Improved High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection Separation of Poly saccharides

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Overview

The goal of this research was to develop a high-performance anion-exchange with pulsed amperometric detection (HPAE-PAD)-based method for separating polymers of poly saccharide with higher degree of polymerization as compared to existing methods.

Introduction

Poly saccharide is a collective name for linear polymers of sialic acid that are covalently bound to proteins as a post-translational modification. It is widely expressed in tissues—bacterial capsules, fish, sea urchin eggs, embryonic tissues, amphibians, animal and human brain, and in a variety of cancers. These sugar chains modulate cell-cell interaction (mainly during embryonic growth), neural plasticity, and tumor metastasis. The major carrier of poly saccharides in mammals is the neural cell adhesion molecule (a glycoprotein that belongs to the immunoglobulin super family). As a linear homopolymer (n = 8 to 100) of sialic acid, these cell surface glycans are highly expressed during embryonic brain development. In postnatal and adult animals their expression is restricted to regions crucial for neuronal and synaptic plasticity. Poly saccharide is capable of neuronal and synaptic plasticity.

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Experimental

Thermo Scientific Dionex ICS-5000 Ion Chromatography system including:
- Gradient Pump
- DC Detector/Chromatography Module
- Electrochemical Detector
- Carbohydrate FFTE Disposable Au Working Electrodes
- Ag/AgCl Reference Electrode
- ASAP Autosampler

Thermo Scientific Chromelon Chromatography Data System software

Samples

Colominic acid (Sigma)
- To prepare a 5 mg/mL solution, weigh out 10 mg of colominic acid and dissolve in 2 mL DI water.
- Freeze the stock solution at -20 ºC until needed.

N-acetylmuramidase and pentamer (Natalca USA)
- Weigh out 1 mg of trimer and dissolve in 1 mL of DI water.
- Freeze stock solution at -20 ºC until needed.

Results

Acetate Gradient Separations

Figure 3. HPAE-PAD chromatographic profile of a commercial sample of colominic acid on a Dionex CarboPac PA 200 column using a gradient of 200 to 1000 mM sodium acetate in 100 mM sodium hydroxide. The DP for n = 3 and 5 were identified by using the retention time of the trimer and pentamer of N-acetylmuramidase.

The Dionex CarboPac PA 200 column is able to resolve colominic acid homologues with DP up to 100 (Figure 2, inset) in 70 minutes. This is an improvement over the maximum DP (up to 60 in 68 min) that could be discerned with a Dionex CarboPac 100 column.

Nitrate Gradient Separations

It has been reported that using nitrate instead of acetate as the pushing agent resulted in better resolution of higher polymers. With the current configuration, our best separation of NeuAc polymers (present in commercial colominic acid) was obtained with sodium nitrate, shown in Figure 3. The maximum DP that can be detected with the nitrate gradient was 140 in 90 min (Figure S8). This is about 60 DP higher than that obtained with a Dionex CarboPac PA 100 column.

Notes
- A gradual drop in peak area (from one injection to the next) was observed when sodium nitrate was used as the pushing agent (data not shown). This loss in response was not observed when an acetate gradient was used, and is most likely due to metal contamination in the sodium nitrate (vendor specification for ion impurity is < 3 ppm on ACS- grade sodium nitrate), which could lead to electrode fouling. Therefore, use of a Thermo Scientific Dionex IonPac MPC™-1 Metal-Free Column is recommended when sodium nitrate is used for this application.

To remove trace metal contamination from high-pH eluents, the Dionex TorrPeak™ MPC-1 column was installed in the anion line prior to the injection valve. As a result, the peak area was higher as compared to when a metal-free column was not used, and over 25 consecutive injections no peak area loss was observed.

Conclusion

The proposed HPAE-PAD method for the analysis of a homologous series of poly saccharides achieves better resolution as compared to existing methods.

With acetate as the pushing agent, changing the linear gradient (Curve 5, Figure 2) to a slightly convex gradient (Curve 4) yields the separation in Figure 4. Although no additional peaks are identified, the spacing between the early-eluting peaks is improved. In the case of nitrate as the pushing agent, the convex gradient was comparable to the linear gradient. The DP information obtained from the previously-mentioned high-resolution method will be very useful for the study of biosynthesis, degradation, and structure-function correlations of poly saccharides. It can also be used for the quality control of related molecules which have the bioengineering potential for increasing stability of enzymes, or in anti viral drugs.

Results and Discussion

The Dionex CarboPac PA 200 column is able to resolve the high DP polymers in a poly saccharide composition as compared to the other columns that have been used for this application. Colominic acid (Sigma) is a collective name for linear polymers of NeuAc that is present in the presence of sodium hydroxide. A HPAE-PAD-based method using the Dionex CarboPac™ PA 100 column and acetate gradien is shown to separate un-modified NeuAc and NeuAcSA with DP up to 60 without the loss of lower DP derivatives of poly saccharide resulted in elution and resolution of the higher polymers (maximum DP resolved was about 20 DP higher), in part because nitrate is a stronger eluent compared to acetate.

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The Dionex CarboPac PA 200 column is able to resolve the high DP polymers in a poly saccharide composition as compared to the other columns that have been used for this application. Colominic acid (Sigma) is a collective name for linear polymers of NeuAc that is present in the presence of sodium hydroxide. A HPAE-PAD-based method using the Dionex CarboPac™ PA 100 column and acetate gradient has been shown to separate un-modified NeuAc and NeuAcSA with DP up to 60 without the loss of lower DP derivatives of poly saccharide resulted in elution and resolution of the higher polymers (maximum DP resolved was about 20 DP higher), in part because nitrate is a stronger eluent compared to acetate.

Figure 1. Colominic Acid

Figure 2. HPAE-PAD chromatographic profile of a commercial sample of colominic acid. Fifty micrograms were injected on a Dionex CarboPac PA 200 column and eluted with nitrate as the pushing agent. Peaks are labeled with the putative n values, although the trimer (n = 3) and pentamer (n = 5) were determined using standards.

Figure 3. HPAE-PAD chromatographic profile of a commercial sample of colominic acid. Fifty micrograms were injected on a Dionex CarboPac PA 200 column and eluted with acetate as the pushing agent. Peaks are labeled with the putative n values, although the trimer (n = 3) and pentamer (n = 5) were determined using standards.

Figure 4. HPAE-PAD chromatographic profile of a commercial sample. Fifty micrograms were injected on a Dionex CarboPac PA 200 column and eluted with nitrate as the pushing agent (Curve 4). Peaks are labeled with the putative n values, although the trimer (n = 3) and pentamer (n = 5) were determined using standards.

References


