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RESEARCH ARTICLE

Derivatization of Dextran for Multiply Charged Ion Formation and Electrospray Ionization Time-of-Flight Mass Spectrometric Analysis

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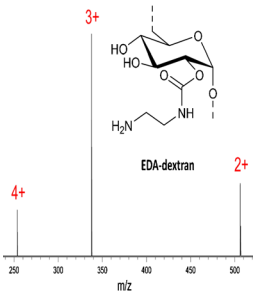

Abstract. We present the use of a simple, one-pot derivatization to allow the polysaccharide dextran to carry multiple positive charges, shifting its molecular weight distribution to a lower *m/z* range. We performed this derivatization because molecular weight measurements of polysaccharides by mass spectrometry are challenging because of their lack of readily ionizable groups. The absence of ionizable groups limits proton abstraction and suppresses proton adduction during the ionization process, producing mass spectra with predominantly singly charged metal adduct ions, thereby limiting the detection of large polysaccharides. To address this challenge, we derivatized dextran T1 (approximately 1 kDa) by attaching ethylenediamine, giving dextran readily ionizable, terminal amine functional groups. The attached ethylenediamine groups facilitated proton adduction during the ionization process in positive ion mode. Using the low molecular weight dextran T1, we tracked the number of ethylenediamine attachments by measuring the mass shift from underivatized to derivatized dextran T1. Using electrospray ionization time-of-flight mass spectrometry, we observed derivatized dextran chains ranging from two to nine glucose residues with between one and four attachments/charges. Our success in shifting derivatized dextran T1 toward the low *m/z* range suggests potential for this derivatization as a viable route for analysis of high molecular weight polysaccharides using electrospray ionization time-of-flight mass spectrometry.

Keywords: Polysaccharide, Dextran, Electrospray ionization, Time-of-flight, Derivatization, Ethylenediamine

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Introduction

The molecular weight analysis of polysaccharides by mass spectrometry (MS) continues to be a challenging task. Compared with the rate of advances in method development for protein analysis, progress in the use of MS for polysaccharide analysis continues to evolve relatively slowly [1]. The most widely used ionization methods for large biomolecules, including polysaccharides, are matrix-assisted laser desorption ionization (MALDI) and electrospray ionization (ESI) coupled with use of time-of-flight (TOF) mass analyzers [2, 3]. These ionization techniques are commonly used for the analysis of large proteins [2] and nucleic acids [4], but both methods have had little success when applied to polysaccharides [5]. The limited success in MS analysis of polysaccharides can be primarily attributed to ionization limitations. Ionization efficiency is low for negative mode ESI because polysaccharides lack the acidic groups typically needed for efficient proton abstraction. Also, for positive mode, ionization efficiency is weak with ESI because polysaccharides have low proton affinity, leading to the more commonly observed metal cation adduct formation [6–8].

Even though both MALDI and ESI have been used for ionization of polysaccharides, MALDI has been the preferred method [1, 9–11]. Unfortunately, MALDI is susceptible to lack of reproducibility because of limited control during the crystallization process, which leads to the formation of "sweet spots" (inhomogeneous distribution of analyte in the matrix, leading to localized high analyte signal intensities), as well as higher likelihood of in-source fragmentation [1, 12, 13]. Although ESI is typically considered poorly suited for analysis of polysaccharides because it is

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less effective for ionization of neutral molecules, and sensitivity decreases as the weight of the molecule increases, it does not suffer from the same drawbacks as MALDI [1, 7, 8, 14].

To increase ionization efficiency, derivatization processes that may allow polysaccharides to carry multiple charges can be used. This would allow MS measurements of multiply charged polysaccharides. Although multiple charges from ESI can be detrimental when complex mixtures are analyzed, this feature could allow high molecular weight polysaccharides to be observed in a low *m/z* region [13]. To date however, there is limited literature regarding the use of derivatization techniques to enhance ionization of polysaccharides with ESI.

We investigated the use of a derivatization technique for dextran because it is a polysaccharide that is difficult to ionize because of the lack of acidic groups for proton abstraction and its low proton affinity for proton adduction. Dextran is a homopolysaccharide of glucose that exhibits primarily α-1,6-glycosidic linkages. This polysaccharide is synthesized by *Leuconostoc*, *Lactobacillus,* and *Streptococcus* bacteria by the enzyme dextransucrase, which catalyzes the synthesis from sucrose giving dextran and fructose as the products [15–17]. Dextran is one of many polysaccharides derived from lactic acid bacteria that has provided a small number of novel products with distinctive properties for food and nonfood use, as well as materials with specific biological properties for the pharmaceutical industry and medical field [15, 18–21].

To encourage ionization of dextran by means of multiple proton adducts for ESI-TOF MS analysis, we used a one-pot derivatization procedure with ethylenediamine that has been previously used as the first step to produce water-soluble dextran–Taxol conjugates [22]. We selected dextran T1 (approximately 1 kDa) because its molecular weight allowed us to track the mass changes and determine the number of ethylenediamine attachments from underivatized to derivatized dextran T1 (EDA-dex-T1). With the attached groups, we took advantage of multiply charged ions typical of ESI to observe a shift of the molecular weight distribution toward the low *m/z* region in the form of ions with multiple charges, depending on the number of successful attachments. To the best of our knowledge, this is the first report of successful use of multiple charges produced via ESI to analyze the polysaccharide dextran in the form of multiple proton adducts.

Experimental

Chemicals

1,1′-Carbonyldiimidazole (CDI) was obtained from Sigma (St Louis, MO, USA). Dextran T1 (average $M_r \sim 1100$) was obtained from Pharmacosmos (Holbaek, Denmark). Spectra/Por dialysis membranes with a molecular weight cutoff of 100–500 Da were obtained from Spectrum Labs (Rancho Domingues, CA, USA). Ethylenediamine (Alfa Aesar), dimethyl sulfoxide (DMSO), liquid chromatography–MS grade methanol, liquid chromatography–MS grade water, herein referred to as methanol and water, were obtained from VWR (Denver, CO, USA). All chemicals were used as received without further purification.

Derivatization Procedure

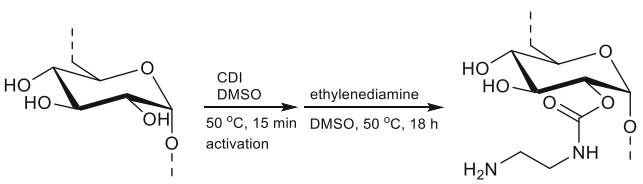
The derivatization of dextran was performed on a half-gram scale, following an established procedure [22] (Scheme 1) in which 0.5 g dextran was dissolved in 6 mL DMSO, to which a solution of 0.2 g 1,1′-carbonyldiimidazole dissolved in 2 mL DMSO was added, and the reaction was allowed to proceed for 15 min at 60 °C to activate the dextran. Following the activation step, 1 mL ethylenediamine was added and the resulting mixture was allowed to react for 18 h at 60 °C. After the reaction reached completion, the mixture was allowed to reach room temperature, and the EDA-dex-T1 was precipitated out of the DMSO with use of excess methanol. The precipitate was then filtered by vacuum filtration and washed with methanol to remove any DMSO still present and impurities soluble in methanol. The solid was then redissolved in Millipore water and dialyzed for 24 h in 0.5% w/v NaCl solution and then for an additional 24 h in 0.01% v/v acetic acid solution. The dialyzed product was then lyophilized for 4 days to recover the purified solid. The recovered solid was stored at -20 °C until analysis.

General Characterization Techniques

NMR spectra were obtained with a Varian Inova 400-MHz Fourier transform NMR spectrometer (Agilent Technologies Inc., Santa Clara, CA, USA). ^{1}H NMR spectra were referenced relative to the solvent (DMSO) and tetramethylsilane. Attenuated total reflectance (ATR) Fourier transform infrared (FT-IR) spectra were collected between 650 and 4000 cm^{-1} with a Nicolet 6700 FT-IR spectrometer (Thermo Electron Corp., Madison, WI, USA) fitted with a Smart iTR ATR sampling accessory and a ZnSe crystal plate.

Mass Spectrometry

ESI-TOF MS All experiments were performed with a 6224 TOF mass spectrometer (Agilent, Palo Alto, CA, USA) equipped with a dual electrospray ion source operated in positive mode. EDA-dex-T1 solutions were prepared at a concentration of 1 mg/mL in 0.1% acetic acid solution. The solutions were directly injected into the ion source (without a preseparation step) at a flow rate of 0.15 mL/min with use of the autosampler on an 1260 Infinity high-performance liquid chromatograph (Agilent, Palo Alto, CA, USA) with an isocratic mobile phase consisting of 90% water and 10%



Scheme 1. Derivatization of dextran. This one-pot derivatization produced ethylenediamine-derivatized dextran with the purpose of giving dextran free terminal amine groups. *CDI* 1,1′-carbonyldiimidazole, *DMSO* dimethyl sulfoxide

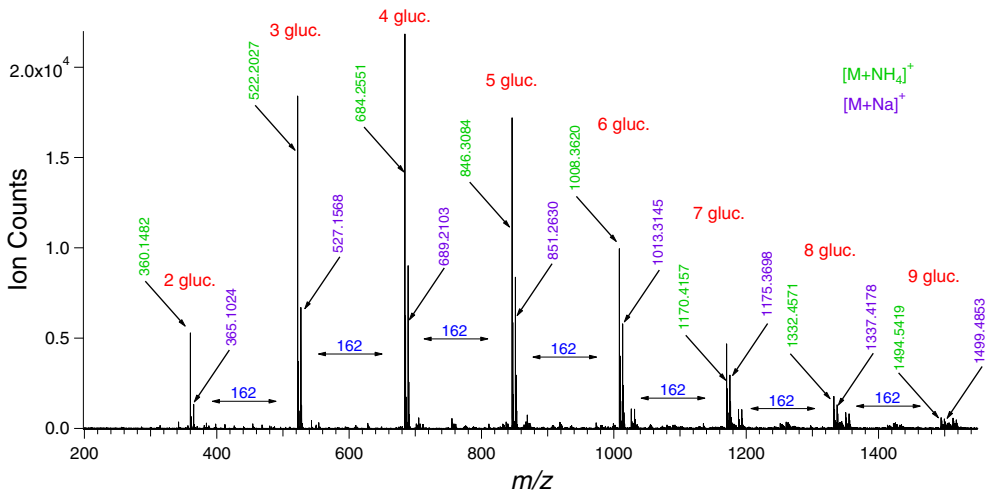


Figure 1. Electrospray ionization (ESI) mass spectrum of dextran T1. The observed mass distribution corresponds to two to nine repeating glucose residue chains with the *m/z* range 360 to 1494 as $[M + NH_4]^+$ ions and the *m/z* range from 365 to 1499 as $[M + Na]^+$ ions. *gluc.* glucose

methanol, both with 0.1% acetic acid. The ion source conditions were as follows: 4.0-kV capillary voltage, 80-V fragmentor voltage, 75-V skimmer voltage, 650-V octopole voltage, 12.5 L/min gas flow (N_2), and 45-psi nebulizer pressure. The detection range was set at 100–3200 *m/z*.

ESI-MS/MS All MS/MS experiments were performed with a Thermo Finnigan linear trap quadrupole (LTQ) equipped with an ESI probe operated in positive mode. Underivatized dextran T1 solutions were prepared at a concentration of 1 mg/mL in a methanol–water (40:60, v/v), and the EDA-dex-T1 solutions were prepared at a concentration of 1 mg/mL in 0.1% acetic acid solution. The solutions were directly injected into the ion source by syringe infusion at a flow rate of 15 µL/min. The following instrument conditions were automatically optimized: electrospray voltage, heated capillary temperature (voltage), tube lens voltage, capillary voltage, sheath gas flow rate, auxiliary gas flow rate and sweep gas flow rate (N_2). The conditions were automatically adjusted with use of the automatic tune function optimizing on ion *m/z* 689 for dextan T1 (*m/z* of 689 corresponds to a four-glucose monomer dextran with a sodium adduct) and on ion *m/z* 753 for EDA-dex-T1 (*m/z* 753 corresponds to a four-glucose monomer dextran with one ethylenediamine attachment and a proton adduct). For the fragmentation studies, the collision-induced dissociation (CID) energy was set as 30 V.

Data Analysis

Data were reported from a minimum of triplicate measurements, unless otherwise noted, and all data were analyzed with Agilent MassHunter Qualitative Analysis B.07.00 (ESI-TOF data) and Thermo Xcalibur Qual Browser 2.2 SP1.48 (ESI-MS/MS data).

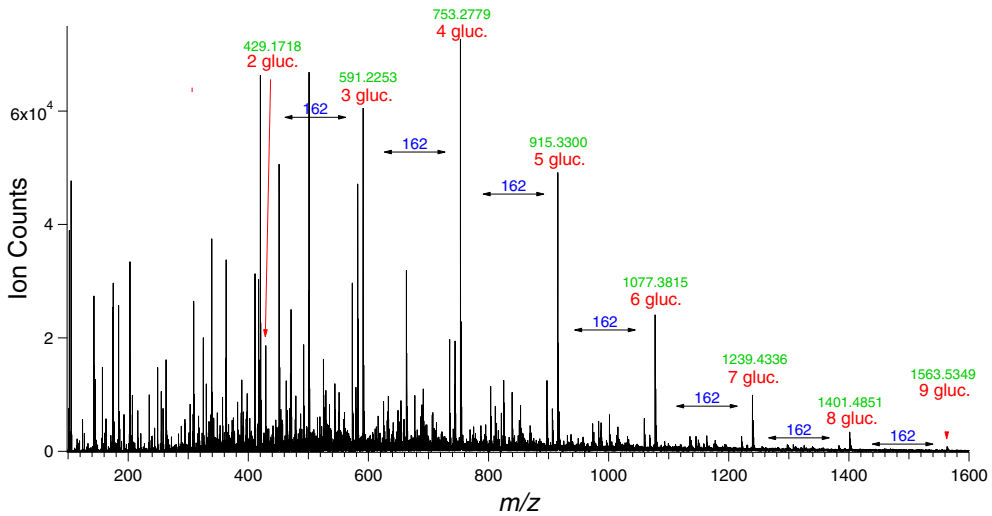


Figure 2. ESI mass spectrum for ethylenediamine-derivatized dextran T1 (EDA-dex-T1). The two to nine glucose residue distribution is shown with an *m/z* range from 429 to 1563 with a mass offset of 86 Da from the dextran T1 distribution. This 86-Da mass increase indicates attachment of a single ethylenediamine to each dextran chain. *gluc.* glucose

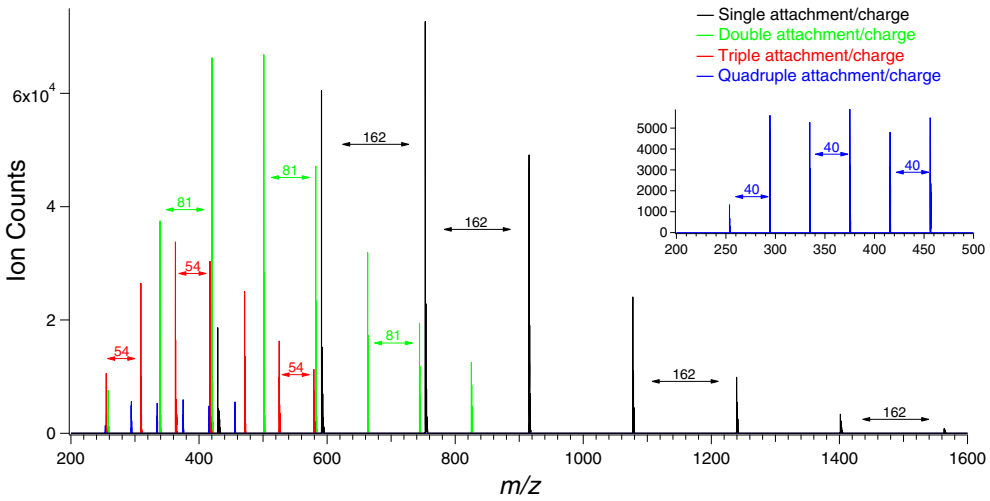


Figure 3. Extracted mass distributions showing single attachment/charge to quadruple attachments/charges. For single attachment/charge, we saw the 162 Da mass increments corresponding to a single repeating glucose residue. We observed similar distribution for double attachments/charges, triple attachments/charges, and quadruple attachments/charges with mass increments of 81, 54, and 40 Da respectively, each corresponding to the mass of a single glucose residue (162 Da) divided by the corresponding number of charges

Results and Discussion

Characterization of EDA-dex-T1

Derivatization of dextran T1 with ethylenediamine resulted in the attachment of $-(C{=}O)NH(CH_2)_2NH_2$ moieties through the formation of carbamate linkages to the polysaccharide as depicted in Scheme 1. We found the ^1H NMR spectrum of EDA-dex-T1 (Figure S1c) to be largely consistent with that of dextran T1 (Figure S1b) with broadened features appearing between 3.71 and 2.88 ppm corresponding to the C_2–C_6 protons. The major feature change between dextran T1 and EDA-dex-T1 was the appearance of two proton shifts at 3.01 and 1.87 ppm, corresponding to the methylene groups from $-(C{=}O)NH(CH_2)_2NH_2$ attachments. Compared with the spectrum for ethylenediamine (Figure S1a), we expected this peak to split into two different peaks and shift downfield because of the carbamate linkage formation. We also performed infrared spectroscopy (ATR FT-IR) on dextran T1 and EDA-dex-T1 (Figure S2), from which we observed the formation of the carbonyl stretch at 1705 cm^{-1}. This corresponds to the carbonyl on the carbamate linkage formed from derivatization of EDA-dex-T1, further confirming the desired product.

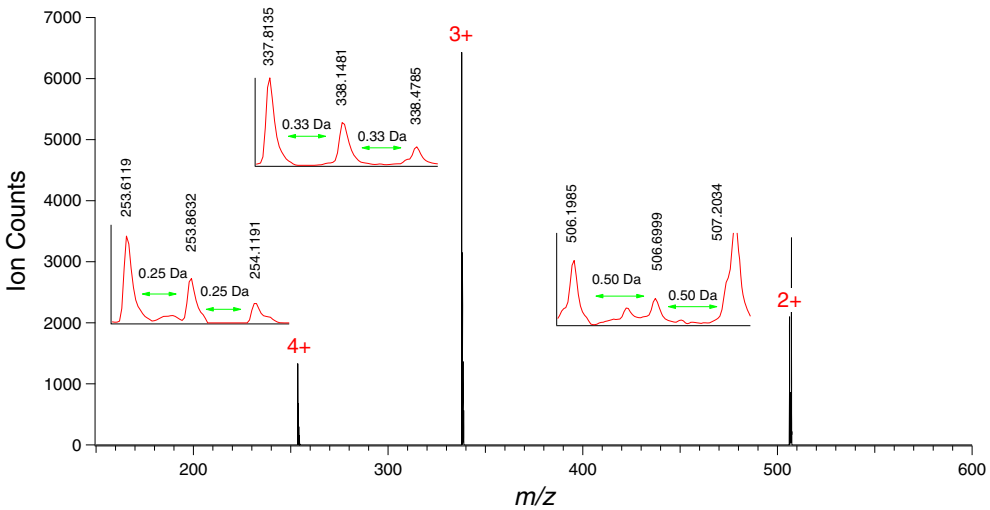


Figure 4. Extracted charge distribution showing the peaks 253, 337, and 506 as 4+, 3+, and 2+ respectively. The deconvoluted mass corresponds to $C_{36}H_{66}N_8O_{25}$, or the four-glucose chain with four ethylenediamine attachments with a mass of 1010 Da. The 0.25-, 0.33-, and 0.50-Da intervals for the quadruply, triply and doubly charged ions are shown in the *insets*. Note that the 507.2034 peak exhibited severe peak overlap

ESI-TOF MS Analysis of dextran T1 and EDA-dex-T1

We quantified the efficiency of derivatization by counting the number of ethylenediamine attachments and comparing the starting mass spectrum of dextran T1 with the spectrum of EDA-dex-T1. In the ESI mass spectrum shown in Figure 1, we observed dextran T1 to range from two to nine repeating glucose residues, corresponding to neutral masses ranging from 342 to 1476 Da as either $[M + NH_4]^+$ ions or $[M + Na]^+$ ions, with peak differences of 162 Da that account for one repeating glucose residue. The collected mass spectrum is similar to previously observed distributions for dextran obtained with ESI in which the observed peaks are primarily a product of Na^+ ion adduct formation [7].

The ESI mass spectrum generated from EDA-dex-T1 is shown in Figure 2. We found that the two to nine glucose unit distribution observed in dextran T1 had been offset by a mass gain of 86 Da, indicating successful derivatization due to $-(C=O)NH(CH_2)_2NH_2$ attachment. We observed ionization to have occurred through the formation of H^+ adducts rather than Na^+ or NH_4^+ adducts, one of the intended purposes of derivatizing dextran. The new observed distribution ranged from *m/z* 429 to *m/z* 1563 in the form of $[M + 86 + H]^+$, which corresponds to a single ethylenediamine attachment onto the dextran.

On further investigation of each ion, we found that some dextran chains had more than one ethylenediamine attachment. A second distribution that ranged from *m/z* 258 to *m/z* 825 corresponded to the same two to nine glucose residue chains, but with two ethylenediamine attachments and two charges (two H^+ adducts). The extracted mass distributions are shown in Figure 3, in which the same results for triple attachments/charges (255 to 579 Da) and quadruple attachments/charges (253 to 455 Da) for the two to nine glucose residue chains are observed. The multiply charged ion distributions for derivatized dextran were the first evidence to show that a polysaccharide can be ionized by means of multiple H^+ adducts. This is remarkably important since we achieved multiple charges on a polysaccharide and shifted the mass distribution toward the lower *m/z* region, the first step toward reducing the problem of low ionization efficiency for polysaccharides.

After further investigation of the ion peaks, we found protein-like charge distributions for EDA-dex-T1. For example, in Figure 4 we show the charge distribution observed for a four-glucose-unit chain with four ethylenediamine attachments. The three ion peaks correspond to *m/z* 253, 337, and 506 as 4+, 3+, and 2+ ions respectively, all corresponding to the formula $C_{36}H_{66}N_8O_{25}$ with a deconvoluted mass of 1010 Da. We determined that these ions were multiply charged from the spectrum as indicated by the 0.25-Da interval for the quadruply charged ion, the 0.33-Da interval for the triply charged ion, and the 0.50-Da interval for the doubly charged ion shown in the insets in Figure 4. The remainder of the combinations of ethylenediamine attachments, charges, and charge distributions are shown in Table 1.

Table 1. Assignment of Several Representative Ions for Ethylenediamine-Derivatized Dextran T1

m/z	Charge, H^+ adduct(s)	Deconvoluted *m/z*	Formula	No. of glucose units	No. of attachments
429.1730	+1	428.1652	$C_{15}H_{28}N_2O_{12}$	2	1
258.1146	+2	514.2136	$C_{18}H_{34}N_4O_{13}$	2	2
591.2256	+1	590.2178	$C_{21}H_{38}N_2O_{17}$	3	1
339.1413	+2	676.2670	$C_{24}H_{44}N_4O_{18}$	3	2
677.2693	+1	676.2615	$C_{24}H_{44}N_4O_{18}$	3	2
255.1129	+3	762.3153	$C_{27}H_{50}N_6O_{19}$	3	3
382.1639	+2	762.3122	$C_{27}H_{50}N_6O_{19}$	3	3
753.2787	+1	752.2709	$C_{27}H_{48}N_2O_{22}$	4	1
420.1676	+2	838.3196	$C_{30}H_{54}N_4O_{23}$	4	2
839.3229	+1	838.3151	$C_{30}H_{54}N_4O_{23}$	4	2
309.1313	+3	924.3705[a]	$C_{33}H_{60}N_6O_{24}$	4	3
463.1909	+2	924.3662	$C_{33}H_{60}N_6O_{24}$	4	3
253.6122	+4	1010.4176	$C_{36}H_{66}N_8O_{25}$	4	4
337.8128	+3	1010.4150	$C_{36}H_{66}N_8O_{25}$	4	4
506.2108	+2	1010.4060	$C_{36}H_{66}N_8O_{25}$	4	4
915.3306	+1	914.3228	$C_{33}H_{58}N_2O_{27}$	5	1
501.1942	+2	1000.3728	$C_{36}H_{64}N_4O_{28}$	5	2
1001.3760	+1	1000.3682	$C_{36}H_{64}N_4O_{28}$	5	2
363.1484	+3	1086.4218	$C_{39}H_{70}N_6O_{29}$	5	3
544.2170	+2	1086.4184	$C_{39}H_{70}N_6O_{29}$	5	3
1087.4209	+1	1086.4131[a]	$C_{39}H_{70}N_6O_{29}$	5	3
294.1300[b]	+4	1172.4888[a]	$C_{42}H_{76}N_8O_{30}$	5	4
391.8306	+3	1172.4684	$C_{42}H_{76}N_8O_{30}$	5	4
1077.3825	+1	1076.3747	$C_{39}H_{68}N_2O_{32}$	6	1
582.2204	+2	1162.4252	$C_{42}H_{74}N_4O_{33}$	6	2
1163.4277	+1	1162.4199	$C_{42}H_{74}N_4O_{33}$	6	2
417.1667	+3	1248.4767	$C_{45}H_{80}N_6O_{34}$	6	3
625.2437	+2	1248.4718	$C_{45}H_{80}N_6O_{34}$	6	3
334.6389	+4	1334.5244	$C_{48}H_{86}N_8O_{35}$	6	4
445.8480	+3	1334.5206	$C_{48}H_{86}N_8O_{35}$	6	4
668.2636	+2	1334.5116[a]	$C_{48}H_{86}N_8O_{35}$	6	4
1239.4347	+1	1238.4269	$C_{45}H_{78}N_2O_{37}$	7	1
663.2468	+2	1324.4780	$C_{48}H_{84}N_4O_{38}$	7	2
1325.4801	+1	1324.4723	$C_{48}H_{84}N_4O_{38}$	7	2
471.1899	+3	1410.5463	$C_{51}H_{90}N_6O_{39}$	7	3
706.2701	+2	1410.5246	$C_{51}H_{90}N_6O_{39}$	7	3
375.1567[b]	+4	1496.5956	$C_{54}H_{96}N_8O_{40}$	7	4
499.8653[b]	+3	1496.5725	$C_{54}H_{96}N_8O_{40}$	7	4
749.2900[b]	+2	1496.5644	$C_{54}H_{96}N_8O_{40}$	7	4
1401.4879	+1	1400.4801	$C_{51}H_{88}N_2O_{42}$	8	1
744.2731	+2	1486.5306	$C_{54}H_{94}N_4O_{43}$	8	2
1487.5321	+1	1486.5243	$C_{54}H_{94}N_4O_{43}$	8	2
525.2026	+3	1572.5844	$C_{57}H_{100}N_6O_{44}$	8	3
787.2975	+2	1572.5794	$C_{57}H_{100}N_6O_{44}$	8	3
415.6656	+4	1658.6312	$C_{60}H_{106}N_8O_{45}$	8	4
1563.5349	+1	1562.5271	$C_{57}H_{98}N_2O_{47}$	9	1
579.2191	+3	1734.6339	$C_{63}H_{110}N_6O_{49}$	9	3
868.3222	+2	1734.6288	$C_{63}H_{110}N_6O_{49}$	9	3
456.1802	+4	1820.6896	$C_{66}H_{116}N_8O_{50}$	9	4

[a]Mass error greater than 5 ppm
[b]Peak overlap

Also, we determined that there is also no evidence that EDA-dex-T1 degraded in the sample derivatization, preparation, or ionization as we did not find the *m/z* 162 or *m/z* 249 ion peaks that would correspond to a single repeating glucose residue or single-attachment glucose residue, respectively, indicative of glycosidic bond cleavage during our ESI-TOF analyses.

ESI-MS/MS of dextran T1 and EDA-dex-T1 Trisaccharides

To further investigate the properties of our derivatized dextran, we collected MS/MS data to determine where the

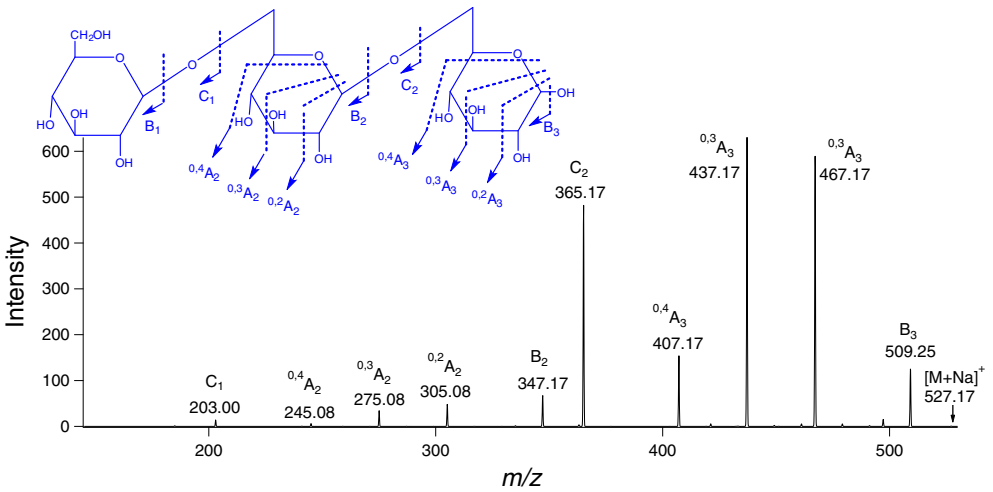


Figure 5. Tandem mass spectrometry (MS/MS) spectrum of dextran T1 trisaccharide

ethylenediamine attachments occurred. We collected fragmentation for a dextran T1 trisaccharide, and then compared this with that of an EDA-dex-T1 trisaccharide with single, double, and triple ethylenediamine attachments. The MS/MS spectrum for the dextran T1 trisaccharide is shown in Figure 5. We assigned the molecular ions and fragment ions using the nomenclature of Domon and Costello [23]. We observed fragment ions that correspond to glycosidic bond cleavages, such as C_1 (*m/z* 203), B_2 (*m/z* 347), and C_2 (*m/z* 365), and cross-ring cleavage fragment ions, $^{0,2}A$, $^{0,3}A$, and $^{0,4}A$, which correspond to the second and third residues from the nonreducing end. All the ions we observed in Figure 5 we found to be largely consistent with the literature [24].

The MS/MS spectrum for the EDA-dex-T1 trisaccharide with a single ethylenediamine attachment is shown in Figure 6. We observed the fragment ions that correspond to glycosidic bond cleavages such as Z_1 (*m/z* 249), Y_1 (*m/z* 267), Z_2 (*m/z* 411), and Y_2 (*m/z* 429). We also identified cross-ring cleavage fragment ions such as $^{0,2}X_1$ (*m/z* 309), $^{0,2}X_2$ (*m/z* 471), and $^{1,5}A_3$ (*m/z* 544), which suggested the location of attachment to be at the C-2 position of the reducing-end glucose monomer. Determination of the exact location of attachment further confirms that ethylenediamine was attached to the dextran trisaccharide and supports our finding that ionization occurred in the form of a proton adduct.

The MS/MS spectra for the EDA-dex-T1 trisaccharide with double and triple ethylenediamine attachments are shown in Figure 7. For EDA-dex-T1 with two attachments (Figure 7a), we observed the fragment ions that correspond to glycosidic bond cleavages: C_1 (*m/z* 353) and C_2 (*m/z* 515). The ions *m/z* 353 and *m/z* 515 indicate that two attachments occurred on the glucose at the nonreducing end, as well as the ions Z_1 (*m/z* 249), Y_1 (*m/z* 411), and *m/z* 573, which suggests that one attachment occurred at the glucose on the reducing end and a second attachment occurred at the glucose on the nonreducing end. This was the first evidence that suggests attachment to be a random occurrence when more than one ethylenediamine is attached to dextran.

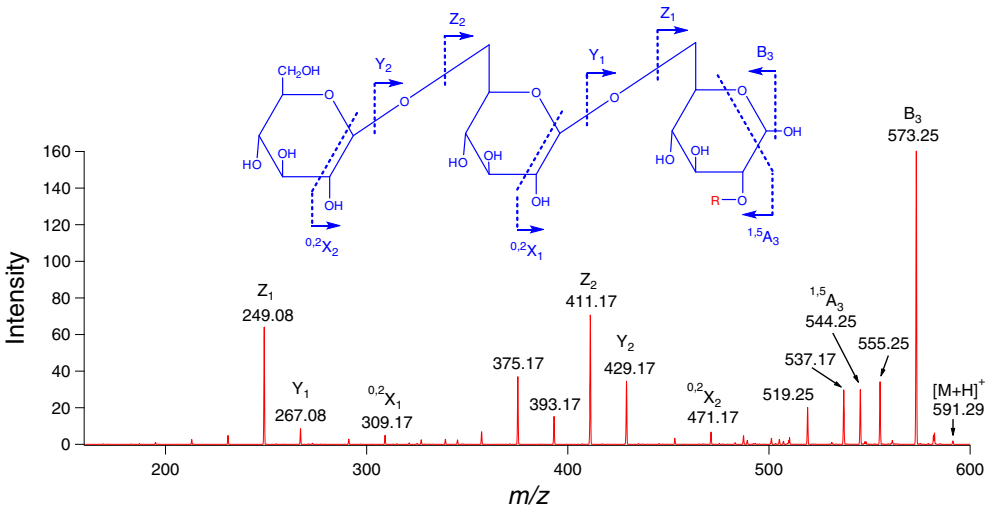


Figure 6. MS/MS spectrum of EDA-dex-T1 trisaccharide with a single attachment as indicated by *m/z* 591. *R* denotes the ethylenediamine attachment (–(C=O)NH$(CH_2)_2NH_2$), which our data suggest to be at the C-2 position of the reducing-end glucose monomer.

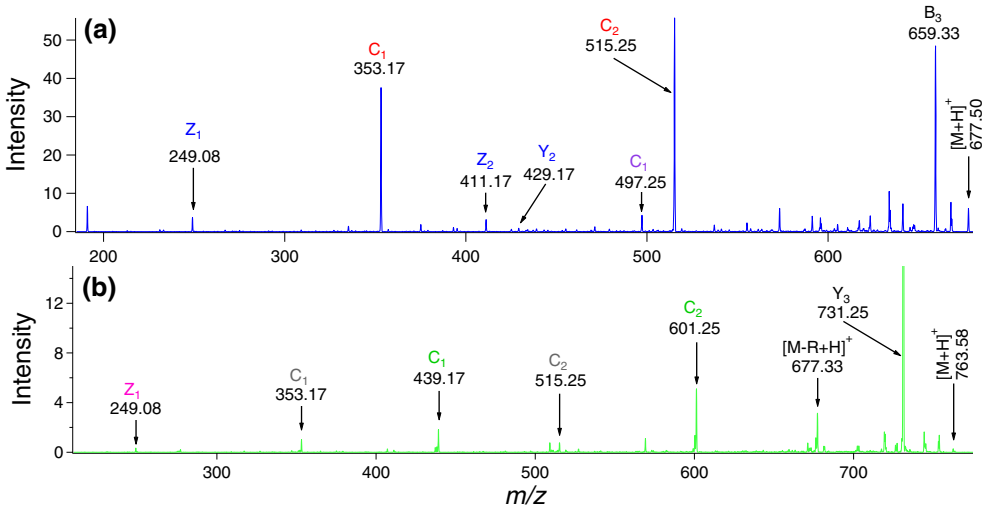


Figure 7. MS/MS spectra of EDA-dex-T1 trisaccharide with double and triple ethylenediamine attachments. **a** EDA-dex-T1 with double ethylenediamine attachments as indicated by *m/z* 677. The fragment ions Z_1, Z_2, and Y_2 in *blue* are indicative of one attachment at the reducing end and one at the nonreducing end. The fragment ions C_1 and C_2 in *red* are indicative of two attachments at the glucose at the nonreducing end and the fragment ion C_1 in *purple* is indicative of one attachment at the middle glucose and a second attachment at the reducing-end glucose. **b** EDA-dex-T1 with triple ethylenediamine attachments as indicated by *m/z* 763. The fragment ion Z_1 in *pink* is indicative of a single attachment at the reducing end and the fragment ions C_1 and C_2 in *green* are indicative of three attachments at the glucose at the nonreducing end. The fragment ions C_1 and C_2 in *gray* are indicative of two attachments at the glucose at the nonreducing end and the third attachment at the reducing-end glucose

For EDA-dex-T1 with three attachments (Figure 7b) we also observed fragment ions that correspond to glycosidic bond cleavages, such as C_1 (*m/z* 353 and 439) and C_2 (*m/z* 515 and 601). The C_1 (*m/z* 439) ion was further indicative of random ethylenediamine attachment since it corresponds to all three attachments occurring at the glucose at the nonreducing end. This further suggests that attachment of multiple ethylenediamine groups to dextran occurs at random available hydroxyl groups on the polysaccharide. Even though attachment of ethylenediamine to dextran appears to be random, our goal of achieving multiple proton adducts was not diminished by this phenomenon as we show in Figures 2 and 3 and Table 1.

Conclusions

This is the first report of a simple, one-pot derivatization of a polysaccharide that allows multiple charge ionization, typical of proteins and glycoproteins, but not previously shown for polysaccharides. ESI-MS is capable of detecting derivatized dextran by allowing multiple charging and thus shifting detection of the polysaccharide toward lower *m/z* ratios. The one-pot derivatization reaction was ideal to attach ethylenediamine groups via carbamate bonds to the polysaccharide, resulting in EDA-dex-T1. Evidence of single attachment/charge to quadruple attachments/charges was shown, as was evidence of protein-like charge distributions with ionization occurring as proton adducts due to the terminal amine groups present in EDA-dex-T1, rather than the metal cation adducts more commonly seen with underivatized dextran. Our MS/MS studies indicate that multiple attachments to dextran after the derivatization step occur at random locations on the polysaccharide. This did not limit EDA-dex-T1's ability to carry multiple charges, and differences in the exact location of the attachments did not impair our primary goal of attaching ethylenediamine groups to encourage ionization to occur by proton adduct formation and achieve multiple charges on a polysaccharide. Collectively, the data obtained from this work show that EDA-dex-T1 is capable of ionizing with multiple proton adducts, thereby shifting the molecular weight distribution to the lower *m/z* ratio detection range. From our findings, the derivatization may be adaptable for use with higher molecular weight dextran. For example, with the assumption that a maximum of four ethylenediamine attachments are possible, theoretically, we could detect dextran with a maximum molecular weight of approximately 12,000, resulting in a measured *m/z* of 3000 with four charges using our current derivatization method and instrument settings.

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