GLYCAN STRUCTURE

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Characterization of the primary structure of glycans in detail is a requirement for further studies of three-dimensional aspects of carbohydrate molecules and glycoconjugates, their dynamics and the interactions with proteins in order to understand function and how to modify the molecules to obtain desired outcomes. In gram-negative bacteria the outer membrane is asymmetric with phospholipids in the inner leaflet and lipopolysaccharides (LPS) in the outer leaflet; the outer membrane is interspersed with proteins. Elucidation of the structure of these LPS is facilitated by chemical analysis, mass spectrometry and NMR spectroscopy. However, by including bioinformatics the structural elucidation process can proceed more rapidly and with higher confidence in the obtained results [1].

To expedite structural [2] as well conformational [3] analysis of carbohydrates by NMR spectroscopy the experiments and the following computer-assisted analysis [4] can be made more efficient by concatenation of NMR modules into a single 2D NMR experiment. In this way the efficiency is improved and results in time-saving due to the fact that two or more experiments share a common recovery delay prior to each subsequent scan of the 2D NMR experiment. This concept has been extended to parallel NOAH (NMR by Ordered Acquisition using ^{1}H-detection) supersequences utilizing sequential, parallel and time-shared acquisitions by which ten spectra can be acquired in a single measurement, referred to as a *p*-NOAH-10 [5]. A NOAH-5 measurement was tailored to produce NMR data for the computer program CASPER [4], which can be used to determine structure of oligo- and polysaccharides. Specifically, the supersequence (BS^{C}S^{J}T/S) consists of five NMR modules, viz., ^{1}H,^{13}C-HMBC, multiplicity-edited ^{1}H,^{13}C-HSQC-COSY, F_2-coupled ^{1}H,^{13}C-HSQC, ^{1}H,^{1}H-TOCSY, and a time-shared multiplicity-edited ^{1}H,^{13}C-HSQC module, covering most of the NMR data used as input to CASPER for a structural elucidation or NMR resonance assignments of an oligosaccharide.

Resonance overlap in NMR spectra of oligosaccharides can be greatly reduced, and resolution improved, by utilizing pure shift methods. Even though many correlations are resolved in ^{1}H,^{13}C-HSQC NMR spectra some may still remain, among other things, due to ^{1}H,^{1}H couplings, though these may be refocused and the resulting pure shift ^{1}H,^{13}C-HSQC NMR spectra are thus devoid of the homonuclear proton-proton couplings. However, peak-picking of cross-peaks in 2D NMR spectra is often time-consuming, limiting the potential of CASPER as an efficient analysis tool. Since pure shift methods aim to collapse multiplets into well-resolved singlets, pure shift data are ideal for use in conjunction with CASPER, allowing for efficient analysis by using automated peak-picking routines [6].

References:

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