VAC.13 - Ultrasound and heterogenous catalysis assisted lenght reduction of *Neisseria meningitidis* serogroup C capsular polyssaccharide

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Introduction:

Neisseria meningitidis C capsular polysaccharide (NMC) is a homopolymer of α–N-acetylneuraminic acid (Neu5Ac) containing α-(2-9) glycosidic linkages; It is an important component in conjugation process used for production of meningococcal serogroup C conjugate vaccine. Its use requires a previous mild oxidation stage, by sodium periodate; leading to the formation of oligosaccharide chains with an average of (10kDa), containing an aldehyde moiety in terminal sugar. The NMR assignment of the aldehyde moieties faces the difficulty of the low relative intensity of these signals (5%). The signal intensity should increase with the reduction of the oligosaccharide length, which favors its observation. The previous reduction of native polysaccharide length, followed by sodium periodate oxidation, appears as an alternative for obtention of a small oligomeric chain, facilitating the observation of hydrogens interaction and determining their sequential connectivities.

Objective:

The aim of this study was the development of a synthetic methodology for the reduction of *Neisseria meningitidis* C capsular polysaccharide length by assisted ultrasound and heterogeneous catalysis, in order to achieve small oligomers, which after oxidation, would generate high-quality NMR *spectra.*

Methodology:

Strong acid cation exchange resin was regenerated with 5% HCl and then dried. To 10mL of PSC solution (10mg/mL) was added 1g of regenerated resin. The mixture was sonicated (40Hz), for 3 and 5 hours (HPSC-3 and HPSC-5), filtered, dialyzed against water and freeze-dried. The resulting polysaccharides were analyzed with TSK gel G5000PW (TOSOH Biosience) column, by exclusion chromatography (SEC), equilibrated with 0.2 mol/L sodium chloride, at a flow rate of 0.5 mL / min. The total and column exclusion volumes were determined by the injection of acetone (58Da) and Blue dextran mass standards (~2,000 kDa). The complete proton and carbon NMR assignments of this compound were determinate by a series of 1D and 2D NMR experiments including (1H, 1H, 1H-COSY, HSQC and HMBC).

Results:

The reduction of polysaccharide length was qualitatively evaluated comparing their Kav values, obtained by SEC analysis. The native PSC (Kav – 0.10) and the hydrolysates (HPSC-3 Kav – 0.62 and HPSC-5 Kav – 0.60) demostrated a significant variation. The ^{1}H NMR data showed that the *O,N*-acetylation were preserved under the applied conditions; the signal of methyl groups were observed at 2.19 to 1.94ppm. HPSC-3 and HPSC-5 were then oxidized by $NaIO_4$ and analyzed by two-dimensional NMR (1H, 1H, 1H, COSY, HSQC). The assignment of aldehyde hydrogen, in its hydrated form, was observed, with good resolution.

Conclusion:

In conclusion, the methodology of polysaccharide hydrolysis assisted by ultrasonic and heterogeneous catalysis is a simple, efficient and selective methodology for oligosaccharides reduction chain. Making it possible to obtention of small oligosaccharide that would facilitate the structural characterization by NMR.

Keywords: Heterogenous catalysis; ultrasound; polysaccharide