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Supramolecular chemical shift reagents inducing conformational transitions: NMR analysis of carbohydrate homooligomer mixtures

Sophie R. Beeren* and Sebastian Meier* 

We introduce the concept of supramolecular chemical shift reagents as a tool to improve signal resolution for the NMR analysis of homooligomers. Non-covalent interactions with the shift reagent can constrain otherwise flexible analytes inducing a conformational transition that results in signal separation. Here we use this approach for the quantitative analysis of a complex homooligomeric glycan mixture.

The analysis of complex mixtures is routinely achieved via the physical separation of components on the basis of differential non-covalent interactions with a stationary phase. Chromatographic and electrophoretic methods can exploit specific interactions with a chosen ligand or utilise the differential partitioning of analytes with a micellar pseudo-stationary phase. NMR spectroscopic analysis of mixtures relies upon differences between the chemical structures of the analytes to achieve chemical shift resolution and therefore the analysis of mixtures of chemically similar analytes, for example homooligomers, is a challenge. In this study, we show that the addition of guests introducing conformational transitions in the analytes is a powerful method to achieve signal dispersion in NMR spectroscopy, whereby the guest functions as a type of non-covalently bound affinity-based diamagnetic chemical shift reagent. Here, therefore, non-covalent interactions are exploited to obtain a spectroscopic separation of mixture components rather than a physical separation, which enables the analysis of complex mixtures in situ.

Fig. 1. Schematic representation of how mixtures of short homooligomeric α(1-4) glucans exist in aqueous solution as unstructured populations with highly overlapped NMR spectra but complexation with suitable amphiphilic shift reagents results in a conformational transition, whereby signals from different length oligomers can be resolved using 1H-13C HSQC spectra.

Improved NMR analysis of complex carbohydrate mixtures may be feasible using complexation with suitable probes. It has been widely recognized that conformationally flexible biomolecules can gain structural definition upon complexation with a suitable interaction partner in a coupled binding and folding reaction, leading to vastly improved chemical shift dispersion. Herein we describe how the resolution and quantification of homooligomeric α-glucans with DP<14 can be achieved using NMR spectroscopy by exploiting the conformational constraints resulting from the interaction of amphiphiles with the hydrophobic aliphatic tail of surfactant-like amphiphiles (Figure 1).

Recently, we have characterized the binding behaviour of amphiphiles to α-glucans by monitoring the 1H chemical shift changes of the amphiphilic guest. The observed chemical shift changes and intermolecular nOes upon binding were consistent with the binding of the guest to the hydrophobic interior of a single-stranded left-handed helix (Figure 1). This mode of solution-state binding mimics that reported for the solid state encapsulation of lipids and surfactants by α-glucans, studied using crystallography and solid-state 13C NMR spectroscopy, and is furthermore supported by
The binding of SDS to oligosaccharides results in a downfield shift in the $\alpha$-anomeric $^1$H signals of the oligosaccharide upon helix formation resulting in a resolution improvement of at least 6-fold in both spectral dimensions. Conventional NMR spectroscopy in the absence of the shift reagent would thus require an at least 6-fold higher magnetic field to reach the same signal resolution. In the presence of SDS, a nearly perfect linear correlation between $^1$H and $^{13}$C chemical shifts is observed (Figure 2b). The linear relationship suggests that the chemical shifts result from a weighted average of the rapid two-state exchange between an unbound population of flexible oligomers and a bound population in which the oligomers are conformationally constrained, where the bound population is greater for longer carbohydrates which bind with higher affinities.

While complexation with SDS results in signal separation in the anomeric spectral region, the assignment of anomeric signals to specific residues of identified oligosaccharides was not possible. The influence of binding to a tailor-made highly water soluble amphiphilic probe HPTS-C$_{16}$ (HPTS = 8-hydroxypyrene-1,3,6-trisulfonic acid trisodium salt), utilised in previous studies$^9$ was therefore investigated. Figure 2d illustrates the additional dispersion of $^1$H signals in the $^1$H dimension resulting from the incorporation of an aromatic head group onto the amphiphile, enabling the resolution of ~160 distinct anomeric signals. HPTS-C$_{16}$ has a dual effect upon binding to oligosaccharides: (1) it causes a conformational transition to the oligosaccharide upon helix formation resulting in a downfield shift in the $^{13}$C dimension and upfield shift in the $^1$H dimension, and (2) it introduces a ring current effect resulting from the aromatic head group, which gives an additional upfield shift in the $^1$H dimension. For comparison, the spectrum of the same oligosaccharide mixture is shown in the presence of the HPTS head group alone, wherein no signal dispersion is seen (Figure 2c); HPTS itself does not bind to oligosaccharides and the ring current of the HPTS head group is only felt when the amphiphile’s tail is present to cause a strong enough binding interaction. Signals from the $\alpha$-glucan mixture in the presence of HPTS-C$_{16}$ were assigned by comparison to complexes of the pure reference compounds for the readily commercially available maltooligosaccharides up to DP 8. The non-linearity of the spectral map in the presence of HPTS-C$_{16}$ suggests an increasing influence of helix formation upon increasing length of the oligosaccharide, but an aromatic ring current effect that is most pronounced at approximately DP 10. This finding can be explained by an increase in the binding strength$^{14}$ and helix definition upon increasing oligosaccharide length ($K_C(DP_5) = 370$ M$^{-1}$ as compared to $K_C(DP_2) = 15000$ M$^{-1}$), concomitant with a reduced average influence of the ring current effect on $^1$H signals, as there are increasingly more $^1$H protons situated at a greater distance from the aromatic head group.

Compared with chromatographic methods for mixture analysis, the presence of several signals per molecular species in NMR spectra may appear to complicate the analysis of mixtures using NMR spectroscopy. However, this level of resolution provides chemical detail for instance for studying binding modes in supramolecular complexes by observing the chemical shift changes of individual sites. While the $\alpha$-anomeric region of the $^1$H-$^{13}$C HSQC spectra shown in

![Fig. 2](image-url)
Figure 2 is highly complex we noticed that the H2, H3 and H5 signals of the β-reducing end glucose unit of different length oligosaccharides in a mixture exhibited particularly well-defined and well-dispersed signals in the presence of HPTS-C8 (ESI Figure S2). Figures 3a, b and f display the three characteristic spectral regions containing signals from for the glucan mixture in the presence of 10 mM HPTS-C4. We suggest that the dispersion in the H dimension results primarily from a ring current effect and influences these protons and especially H5β and H5β due to their proximity to the aromatic head group: H5β, H3, H5 and H6 point towards the center of the helix when an oligosaccharide wraps around a hydrophobic guest.9

The importance of amphiphile tail length for the observation of this phenomenon was investigated by varying the length from C4 to C8, C and C (Figure 3c-f and ESI Figure S4). While the C4 amphiphile was as effective as C8 at dispersing the β-reducing end oligosaccharide signals, the amphiphile with a C8 aliphatic tail did not induce any significant signal dispersion possibly due to its reduced hydrophobicity and therefore poor binding affinity. Meanwhile the C8 amphiphile could disperse the signals up to a maximum oligosaccharide length of DP 10, whereafter signals for longer α-glucans were overlapped. We expect that this signal overlap is due to the fact that 10 glucose units are sufficient to wrap around an alkane with 8 carbon units and each additional glucose unit therefore has a minimal effect on the strength of the binding interaction and on the proximity of reducing end and the HPTS moiety. A similar length limit of resolution most likely exists for the C8 and C amphiphiles but this limit occurs at DP>13. The observed upper limit of DP 13 is a consequence of the low concentration of longer glucans in the mixture and thus a limitation by sensitivity rather than resolution. Signal resolution increases with increasing concentration of the added amphiphile relative to the analyte concentration and 10 mM was found to give sufficiently good signal dispersion for this 10 mg/ml oligosaccharide sample. Accordingly, lower-field NMR instrumentation (below 800 MHz) would be expected to suffice for resolving the mixture up to dp 13 in the case of sufficiently sensitive NMR instrumentation and additional amphiphile could be added as required.

Through the enhanced resolution of oligosaccharide signals in HSQC provided by the addition of our amphiphilic shift reagent, in situ quantification of oligosaccharide concentrations was made possible. Signals from the β-reducing end were integrated and the relative concentrations in the mixture were compared to chromatographic quantifications obtained using UPLC on the analyte mixture pre-derivatised with a 2-aminobenzamide fluorescent label at the reducing end (Figure 4 and ESI Figure S5). UPLC quantifications were directly compared to HSQC signal volumes for signals with DP>14. Even in the absence of correction for the different relaxation behaviour of the HSQC signals from α-glucans with different length, a convincing agreement was found between HSQC and UPLC determinations. The underestimation of HSQC signal areas for longer α-glucans is consistent with increasing relaxation losses for the longer analytes. The agreement could be further improved by the use of standard curves for quantification or by HSQC experiments that account for the differential loss of magnetization during polarization transfer pathways.15

In conclusion, we have described how the binding of tailor-made amphiphiles by mixtures of α(1-4) glucans imposes structural definition on the otherwise conformationally flexible oligosaccharides resulting in a length-dependent chemical shift change. As a consequence of the increased resolution of signals in 1H-13C HSQC spectra, the quantitative in situ analysis of a complex α-glucan mixture is made possible using NMR spectroscopy. Surfactants have previously been used in matrix-assisted diffusion ordered spectroscopy (DOSY) to affect the diffusion properties of mixture compounds and resolve them through micellar partitioning.14 Here we have shown that surfactants can also be directly used for their specific interaction as complexing agents to distinguish different analyte sizes through differential chemical shift changes resultant upon binding induced conformational transitions. We propose that a range of carefully selected supramolecular shift reagents could play a role in the NMR-based mixture analysis of homooligomers generally, and work towards this goal is on-going.

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Notes and references