International Journal of Mass Spectrometry 402 (2016) 57–65

Contents lists available at ScienceDirect

International Journal of Mass Spectrometry

journal homepage: www.elsevier.com/locate/ijms

Relative ion intensities of maltooligosaccharide ethers in electrospray ionization ion trap mass spectrometry: A quantitative evaluation

Sheetal Gangula [a], Manfred Nimtz [b], Petra Mischnick [a,*]

[a] Technische Universität Braunschweig, Institute of Food Chemistry, Schleinitzstr. 20, D-38106 Braunschweig, Germany
[b] Helmholtz-Centre for Infection Research, Inhoffenstraße 7, D-38124 Braunschweig, Germany

ARTICLE INFO

Article history:
Received 14 October 2015
Received in revised form 11 January 2016
Accepted 20 January 2016
Available online 19 March 2016

Keywords:
Electrospray ionization
Relative ion intensities
Quantitative MS analysis
Maltooligosaccharide ethers

ABSTRACT

Electrospray ionization coupled with mass spectrometry (ESI-MS) is an important analytical technique for the analysis of polysaccharide derivatives. It can be used to analyze substitution patterns in mixtures of oligomers to recognize heterogeneities, bimodality or blocky sequences. Quantitative analysis is hampered, however, by the fact that compounds of different chemistry and molar mass usually show different ionization efficiencies. In order to study the parameters of influence and to understand whether relative ion intensities in MS follow certain patterns. We analyzed fully *O*-methylated and fully *O*-perdeuteriomethylated oligosaccharides as two extreme cases of possible mixed *O*-Me-/*O*-Me-d_3 by ESI-MS under defined conditions. Binary and complex mixtures of maltooligosaccharide ethers from DP 1 to 7 by were prepared from partially hydrolyzed per-*O*-methyl-β-cyclodextrin (Me-β-CD) and per-*O*-deuteromethyl-β-cyclodextrin (Me-d_3-β-CD). Reference data for their real compositions were obtained after reductive amination by HPLC–UV. Within the applicable concentration range and with properly adjusted instrumental parameters, relative sensitivities (Me-d_3/Me) in ESI-MS remained constant for a respective DP. With increasing DP, the relative sensitivity coefficients decreased from 1.01 for DP 2 to 0.78 for DP 6 for the binary mixtures and showed similar behavior for complex mixtures. The decrease in relative sensitivity coefficients for corresponding Me-d_3 and Me derivatives has been overcome by *m*-amino-benzoic acid labeling and ESI-MS in negative mode or by measuring the original mixtures in nano-ESI-MS. Thus, discrimination probably caused by differences in surface activity and electrophoretic mobility can be leveled by an appropriate tag or formation of smaller droplets as in nano-ESI.

1. Introduction

Naturally occurring polysaccharides such as cellulose and starch, are modified by esterification or etherification of their free hydroxyl groups for various applications [1]. Such derivatives are employed in the construction area, for pharmaceuticals, food, cosmetics, and in the packaging industries, to name but a few applications. The physicochemical properties of these chemically modified biopolymers such as solubility, biodegradability, viscosity, or thermoreversible gelation, depend not only on the type of substituent and the average degree of substitution (DS), but also on the distribution in the glucosyl unit both along and over the macromolecule chains [2–4]. The molar ratios of the constituents varying in position and number of substituents are usually determined by NMR spectroscopy, GLC, CE or HPLC analysis of the depolymerized sample [5]. To gain information on the substitution pattern on the different structural levels, random degradation followed by mass spectrometric analysis of the DS profiles of sets of oligomers and comparison of these to a statistical model is applied [6–9]. To prevent discrimination during hydrolysis and subsequent MS analysis, methyl celluloses are permethylated with deuteromethyl iodide, resulting in chemically uniform polymers. MALDI-ToF-MS and ESI-IT-MS are applied as well, while the latter provides the advantage of easily being coupled with liquid chromatography (LC–ESI-IT-MS) and thus automation [10]. In the case of other MCs, for instance, *O*-ethyl-*O*-methyl glucans, this approach is hampered, however, by the fact that relative ion abundance in mass spectra does not necessarily represent the actual molar composition of the sample. Here, the compounds of lower polarity are detected with higher intensity, which causes distortion of MS

Abbreviations: BM, binary mixture; CM, complex mixture; CRM, charge residue model; DMSO, dimethyl sulfoxide; DP, degree of polymerization; DS, degree of substitution; GLC, gas liquid chromatography; HPLC, high performance liquid chromatography; IEM, ion evaporation model; IT, ion trap; *m*ABA, *meta*-amino benzoic acid; Me, methylated oligomer; Me-d_3, deuteromethylated oligomer; Rel. Int., relative ion intensities; MR, molar ratio; RP, reverse phase; TLC, thin layer chromatography; TM, target mass; *U*, uncertainty.

* Corresponding author.
E-mail address: p.mischnick@tu-braunschweig.de (P. Mischnick).

http://dx.doi.org/10.1016/j.ijms.2016.01.009
1387-3806/

profiles. *O*-Methyl-*O*-methyl-d_3 oligomers are chemically more similar and thus ESI-IT-MS data can be used for quantitative analysis. In monomer analysis regioisomeric *O*-methyl-*O*-methyl-d_3-glucose derivatives cannot be separated, though, while *O*-ethyl-*O*-methyl homologs can. Thus, it is of interest to extend the 'oligomer analysis' to chemically more different mixed ethers. As a prerequisite, the influence of the chemistry and molar mass (DP) on relative ion intensities in ESI-IT-MS needs to be investigated, starting with the well-known system *O*-Me/*O*-Me-d_3 as a reference. In a subsequent paper we will present the results for chemically more different glucooligosaccharide ethers.

Mechanism of formation of gas phase ions from solution in ESI is of interest for many researchers. This process is described in three major stages [11]. The sample solution is infused into a high electric field; a 'Taylor cone' is formed at the tip of the needle. Consequently, charged droplets are expelled [12,13], and from these droplets gas phase ions are formed. Two main theories are widely accepted and have been experimentally investigated with respect to the mechanism of formation of gas phase ions from these charged droplets: the ion evaporation model (IEM) and the charge residue model (CRM) [14–16]. Solvated ions diffuse to and escape from the droplet surface. The ion yields of analyte molecules depend on their basicity or cation complexation ability (positive mode), polarity and surface activity of the analyte, its electrophoretic mobility and (de)solvation energy [17]. Therefore, it is plausible that even a small structural change can cause huge differences in relative ion intensities in ESI-MS. For quantifying the molar ratios of analytes from relative ion intensities, their dependence on sample composition and concentration must also be considered. Furthermore, with respect to instrumental parameters, flow rate [18] is also critical, because lower flow rates produce smaller droplets. Hence diffusion of analyte molecules to the surface of the droplet is affected and number of Coulomb explosions as well. Therefore, nano-ESI-MS [19] was applied for some selected samples. Finally, transmission of ions to the mass analyzer, in our case an ion trap, is affected by skimmer voltages and further parameters [20].

To gain insight into the influence of all these parameters on relative ion intensities, fundamental studies of ESI-IT-MS with oligosaccharide derivatives are required. For the preparation of defined sample solutions, cyclodextrins rather than polysaccharides were chosen. β-Cyclodextrin was permethylated and perdeuteromethylated, respectively. Corresponding 2,3,6-tri-*O*-substituted maltooligosaccharides of DP 1–7 were obtained by partial hydrolysis and used for the preparation of mixed ethers. These were systematically studied by ESI-IT-MS. From our experience with mixed *O*-Me-*O*-Me-d_3 oligosaccharides we expected close to equal ion intensities in the mass spectrum referred to equimolar concentration. Using this as a basis, we studied the behavior of uniformly *O*-methyl and corresponding *O*-methyl-d_3 maltooligosaccharides of DP 2–6 in ESI-IT-MS, which represent the two extreme cases in oligomer analysis of partial hydrolysates of perdeuteromethylated methyl glucans [10]. It is our long-term goal to establish whether the differences in ion yields of homolog series of oligosaccharide derivatives follow certain rules, which would allow correcting the data of a measurement of a mixture of unknown composition for quantification of molar ratio from relative ion intensities.

2. Experimental

2.1. Reagents and materials

All chemicals purchased were of highest purity and used without further purification. Iodomethane (MeI), iodomethane-d_3 (Me-d_3-I), *m*-aminobenzoic acid (*m*ABA) and 2-picoline borane were purchased from Sigma Aldrich. Solvents used for ESI-MS were LC–MS grade and were purchased from Fluka.

2.2. Synthesis

Per-*O*-methylated-β-cyclodextrin (Me-β-CD) and per-*O*-deuteromethylated-β-cyclodextrin (Me-d_3-β-CD) were synthesized according to Ciucanu and Kerek [21] in DMSO with NaOH/MeI or MeI-d_3, respectively. Products were purified by re-crystallization from acetone. Completeness of alkylation was checked by the absence of OH-absorption >3000 cm^{-1} by ATR-IR spectroscopy. ^{1}H NMR spectra showed only a single doublet peak for H-1 confirming the uniformity and were in agreement with the literature [22]. GLC analysis was performed after total hydrolysis, reduction and acetylation [23], DS was 2.99. In ESI-MS only peaks from completely alkylated cyclodextrins 1451.6 $[M+Na]^+$, 737.4 $[M+2Na]^{2+}$ for Me-β-CD and 1515.1 $[M+Na]^+$, 769.1 $[M+2Na]^{2+}$ for Me-d_3-β-CD, were detected.

2.3. Partial hydrolysis

Peralkylated-β-cyclodextrins were submitted to partial hydrolysis in a 1 mL V-Vial. Ca. 2 mg in 1 mL of 1 M TFA was heated at 120 °C for 21 (Me-β-CD) and 15 min (Me-d_3-β-CD), respectively. After cooling to room temperature, aqueous acid was removed in a stream of nitrogen and finally co-distilled with toluene and the remainder evaporated to dryness. The obtained *O*-methylated maltooligosaccharides of DP 1–7 were dissolved in 1 mL of methanol. Composition with respect to DP was determined after reductive amination (Section 2.4) by HPLC–UV–MS (Section 2.5) for each sample.

2.4. Reductive amination

The *O*-methylated maltooligosaccharides obtained by partial hydrolysis (Section 2.2) were labeled [24] with *m*-aminobenzoic acid (*m*ABA). To ca. 2 mg of the substrate in 0.5 mL MeOH, 0.3 mL *m*ABA in methanol (containing 2.6 mg/19 μmol *m*ABA) and 0.15 mL glacial acetic acid was added, and the mixture was heated to 40 °C for 30 min. Subsequently, 50 μL of 2-picoline borane in MeOH (containing 1.7 mg; ~19 μmol) was added, and the mixture was heated to 40 °C for 45 min. Subsequently the solvent was removed in a stream of nitrogen and the residue was dissolved in 1 mL of MeOH. The completeness of labeling was determined by TLC and by ESI-IT-MS in positive ion mode for the presence of unlabeled sample.

2.5. HPLC–UV–MS

LC–MS separations were carried out on an Agilent HPLC system consisting of a binary pump (1100 series) and a DAD (G1312A). A RP-C_{18}-column (Phenomenex, Gemini, 250 × 4.60 mm, 5 μm) was used. The mobile phase consisted of acetonitrile (ACN) and water with 1% added acetic acid [10]. Three different linear gradient systems were used starting with H_2O/ACN 90/10, 80/20 or 70/30 (v/v) at t = 0–100% ACN at t = 50 min, respectively. If not otherwise stated, sample concentration was 0.2 mg mL^{-1} in MeOH. Flow rate was set to 0.4 mL min^{-1}. UV absorption was recorded at 330 nm [10]. Mass spectra were obtained by coupling with ESI-IT-MS (Section 2.7.1). Spectra were recorded in both, positive and negative ion modes. The parameters were the same for syringe pump infusions except for the solvent (eluent of HPLC), nitrogen dry gas (9 L min^{-1}, 365 °C) and nebulizer gas (40 psi).