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Foreword

The past decade has seen remarkable advances in both of the major types of instrumentation used in carbohydrate structure analysis: mass spectrometry and n.m.r. spectroscopy. In this Thematic Issue of *Carbohydrate Research*, we present a selection of invited papers that are representative of the new and exciting trends in the application of these methods to carbohydrate-containing biopolymers.

The introduction and wider availability of high-field instruments, developments in two- and multi-dimensional techniques, spin-locked experiments (such as HOHAHA and ROESY), increasingly sophisticated electronics, and the availability of unprecedented computing power have transformed n.m.r. spectroscopy and produced an explosion in the amount of information that can be derived from the spectra. Although many of these developments were driven by the demands of work with proteins and nucleic acids, applications to carbohydrate systems soon followed. No longer need the majority of the resonances remain unassigned and unproductive. Full spectral assignments are now available from 2D methods, or their 1D equivalents, for increasingly complex systems. Sensitivity and dispersion have been increased by the use of higher fields, improved electronics, novel techniques, and inverse detection. Nuclear Overhauser enhancement has been exploited to define spatial relationships between and within sugar residues. Coupling constants, both homo- and hetero-nuclear, can be accurately determined in two-dimensional experiments even when both resonances are unresolved.

The structures of the repeating units of polysaccharides, determined primarily by n.m.r. spectroscopy of the intact material of high molecular weight, are frequently reported. In the process, we have become aware of the extraordinary diversity of sugar residues and types of substitution that are present in Nature. However, in carbohydrate systems, such as the sugar chains of glycoproteins and the polysaccharides of plants and animals, heterogeneity is the norm and is an important factor in the modulation of biological activity. Because of their complexity, these polysaccharides are difficult to characterise directly, and the preparation and analysis of constituent oligosaccharides enable many subtle assignments that can be applied to the intact system.

With the wealth of data available from modern n.m.r. experiments, studies of conformation and dynamics have become increasingly important. Although n.O.e.s and coupling constants remain the major data, several novel correlations have recently been recognised and are being developed as tools for the future. Three-dimensional models of oligo- and poly-saccharides can be built from these constraints, and refined by the methods of molecular mechanics and molecular dynamics, and by spectral simulations. The high accuracy with which the spectral data can be determined shows that the static models for carbohydrate conformation are incomplete, and that the dynamics of these systems must be taken into account. Again, simulations by molecular dynamics play a vital role in developing our understanding of this problem.

Speculations about future trends are always personal and a temptation to fate, but some clues about the development of n.m.r. spectroscopy of carbohydrates may be found in recent work on proteins and nucleic acids. On the instrumental side, field-gradient probes offer increased sensitivity in two- and multi-dimensional experiments and a reduction in spectral artefacts. The power of three-dimensional methods to resolve overlapping resonances is now clear, but the heteronuclear 3D experiments are usually carried out with isotopically labelled samples, and improved synthetic and biosynthetic routes to such carbohydrates will be needed. Shaped pulses have been used to improve selectivity and have allowed the development of 1D equivalents of the 2D experiments; the 2D equivalents of 3D experiments are now appearing and offer further spectral simplification. Recent work on proteins has emphasised how the reliability of the final structures improves with an increase in the constraints applied. Although carbohydrates give notoriously few inter-residue interactions, methods are becoming available to maximise the data available. The ^{1}H-n.m.r. spectra of underivatised oligosaccharides or polysaccharides have usually been obtained on solutions in deuterium oxide, but the value of the hydroxyl and amide protons as probes of conformation and hydrogen bonding should not be underestimated. Some of these data can be obtained from solutions in dimethyl sulfoxide, but techniques to probe hydrogen bonding in aqueous solvents will become increasingly important.

Many recent n.m.r. studies of proteins and nucleic acids have investigated the dynamics of these systems. Large amounts of data are available from homo- and hetero-nuclear n.O.e.s, relaxation rates, and time-avaraged coupling constants. The relaxation rates of less sensitive nuclei can be probed at higher sensitivity by inverse detection in the proton domain, which improves sensitivity, and relaxation experiments in the rotating frame (T_1) opens windows into otherwise inaccessible time scales. A detailed understanding of the dynamics of polysaccharide systems from n.m.r. data is an exciting prospect for the future.

The biological activity of many carbohydrates arises from their interaction with a protein, whether covalently attached as in glycoproteins or as ligands. Using isotopically labelled ligands, half-filtered experiments have been used to collect the proton spectrum of the bound ligand whilst eliminating resonances from the protein, or to highlight those n.O.e.s arising from interactions of the ligand with the protein. A detailed knowledge of the conformation of the bound ligand and its interactions with the protein will be enable old hypotheses to be tested, current structure–activity relationships to be explained, and a new era of carbohydrate biochemistry to begin.

The m.s. papers in this Issue illustrate the wide variety of techniques currently being exploited in the analysis of carbohydrate structures whilst, at the same time, emphasising the pre-eminence of f.a.b.-m.s. which is the method used in more than half of the studies. It is now just over a decade since f.a.b.-m.s. was first introduced and its immense potential for solving the structures of minute quantities of complex carbohydrates is still not fully realised. New strategies and novel applications continue to be introduced, as exemplified by the variety of research reported herein.

F.a.b.-m.s. has an unrivalled track-record for solving a broad spectrum of

carbohydrate structural problems. It gives information on molecular weight which is especially valuable for analysing mixtures and for detecting such functional groups as methyl, acyl, phosphate, sulphate, *etc.*, whilst data on fragments ions are important for detailed structure assignment. A variety of strategies have been devised to maximise the information content of f.a.b. spectra. Whilst the sequencing-capabilities of f.a.b.-m.s. have been very widely exploited over the past decade, it is only relatively recently that the potential of f.a.b.-m.s. for providing linkage and stereochemical information has been explored in any depth. F.a.b.-m.s. strategies described in this Issue include the well established use of derivatisation to direct fragmentation, the analysis of products of oxidative cleavage, and the application of collisional activation technology to enhance fragmentation.

Whilst f.a.b.-m.s. is undoubtedly a very powerful tool, it should be remembered that the less glamorous 'old-fashioned' techniques of e.i.- and c.i.-m.s. continue to play a vital role in the analysis of carbohydrate structures. For example, a large proportion of these analyses are underpinned by linkage and compositional studies performed by g.l.c.–m.s., experimental methods for which were devised in the sixties and seventies and have remained largerly unaltered in the last decade. G.l.c.–m.s. remains the method of choice for linkage analysis in most laboratories despite continuing attempts to replace it with newer m.s. technologies. It will be interesting to see wether electrospray(e.s.)-m.s. studies of the type reported herein will become a genuinely viable alternative to g.l.c.–m.s.. In any event, irrespective of its applicability to linkage analysis, e.s.-m.s. is certain to have a bright future in carbohydrate structure analysis, especially for such biopolymers as glycoproteins where its potential for providing molecular weight data on the intact molecule is an exciting prospect for future work aimed at elucidating the biological purpose of the existence of multiple glycoforms.

At the beginning of the 1980's, very little m.s. data were being recorded above masses of about 2,000 Da and hundreds of micrograms of sample were required for most analyses. At the beginning of the 1990's, sector-type mass spectrometers operate routinely up to 15,000 Da at full sensitivity and it is normal to work with low to sub-microgram quantities of materials. Direct analyses of intact biopolymers of high molecular weight (up to more than 100,000 Da) are now feasible using electrospray and laser methods of ionisation although, as yet, the number of applications of these techniques to carbohydrates is relatively small. The next few years should see a realisation of the true potential of these instruments for high-mass work in the carbohydrate area. A flurry of recent activity in the field of array detection now permits sensitivity improvements of up to two orders of magnitude on the magnetic-sector instruments. With all these exciting developments, it is not unreasonable to speculate that a decade from now the challenging problems of today will have become routine at the nanogram level and the detection of molecular ions of 100,000 Da or more will be unremarkable.

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