UDC 541.61.614 DOI: 10.25808/08697698.2018.202.6S.007

K.V. BELOKOZOVA, O.S. MALYARENKO, S. D. ANASTYUK

Mass spectrometry of sulfated laminaran derivatives, obtained by autohydrolysis in heavy-oxygen water

Key words: mass-spectrometry, laminaran, fucoidan, autohydrolysis, derivatization, heavy-oxygen water.

The mass spectrometry (MS) is a rapidly-developing, fast, sensitive and accurate instrumental method of molecular weight measurement. It is capable of measuring not only weights (MW) of molecular (parent) ions but also MW of their fragments (daughter ions) using a tandem (MS/MS) mode of operation. By the analysis of fragmentation pathways it is possible to establish structural features of the parent molecule. The structural analysis of native and modified carbohydrates is only small part of the application area of MS, but the important one, since the direct instrumental sequencing of carbohydrates is an unsolved problem. It is essentially to acquire information on fragmentation patterns of different samples of carbohydrates with known/defined structural features, especially if these samples are interesting due to their biological activities.

Recently, we have described a procedure modification of the biologically active complex sulfated polysaccharide – fucoidan. It was found that method of decomposition, an autohydrolysis (an autocatalysis of mild acid hydrolysis where own sulfate groups of the molecules act like the source of acid) was able to lower the molecular weight of fucoidan from *S. cichorioides* and to selectively remove the sulfate groups from C-2 positions of Fuc residues without loss of biological activity. This method was further improved: we used heavy-oxygen water for autohydrolysis of known fucoidan samples for labeling the reducing end with ^{18}O. Fragments were analyzed by electrospray ionization mass spectrometry (ESIMS/MS). The labeling improved our abilities for exact assignment of fragment ions: new fragmentation pathway was described: the substitution at C-4 generated $^{2,4}A$-type ion (following nomenclature, suggested by Domon and Costello).

In present work we analyzed fragments of sulfated laminaran DdLS from brown alga *Dictyota dichotoma* during autohydrolysis in $H_2{}^{18}O$. Laminaran is 1,3;1,6-linked beta-D-glucan 1,3:1,6-linkage ratio 3:1, which was extracted, purified and sulfated with a pyridine solution of chlorosulfonic acid and dimethylformamide as described previously. The MWs of DdLS was analyzed during autohydrolysis (60 °C) using high-performance size-exclusion chromatography: the MW ranged from (intact) 12.5 kDa, 9.5 kDa at 24 h of autohydrolysis, 8.5 kDa at 48h and 6.4 kDa at 72 h. During the reaction the low MW fragments (1.2 Da - 2 kDa) were accumulated. ESIMS/MS analysis was capable of observing structural features of oligosaccharides only after

* BELOKOZOVA Kristina Vladimirovna – Bachelor in chemical sciences, Laboratory Assistant, MALYARENKO Olesya Sergeevna – PhD, Researcher, ANASTYUK Stanislav Dmitrievich – PhD, Researcher (G.B. Elyakov Pacific Institute of Bioorganic Chemistry, FEB RAS, Vladivostok, Russia). *E-mail: kristina_ars@bk.ru

The work was supported by the Grant No. 16-13-10185 from the RSF (Russian Science Foundation).

additional mild acid hydrolysis in TFA (1N, $H_2{}^{18}O$) at 80 °C for 4 h. ESIMS/MS of disulfated glucose at *m/z* 169.99^{2-} revealed sulfation at C-2, C-4 and/or C-6. $^{0,3}X$ ion signal at 171.00 indicated sulfation at C-3. Probably, these residues were terminal or were single 1:6-branches. ESIMS/MS of a trisulfated glucobiose at *m/z* 193.66^{3-} was also informative: we observed [Glc-(1,3)-2,3,6-tri-OSO_3]$^{3-}$, [Glc-2-OSO_3(1,6)-3,4-di-OSO_3]$^{3-}$, [Glc-2,3-di-OSO_3(1,6)-3-OSO_3]$^{3-}$ structural variants. Again, due to the labeling at the reducing end, we were able to distinguish between different fragment ions which could had same *m/z* without a label.

The estimation of MW of laminaran and its derivatives was performed in the center for collective use of scientific equipment “Far Eastern Center of Structural Studies”.