Examining Cinderella

The 7th Harden Discussion Meeting on 'Physical Methods for Glycopolymer Characterisation', Eynsham Hall, 11 - 13 September 1992

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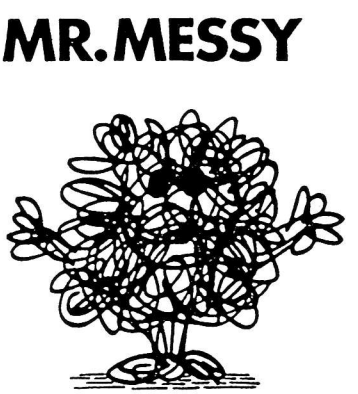
"POLYHYDROXY COMPOUNDS have for long been the Cinderellas of physical chemistry" said Felix Franks at a Techniques Group colloquium in 1988[1]. What he was saying was that polysaccharides and related glycopolymers have always been the poor relation, in terms of structure and physicochemical properties in solution compared to other classes of macromolecule, in particular — of course — proteins and nucleic acids. He also identified the growing interest in glycobiology and his comments stimulated one of the organisers to think about pulling together for a weekend under one roof, experts in the physical chemistry of polysaccharides and glycoconjugates. The eventual result, some four years later, was the 7th Harden Discussion Meeting, 'Physical Methods for Glycopolymer Characterisation' organised by myself, K. Vårum, S. Paoletti and E. Hounsell.

There were 22 invited speakers (8 UK, 4 Italian, 3 Norwegian, 2 German, 2 USA and 1 each from Austria, France and Belgium) one of whom was also a discussion leader, and 5 other discussion leaders (from Italy, Sweden, Norway and the UK (2)). Besides the invited people there was room for another 37 registrants (many of them at the postdoc. or postgraduate level, together with representatives from industry), from 8 countries.

In the Opening Address it was explained how the meeting came about and its purpose. The 'Cinderella' theme for glycopolymers was first of all explained along the lines that our knowledge of the structure and behaviour of these molecules is still some two decades behind that of the more "fashionable" molecules of biochemistry (namely proteins and nucleic acids), and for reasons largely deriving from the difficult heterogenous nature of glycopolymers. The meeting was therefore constructed to help address this by considering a range of ideas and expertise together. The aim of the meeting was thus stated to be the exchange of ideas and protocols between the polysaccharide and glycoprotein communities who so often in the past have been totally unaware of significant developments in methodology in each other's field.

The meeting was configured so that the so-called "fine structure" of "high resolution" techniques (X-ray diffraction, electron microscopy, NMR, mass spectrometry, molecular modelling, circular dichroism) were considered first, building through to the gross conformation, size distribution and interaction probes (gel electrophoresis, chromatography, ultracentrifugation, rheology, light and X-ray scattering and other thermodynamic probes). The first two sessions on Friday evening (with talks by Stokke, Sheenan, Atkins, Rizzo and Laggner) considered the classical "solid state" techniques of X-ray diffraction and electron microscopy. X-ray diffraction focussed on crystallography (where possible) and fibre diffraction, as well as considering the potential of X-ray spectroscopy and the availability of new synchrotron X-ray sources in Europe — particularly one in Trieste. The two talks on Electron Microscopy involved an interesting exchange of ideas between the polysaccharide and glycoprotein speakers.



(Courtesy of the Hargreaves Organisation)

A typical glycopolymer.....

On Saturday morning (with talks by van Halbeek, Grasdalen, Torri and short presentations by Gidley, Oreste and Andersen) NMR took the limelight and the different lines of attack applied to glycoproteins, heteropolysaccharides and sulphated glycosaminoglycans and related polysaccharides could be compared and contrasted. The afternoon session on Saturday (with talks by Dell, Morris, Brant and short presentations by Mulloy, Wait, Williamson, Foster, Gidley and Skjåk-Braek) focussed on the range of other fine structure probes, and besides circular dichroism, molecular mechanics/dynamics, and infra red probes, the power of the Mass Spectrometer caught delegates' imagination.

The Sunday morning session — starting at 8.30a.m.! — including talks by Gallagher, Berth, Harding, Ross-Murphy, Hardingham and short presentations by de Smedt, Medin, White and Jumel) considered first electrophoresis probes, showing in particular how these have enhanced our understanding of the structure of heparin sulphate. Gel chromatography was then examined demonstrating the importance of using an absolute molecular weight detector whenever possible: although a light-scattering photometer was the most convenient, extreme care was still necessary when dealing with macromolecules like pectin which can be chemically as well as physically heterogenous. Ultracentrifugation (an old, vastly underrated but powerful — particularly for interaction studies — relic now reborn, so says this totally unbiased meeting co-organiser!) was scrutinised. With the two talks on Rheology, again there was an interesting exchange of ideas between the polysaccharide and glycoconjugate speakers.

The final session on Sunday afternoon saw two talks (by Dautzenberg and Reynaers) on scattering techniques, one more theoretical (focussing again on the importance of care in interpreting data from certain polysaccharide systems

because of aggregation and heterogeneity problems), the other more experimental (showing how various scattering techniques have contributed in particular to our understanding of the structure of carrageenans). The final two talks (by Cesaro and Vårum) together with a short presentation by Anthonsen, covered molecular association phenomena — particularly involving interpretation of light scattering data and thermodynamic considerations particularly applied to glycopolymer polyelectrolytes. The Final Round-up, at 4.30p.m. on the Sunday, confirmed that the goals stated in the Opening Address had been achieved.

This being the third meeting I have helped organise in four years, it was a considerable relief to be supported in a substantial way by the Harden Committee to such an extent that the pursuit of extensive external sponsorship was not the overriding concern this time, although the substantial support from Fidia Ltd. and the Norwegian Chemical Society (Macromolecules Division) proved invaluable and enabled substantial bursaries to be offered to the younger scientists attending. And, as the organisers of the 6th Harden Discussion Meeting (A. D. B. Malcolm and R. Cotton) found[2], Eynsham Hall provided an ideal location for this type of occasion, with everybody feeling relaxed, happy and in a mood for hard work: an ideal recipe for good discussion. Although the schedule was extremely crowded scientifically (Friday's session finished at 10.00p.m.) there was still room for 4 separate sessions of short presentations which were highly successful. The Madrigal performance on Saturday evening given by a group from Worcester Cathedral was greatly enjoyed, as was the dinner in which an Italian-Norwegian "flavour" was highly evident.

But this was more than just a mutual "pat-on-the-back" exercise between the glycoconjugate and polysaccharide communities and a number of points became very clear during the meeting:

1. The value of getting the polysaccharide and glycoconjugate communities together to discuss/exchange ideas and experimental protocols

2. The need for extreme care when handling and interpreting data from these substances (this was stressed particularly by the polysaccharide community)

3. In return it was clear that workers on polysaccharides could learn from the detailed biochemistry which the glycoconjugate people have had — by necessity — to apply to their samples. The need to check for any free protein/nucleic acid etc. contamination by, for example, isopycenic density gradient centrifugation and chromatographic procedures was evident (Poly-saccharide Community: take note!).

4. Perhaps the most overriding message that came across was the need to gather information from more than one independent technique before firm conclusions about the size, structure or interaction properties of a macromolecule could be made: a comment which applies to more than just Cinderella molecules. ◆

References

1. Franks, F. (1988) in "Dynamic Properties of Biomolecular Assemblies" (Harding, S. E. & Rowe, A. J. eds.), pp224, Royal Society of Chemistry, Cambridge UK.
2. Malcolm, A. D. B., (1992), *The Biochemist*, 13 (5), pp22-23.

How Carbohydrates Cross the Membranes of *Escherichia coli*

A report of the Biochemical Society Lecture to the Edinburgh Microbiology Society, given by Professor Sir Hans Kornberg, November 1992.

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JAQUES MONOD once said "What is true for *E. coli* may also be true for elephants" and although this has been shown to be partially true for some features of the cell, it is not considered so for membrane transport. However, despite the fact that *E. coli* possesses an outer membrane and so a periplasm, it is possible to demonstrate some similarities between *E. coli* and animal cells in their carbohydrate transport systems.

E. coli sugar transport involves the sugar penetrating the outer membrane (which is permeable to small carbohydrates), crossing the periplasm and passing through the cell membrane. Membrane spanning proteins can control both the specificity and rate of carbohydrate transport across the cell membrane. There are three ways in which sugar can cross the membrane: by facilitated diffusion; active transport; or group translocation. An example of the former operates in the liver: a liver cell has a very low glucose concentration (in comparison to that in the blood, which is about 5mM) and thus the transmembrane protein need only act as a gate to allow glucose along a concentration gradient. However, for most of the time an *E. coli* cell in the gut has very low levels of food available, due to the large numbers of bacteria present and therefore an energy utilising process is required to transport sugars against a concentration gradient. The process is known as active transport and this energy conversion usually involves phosphorylation.

Two main types of active transport are found in *E. coli*. One type involves a system where periplasmic soluble binding proteins bind to sugars. These proteins are usually 35-60kdal in size and exhibit very tight binding to their specific sugar (typically kD 10^{-9} M). The proteins transport the sugar to transmembrane proteins, where a conformational change is likely to occur in the binding protein allowing it to release the sugar. The transmembrane protein can then translocate the sugar across the membrane using energy derived from ATP hydrolysis via a cystolic protein.

The different periplasmic sugar binding proteins show similarities in structural features, such as the pocket in the protein where the sugar binds to multiple amino acid groups via the hydroxyls on the particular sugar. This gives the tight and specific binding. There are a