac PA-10 column (4 × 250 mm, Dionex, Sunnyvale, CA) at a flow rate of 1 mL/min. The analysis was carried out at 30 °C with a multistep gradient, in which the mobile phase A was 100 mM NaOH, and mobile phase B was 1 M NaOAc in 100 mM NaOH. The isocratic mobile phase A (100 mM NaOH) was eluted for first 35 min, and a linear gradient from 0% to 12% of mobile phase B (1 M NaOAc in 100 mM NaOH) was eluted during the next 20 min. The PAD data was acquired and analyzed with software MagIC Net 2.4 (Herisau, Switzerland).

2.4. Desalting

The eluent after the column is split using a T-piece, in which 70% of the eluent went to PAD (0.7 mL/min), and 30% of the eluent (0.3 mL/min) went to the desalter, based on a suitable MS flow rate. The ratio of splitting was selected based on the backpressures from these two pathways, which were adjusted by selecting the diameters and lengths of tubes. An ion suppressor MSM-HC Rotor A (Metrohm, Switzerland) was used to remove sodium before MS analysis. There were three cation-exchange cartridge units installed in this column array. For continuous operation of the desalter, the rotor rotates every 20 min. While one cartridge was used for suppression, a regeneration step was performed on the second one with dilute sulfuric acid (~400 mmol/L) at a flow rate of 1 mL/min. The third cartridge was rinsed automatically with pure water during this time. Thus, a freshly regenerated suppressor cartridge was always available. The eluent from the desalter with the cation-removed went directly to the ESI-MS. A schematic diagram of system is shown in Fig. 1.

2.5. MS parameters

Nitrogen gas was used in the nebulizer at a pressure of 40 psi as drying gas. The spray voltage was 3.5 kV and a flow of nitrogen gas of 10 L/min at 350 °C assisted in the drying process. The fragment voltage was set to 20 V. A full MS scan between 100 and 2000 m/z was performed. The collision-induced dissociation (CID) energies used in MS/MS to dissociate disaccharide/trisaccharide, tetrasaccharide/pentasaccharide and hexasaccharide were set as 15–30 V according to the molecular mass. All data were acquired at negative ion mode with MassHunter 6.0 (Agilent Technologies).

3. Results and discussion

3.1. HAPEC-PAD analysis

The aliquots taken from digestion at different times were analyzed with the optimized HPAEC method (Fig. 2). The aliquot taken at 1 h showed more than 24 peaks in its chromatogram corresponding to dextran oligosaccharides of degree of polymerization (dp) 2 to dp25, which were confirmed with simultaneous MS analysis. Oligosaccharides with larger dp were observed at longer retention times (RT). These peaks showed a regular pattern of spacing implying they had the same structural properties and were primarily derived from the 1–6 linked main (backbone) chains, consistent with our previous analysis of this polysaccharide [28]. In the chromatogram of the aliquot taken at the 3 h time point, the peaks corresponding to the larger oligosaccharides, dp > 6, were of low intensity, the peaks corresponding to the smaller oligosaccharides, dp2, 3 and 4, dominated the chromatogram, and a peak corresponding to the monosaccharide glucose (dp1) was present. Moreover, additional peaks, not showing a regular pattern of spacing, were observed from 25 to 35 min (Fig. 2 insert). These could correspond to oligosaccharides with the linkages other than 1–6 and/or to branched structures. After a 12 h digestion, the peaks corresponding to dp1 and 2 dominate the chromatogram, most of the regularly spaced oligosaccharide peaks have disappeared, and some of the irregularly spaced peaks, corresponding to oligosaccharides with other linkages and/or branches, are present at higher intensity. After 24 h digestion, the intensities of some of the irregularly spaced peaks also decreased, *i.e.*, peaks between 30 and 33 min, and some increased, *i.e.*, peaks between 28 and 30 min. The PAD profile of the 36 h aliquot was identical to that obtained on 24 h aliquot (data not shown). These results suggest that all of the digestible domains within dextran are broken down within 24 h, and some oligosaccharides are resistant to enzymatic digestion and accumulate in the final digestion product. The major enzyme-resistant oligosaccharides are labeled as a through h in Fig. 2 insert. The small peak observed at 24.9 min in Fig. 2 is the remaining 1–6 linked final tetrasaccharide (dp4) product. The broad peak present

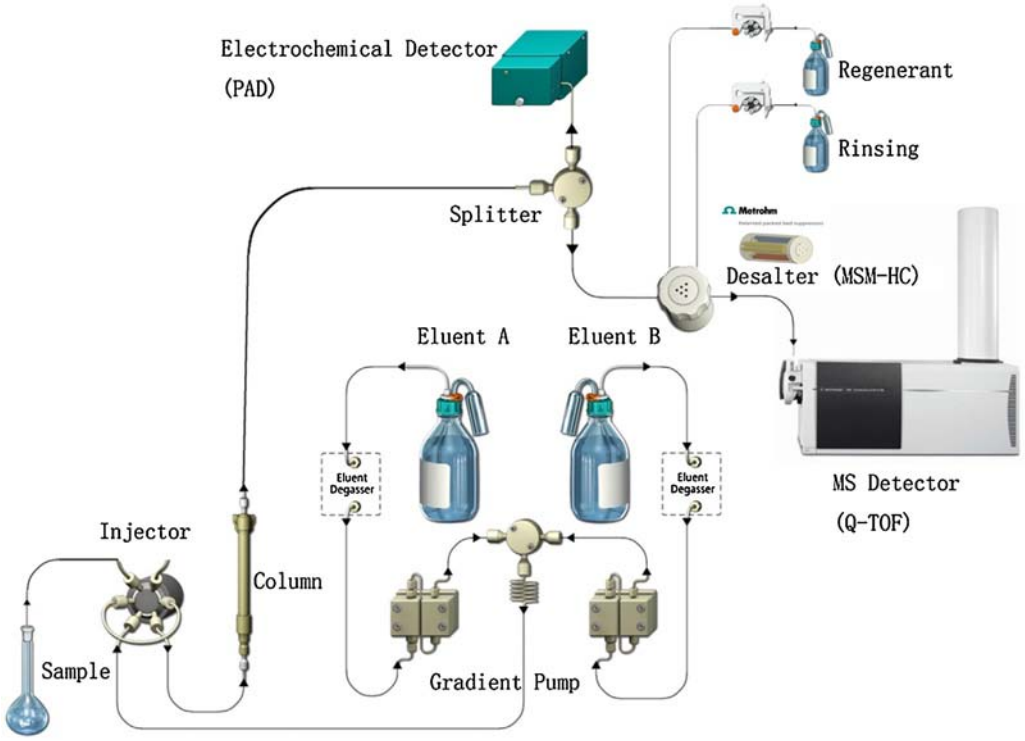
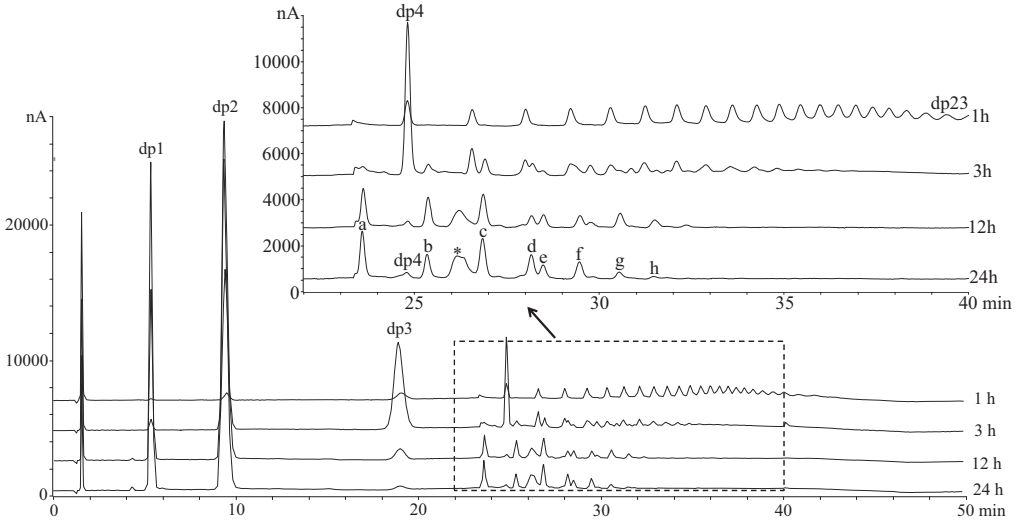


Fig. 1. Schematic of HPAEC-PAD-MS system.

at 26.0–26.5 min in the final product mixture is a non-carbohydrate impurity. No specific oligosaccharide signal was observed in the following MS analysis of this peak and the intensity of MS signal is low in negative mode. It was possibly peptide degradents coming from enzyme. The MS spectrum of this peak was shown in supplementary data (Figure S1). Overall, the resolution of enzyme digested dextran oligosaccharides obtained using HPAEC was much higher than that obtained with previous HILIC method [28].

3.2. Online MS and MS/MS analysis

The conditions used in ESI require optimization to accurately analyze oligosaccharides because of the presence of residual acetic acid in the aqueous eluent after cation-exchange desalter. The fragment voltage, the most important factor for ionizing these sugars properly, was optimized to 20 V. Details of the optimized conditions are presented in the Supplementary data (Figure S2).



dp, degree of polymerization
a-h, peaks of enzyme-resistant oligosaccharides
asterisk, non-carbohydrate peak

Fig. 2. Overlaid chromatograms of enzyme digested dextran oligosaccharides taken from 1, 3, 12 and 24 h aliquots detected with PAD. dp, degree of polymerization. a–h, peaks of enzyme-resistant oligosaccharides. asterisk, non-carbohydrate peak.

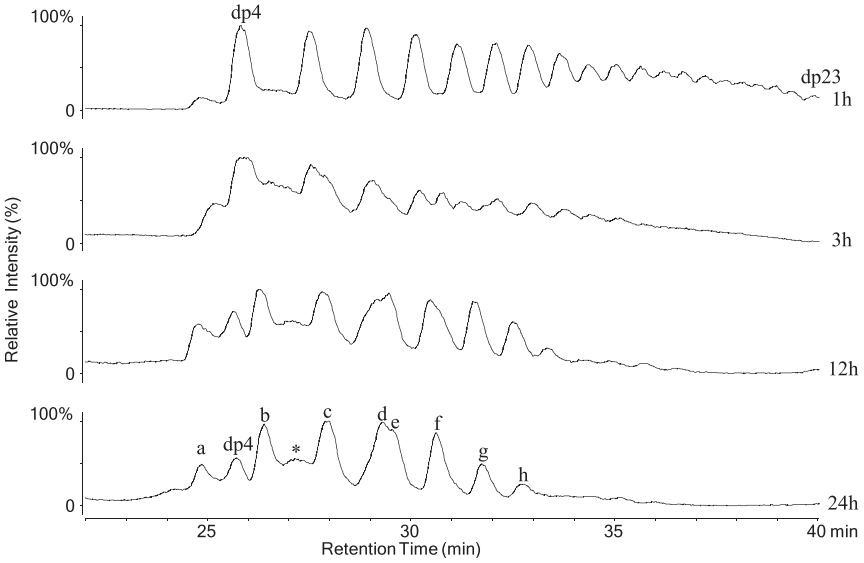


Fig. 3. Overlaid TICs of enzyme digested dextran oligosaccharides taken from 1, 3, 12 and 24 h aliquots detected with MS. dp, degree of polymerization. a–h, peaks of enzyme-resistant oligosaccharides. asterisk, non-carbohydrate peak.

The aliquots, taken at different digestion time points, were analyzed by MS and MS/MS in parallel with PAD. The total ion chromatograms (TIC) of these aliquots are shown in Fig. 3. Because of the existing of the dead volume (<250 μL) in desalter, the peaks in the TICs appeared to be broader than those in the PAD chromatograms. Oligosaccharides of up to dp23 were resolved in the TIC of 1 h time point. The mass spectra of dp2 to dp23 are shown in Fig. 4. Singly charged molecular ions ($[M-H]^-$) of dp2–dp10 were observed as major signals in each spectrum at m/z 341, 503, 665, 827, 989, 1151, 1313, 1475 and 1637. Doubly charged molecular ions $[M-2H]^{2-}$ dominated the spectra of dp11–dp23 and were observed at m/z 899, 980, 1061, 1142, 1223, 1304, 1385, 1466, 1547, 1628, 1628, 1709, 1790 and 1871, respectively. In addition, adducts molecular ions containing two water molecules, $[M+2H_2O-H]^-$ or $[M+2H_2O-2H]^{2-}$, were always present along with $[M-H]^-$ or $[M-2H]^{2-}$ as a result of the aqueous media and low fragment voltage that was used. The TIC of each sample was similar to its PAD profile. Some oligosaccharides were present in the first aliquot, new oligosaccharides appeared after 3 h digestion; and several oligosaccharides were resistant to enzyme digestion and accumulated in the final product. The dp of enzyme resistant dextran oligosaccharides were confirmed with MS analysis, in which peaks a and c both corresponded to tetrasaccharides (dp4), peaks b, d and e corresponded to pentasaccharides (dp5), and peaks f, g and h are corresponded to hexasaccharide (dp6), heptasaccharide (dp7) and octasaccharide (dp8), respectively. The resolution obtained with HPAEC-MS was considerably higher than observed using our previously developed HILIC method [28]. Oligosaccharides having the same dp but with different linkages were separated. Some of these could even be resolved with baseline separation. Compared to results obtained using HILIC [28], one additional pentasaccharide could be detected and identified in the enzymatic digestion final product mixture using HPAEC. The sequence analysis by MS/MS on these well-separated oligosaccharides was next undertaken.

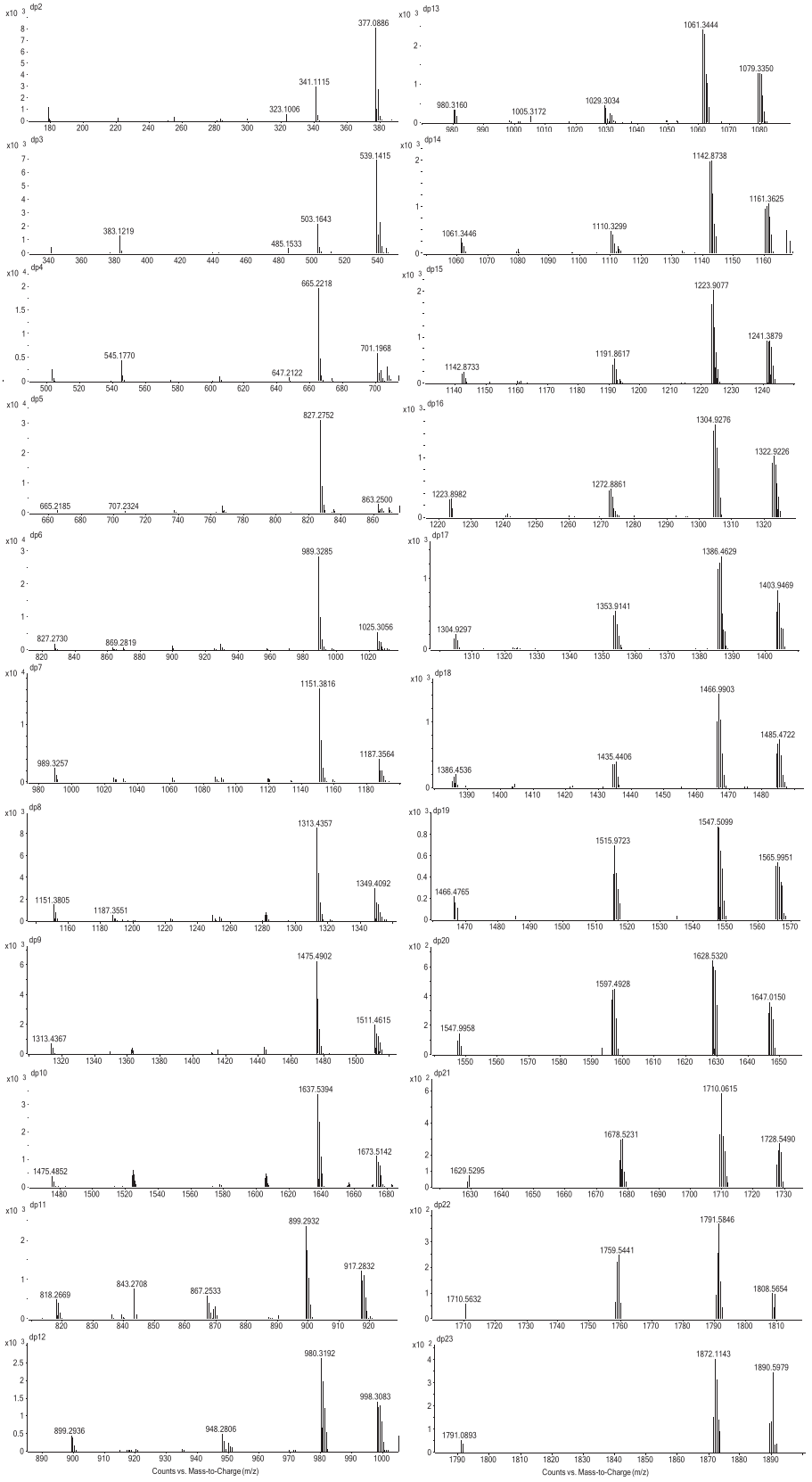
The nomenclature for describing the MS/MS fragmentation of dextran oligosaccharides has been previously described [28]. The presence of a series of cross ring cleavage ions, $^{0,2}A$, $^{0,3}A$ and $^{0,4}A$, is consistent with a 1–6 linkage, the presence of cross ring cleavage ion, $^{2,5}A$, indicates a 1–4 linkage, and the absence of cross-ring cleavage ions suggests a 1–3 linkage. The spectra are presented in supplementary data (Figure S3). The sequences of most of the oligosaccharides present in the enzymatic digestion final product are provided in Table 1. These included a new pentasaccharide with a 1–3 linkage in the center of the oligosaccharide. The MS/MS peaks for dp7 and 8 were of insufficient intensity to be assigned.

Table 1
The enzyme-resistant structures detected with HPAEC-MS/MS.

Peaks	Structures
a	Glc 1–4 Glc 1–6 Glc 1–6 Glc
b	Glc 1–6 (Glc1–4) Glc 1–6 Glc 1–6 Glc
c	Glc 1–3 Glc 1–6 Glc 1–6 Glc
d	Glc 1–6 Glc 1–3 Glc 1–6 Glc 1–6 Glc
e	Glc 1–3 Glc 1–6 Glc 1–6 Glc 1–6 Glc
f	Glc 1–6 Glc 1–3 Glc 1–6 Glc 1–6 Glc 1–6 Glc

3.3. Branch degree quantitation

PAD analysis using a gold electrode is a well-established method for analyzing carbohydrates. The current produced from an oxidation reaction at the reducing end of carbohydrate makes its detection highly sensitive and quantitative over a wide range of concentrations [29–31]. The molar composition of oligosaccharides can be calculated from the ratio of the peak integrations obtained with PAD. The peaks in the chromatogram of the enzymatically digested final products were unambiguously identified as enzyme-resistant oligosaccharide domains having 1–3 or 1–4 linkages by MS and MS/MS analysis. The degree of 1–3 and 1–4 branching of dextran was calculated using the equations: $(^{1-3}I_4+\ldots+^{1-3}I_n)/(I_1\times1+\ldots+I_n\times n)$ and $(^{1-4}I_4+\ldots+^{1-4}I_n)/(I_1\times1+\ldots+I_n\times n)$, respectively, in which I represents the PAD integration of each peak, the labels at the left superscript represent the linkage patterns, and the numbers at



dp, drgree of polymerization

Fig. 4. MS spectra of dextran oligosaccharides from dp2 to dp23. dp, degree of polymerization.

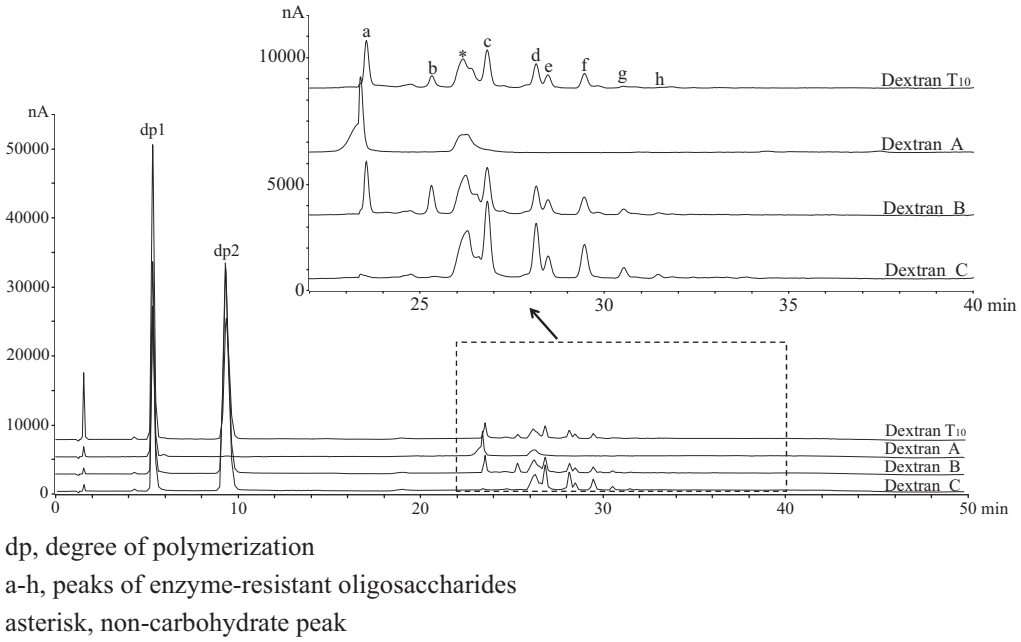


Fig. 5. Overlaid chromatograms of enzymatic digestion final products from four dextrans with PAD. dp, degree of polymerization. a–h, peaks of enzyme-resistant oligosaccharides. asterisk, non-carbohydrate peak.

Table 2
Degree of branching of different dextran samples.

Samples	Degree of branching (%)		
	1–3	1–4	Total
Dextran T_{10}	3.21%	1.61%	4.82%
Dextran A	n.d.	9.80%	9.80%
Dextran B	3.00%	1.74%	4.74%
Dextran C	4.74%	0.10%	4.85%

n.d., not detected.

right subscript correspond to the dp of the oligosaccharide. Since only one reducing end is present in each oligosaccharide and it is detected and quantitated by PAD, the sum of $I_n \times n$ in denominator corresponds to the total number of sugar residues in dextran. As only one specific linkage pattern other than 1–6 was observed in each oligosaccharide present in the final product mixture, the number of each oligosaccharide equals to the corresponding number of each specific linkage. Thus, the sum of $^{1-3}I_n$ or $^{1-4}I_n$ in numerator corresponds to the number of 1–3 and 1–4 linkages in the dextran chain, respectively.

The degree of 1–3 and 1–4 branching in dextran T-10 was calculated using the equations presented above. An additional three commercial dextrans were similarly digested, and their final products were then similarly analyzed using HPAEC-PAD-MS/MS method. The sequence of each oligosaccharide, present in the final product mixture, was confirmed and the degree of branching was analyzed. The overlaid PAD chromatograms of those four dextrans are shown in Fig. 5. The degree of 1–3, 1–4 branching, and the total branching were calculated and are presented in Table 2. The total degree of branching in dextran T10, and dextrans A and B are similar (~5%), but the degree of 1–4 branching in dextran A was only 0.1%, much lower than that detected in dextran T10 and dextran B, and correspondingly, the degree of 1–3 branching in dextran A was significantly higher than that detected in dextran T10 and dextran B. The dextran C seems totally different from all of the other dextrans studied. The 1–4 linkage dominated all branching at 10% and no 1–3 linkages were detected.

4. Conclusions

In this work a method with HPAEC-PAD-MS/MS was developed for the qualitative and quantitative analysis of dextran branching. In this method, the oligosaccharides were efficiently separated with an HPAEC column, and analytes were detected, in parallel, by both PAD and MS. The cation-exchange desalter removes the nonvolatile salts after separation affording an MS compatible eluent. MS and MS/MS provide structural information on the oligosaccharides and PAD provides quantitative data. Four dextrans from different sources were analyzed using this method. Their different branching patterns were elucidated. In conclusion, this method provides comprehensive approach for the analysis of dextran branching patterns and should be useful in the quality control new applications of dextran.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chroma.2015.10.064.

References

[1] N.H. Maina, M. Tenkanen, H. Maaheimo, R. Juvonen, L. Virkki, NMR spectroscopic analysis of exopolysaccharides produced by Leuconostoc citreum and Weissella confuse, Carbohyd. Res. 343 (2008) 1446–1455.
[2] M. Naessens, A. Cerdobbel, W. Soetaert, E.J. Vandamme, Leuconostoc dextransucrase and dextran: production, properties and applications, J, Chem. Technol. Biotechnol. 80 (2005) 845–860.

[3] N.H. Main, L. Virkki, H. Pynnönen, H. Maaheimo, M. Tenkanen, Structural analysis of enzyme-resistant isomaltooligosaccharides reveals the elongation of α-(1, 3)-linked branches in Weissella confuse dextran, Biomacromolecules 12 (2011) 409–418.
[4] J.F. Robyt, S.Y. Lee, J.H. Lee, Y.M. Kim, Dextran molecular size and degree of branching as a function of sucrose concentration, pH, and temperature of reaction of Leuconostoc mesenteroides B-512 FMCM dextransucrase, Carbohyd. Res. 338 (2003) 1183–1189.
[5] M. Naessens, A. Cerdobbel, W. Soetaert, E.J. Vandamme, Leuconostoc dextransucrase and dextran: production, properties and applications, J. Chem. Technol. Biotechnol. 80 (2005) 845–860.
[6] S. Ahmad, R.F. Tester, A. Corbett, J. Karkalas, Dextran and 5-aminosalicylic acid (5-ASA) conjugates: synthesis, characterisation and enzymic hydrolysis, Carbohyd. Res. 341 (2006) 2694–2701.
[7] S. Patel, N. Kasoju, U. Bora, A. Goyal, Structural analysis and biomedical applications of dextran produced by a new isolate Pediococcus pentosaceus screened from biodiversity hot spot Assam, Bioresource. Tech. 101 (2010) 6852–6855.
[8] T.R. Shamala, M.S. Prasad, Preliminary studies on the production of high and low viscosity dextran by Leuconostoc spp, Process. Biochem. 30 (1995) 237–241.
[9] E.J. Bourne, D.H. Hutson, H. Weigel, Studies on dextrans and dextranases, Biochem. J. 85 (1962) 158–163.
[10] M. Bashari, C. Lagnika, D. Ocen, H. Chen, J. Wang, X. Xu, Z. Jin, Separation and characterization of dextran extracted from deteriorated sugarcane, Int. J. Biol. Macromol. 59 (2013) 246–254.
[11] G. Arturson, G. Wallenius, the intravascular persistence of dextran of different molecular sizes in normal humans, Scand. J. Clin. Lab. Inv. 1 (1964) 76–80.
[12] A. Aman, N.N. Siddiqui, S.A.U. Qader, Characterization and potential applications of high molecular weight dextran produced by Leuconostoc mesenteroides AA1, Carbohyd. Polym. 87 (2012) 910–915.
[13] T. Heinze, T. Liebert, B. Heublein, Stephanie Hornig, Functional polymers based on dextran, Adv. Polym. Sci. 205 (2006) 199–291.
[14] N.N. Siddiqui, A. Aman, A. Silipo, S.A.U. Qader, A. Molinaro, Structural analysis and characterization of dextran produced by wild and mutant strains of Leuconostoc mesenteroides, Carbohyd. Polym. 99 (2014) 331–338.
[15] M.H.P.B. Vettori, S.M.M. Franchetti, J. Contiero, Structural characterization of a new dextran with a low degree of branching produced by Leuconostoc mesenteroides FT045B dextransucrase, Carbohyd. Polym. 88 (2012) 1440–1444.
[16] I. Benzeval, A. Bowyer, J. Hubble, The influence of degree-of-branching and molecular mass on the interaction between dextran and Concanavalin A in hydrogel preparations intended for insulin release, Eur. J. Pharm. Biopharm. 80 (2012) 143–148.
[17] M.U. Roslund, P. Tähtinen, M. Niemitzc, R. Sjöholm, Complete assignments of the 1H and 13C chemical shifts and JH,H coupling constants in NMR spectra of D-glucopyranose and all D-glucopyranosyl-D-glucopyranosides, Carbohyd. Res. 343 (2008) 101–112.
[18] R.K. Purama, P. Goswami, A.T. Khan, A. Goyal, Structural analysis and properties of dextran produced by Leuconostoc mesenteroides NRRL B-640, Carbohyd. Polym. 76 (2009) 30–35.
[19] S.S. Leeuwen, B.R. Leeflang, G.J. Gerwig, J.P. Kamerling, Development of a 1H NMR structural-reporter-group concept for the primary structural characterisation of α-D-glucans, Carbohyd. Res. 343 (2008) 1114–1119.
[20] I. Ciucanu, Per-O-methylation reaction for structural analysis of carbohydrates by mass spectrometry, Anal. Chim. Acta 576 (2006) 147–155.
[21] H. Bjorndal, C.G. Hellerqvist, B. Lindberg, S. Svensson, Gas-liquid chromatography and mass spectrometry in methylation analysis of polysaccharides, Angew. Chem. Int. Edit. 9 (1970) 610–619.
[22] I. Ciucanu, R. Caprita, Per-O-methylation of neutral carbohydrates directly from aqueous samples for gas chromatography and mass spectrometry analysis, Anal. Chim. Acta 585 (2007) 81–85.
[23] A.I. Ruiz-Matutea, O. Hernández-Hernández, S. Rodríguez-Sánchez, M.L. Sanz, I. Martínez-Castro, Derivatization of carbohydrates for GC and GC–MS analyses, J. Chromatogr. B 879 (2011) 1226–1240.
[24] I. Ciucanu, C.E. Costello, Elimination of oxidative degradation during the per-O-methylation of carbohydrates, J. Am. Chem. Soc. 125 (2003) 16213–16219.
[25] N. Viseux, C.E. Costello, B. Domon, Post-source decay mass spectrometry: optimized calibration procedure and structural characterization of permethylated oligosaccharides, J. Mass. Spectrom. 34 (1999) 364–376.
[26] P. Kang, Y. Mechref, I. Klouckova, M.V. Novotny, Solid-phase permethylation of glycans for mass spectrometric analysis, Rapid Commun. Mass. Sp. 19 (2005) 3421–3428.
[27] J.Ø. Duus, C.H. Gotfredsen, K. Bock, Carbohydrate structural determination by NMR spectroscopy: modern methods and limitations, Chem. Rev. 100 (2000) 4589–4614.
[28] L. Yi, X. Sun, K. Du, Y. Ouyang, C. Wu, N. Xu, R.J. Linhardt, Z. Zhang, UP-HILIC-MS/MS to determine the action pattern of Penicillium sp. Dextranase, J. Am. Soc. Mass. Spectr. 26 (2015) 1174–1185.
[29] Z. Zhang, N. Khan, K. Nunez, E. Chess, C. Szabo, Complete monosaccharide analysis by high performance anion exchange chromatography with pulsed amperometric detection, Anal. Chem. 84 (2012) 4104–4110.
[30] T.R.I. Cataldi, C. Campa, G.E.D. Benedetto, Carbohydrate analysis by high-performance anion-exchange chromatography with pulsed amperometric detection: the potential is still growing, Fresen, J. Anal. Chem. 368 (2000) 739–758.
[31] J.R. Thayer, J.S. Rohrer, N. Avdalovic, R.P. Gearing, Improvements to in-line desalting of oligosaccharides separated by high-pH anion exchange chromatography with pulsed amperometric detection, Anal. Biochem. 256 (1998) 207–216.
[32] C. Guignard, L. Jouve, M.B.B. Triboulot, E. Dreyer, J. Franc, O. Hausman, L. Hoffmann, Analysis of carbohydrates in plants by high-performance anion-exchange chromatography coupled with electrospray mass spectrometry, J. Chromatogr. A 1085 (2005) 137–142.
[33] C. Bruggink, R. Maurer, H. Herrmann, S. Cavalli, F. Hoefle, Analysis of carbohydrates by anion exchange chromatography and mass spectrometry, J. Chromatogr. A 1085 (2005) 104–109.