U. S. Department of Energy-Sponsored
Workshop to Establish Databases of Carbohydrate Spectra
August 18-20, 1995, Seattle, Washington

Venue, sponsor, and organizers of the workshop. The workshop convened in the Westin Hotel in Seattle during the two days directly preceding the XIII International Symposium on Glycoconjugates held at the same hotel. The workshop was sponsored by the U. S. Department of Energy and organized by the Complex Carbohydrate Research Center of the University of Georgia (USA) and the Department of Bio-Organic Chemistry of the University of Utrecht (The Netherlands).

Purpose and objectives of the workshop. The workshop was organized to formulate guidelines for establishing spectral databases of complex carbohydrates. The databases will enable the scientific community to avoid the great waste of research effort and funds that frequently occurs when carbohydrate chemists are forced to duplicate the structural characterization of previously characterized complex carbohydrates. Chemists waste their effort on repetitive characterizations because in the absence of spectral databases they are unaware they are analyzing a known molecule until they have completely determined its structure. Chemists will be able to avoid much of this wasted effort when the collections of mass and of nuclear magnetic resonance (NMR) spectra initiated at the workshop are subsequently developed into searchable databases. Then scientists only need query the databases with the spectrum or with information defining the spectrum of an unidentified carbohydrate to find out if it has been previously characterized.

Elucidating the functions of complex carbohydrates depends on knowledge of their structures. There has been a great increase in the level of interest in the structures and functions of complex carbohydrates. Much of this interest stems from the increasing recognition that the asparagine-linked (*N*-linked) and serine- or threonine-linked (*O*-linked) carbohydrate chains of glycoproteins, the carbohydrate chains of glycolipids, and oligosaccharide fragments of glycosaminoglycans are involved in many of the most important diseases of man, including autoimmune diseases, inflammatory diseases, cancer, and inherited storage diseases. Complex carbohydrates are also strategically involved in many of the normal physiological functions and developmental processes of the cells of all organisms.

The importance of complex carbohydrates in medicine has drawn many industrial scientists to join the expanding ranks of academic scientists studying these molecules. Industrial efforts are also motivated by the need to identify and control the structures of the carbohydrate side chains of glycoproteins, for that is a major requirement receiving regulatory approval of recombinant glycoproteins as pharmaceuticals. A variety of other types of complex carbohydrates, including glycosaminoglycan fragments, gangliosides, saponins, cell wall polysaccharides, and lipopolysaccharides, show promise as pharmaceuticals that, like glycoproteins, require careful characterization and quality control during their manufacture in order to be approved by government regulatory agencies. Efforts are also under way in many laboratories to identify those portions of bioactive carbohydrates that interact with receptor proteins (lectins and selectins). These efforts have been extended to chemically and enzymatically synthesizing the binding epitopes (ligands) of the receptors as well as synthesizing mimetics of the ligands with the goal of developing pharmaceuticals that inhibit harmful oligosaccharide-protein interactions. Complex carbohydrates are also signal molecules (oligosaccharins) that determine the outcome of interactions between plants and microbial pathogens, and again, characterization of their receptors and the ligands of the receptors will lead to commercial products.

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Considerable progress has been made in developing methods for the structural analysis of complex carbohydrates. About 500 μg of a nearly homogeneous oligosaccharide is now required to elucidate the structure of most asparagine-linked (*N*-linked) oligosaccharides, and each such structural analysis requires about one manmonth of effort by a highly trained specialist. Unfortunately, there are relatively few individuals with the requisite training to conduct such analyses. Furthermore, much of the effort of the few chemists capable of determining the structures of complex carbohydrates is wasted because, for example, about 90% of the *N*-linked oligosaccharides that they characterize have been previously characterized. The analyst often determines that the oligosaccharide under study had been previously characterized when he or she completes the structural analysis and uses the CarbBank search routine to compare the deciphered structure to the structures contained in the Complex Carbohydrate Structure Database (CCSD).

The Complex Carbohydrate Structure Database (CCSD). The great interest in complex carbohydrates has led to an explosion in the number of published complex carbohydrate structures. Most of the published structures of complex carbohydrates are contained in the CCSD, a database organized by a 1986 workshop that like the present workshop was sponsored by the U.S. Department of Energy. The success of the CCSD has depended on the cooperation of investigators from many countries and on the financial contributions of agencies from a number of these countries.

The CCSD is a searchable database designed to contain the structures of all published complex carbohydrates together with text files that provide such information as the citation of the publication containing the structural characterization, the biological source of the carbohydrate, and for example, the associated protein and point of attachment of the carbohydrate when the carbohydrate is part of a glycoprotein. Many of the structures in the CCSD have been reported in the literature multiple times. Each such report is a separate, cross-referenced record in the CCSD. The latest release of the CCSD contains more than 42,000 records, each assigned a unique accession number. The workshop participants recognized that the task of developing spectral databases will be simplified by attaching the CCSD accession number to each spectrum, which provides the analyst access to the structure and associated text information of the complex carbohydrate that generated the spectrum.

Relatively few spectra are required to generate a useful database. The number of records in the CCSD is increasing rapidly. In 1995 alone, 15,000 to 20,000 records were published, which is about four times as many as were published just four years ago. Most of the structures of complex carbohydrates published today are identical to structures already contained in the CCSD that were isolated from other organisms, tissues, cells, or glycoconjugates. Since each substance entered into the CCSD is assigned its own accession number, a single oligosaccharide has as many accession numbers as the number of times the oligosaccharide is listed in the database. To facilitate the development of collections of spectra, the CarbBank/CCSD administrators have agreed to attach a substance identifier number which will remain invariant for all entries of a substance in the CCSD. The substance identifier number will lead the user to all CCSD records of the substance.

The workshop participants agreed that a separate collection of spectra should be formed for each class of complex carbohydrate. Thus the spectra of *N*-linked carbohydrate side chains of glycoproteins will be stored in one collection and the spectra of *O*-linked (serine-/threonine-linked) side chains in another. The spectra of glycolipids, glycosphosphoinositols, glycosaminoglycans, bacterial polysaccharides, lipopolysaccharides, saponins, etc., will each be located in a separate collection. Partitioning in this manner will enable the databases to be managed by curators who are experts on the spectra in their collections and will reduce the number of spectra in each collection, further simplifying the work of the curators.

The collections will often be further divided into those that contain mass spectra and those that contain NMR spectra, again making the responsibilities of curators more manageable. However, any one complex carbohydrate may have a variety of types of mass spectra or NMR spectra associated with it. The mass spectra might include fast atom bombardment, electrospray, matrix-assisted laser-desorption, electron impact and/or chemical ionization spectrometry. Separate collections or databases will be organized for each type of low molecular weight compound, such as the partially methylated alditol acetates that are widely used to determine glycosyl linkage compositions. Standard protocols will be distributed (see below) for acquiring mass spectra of low molecular weight carbohydrates so that the spectra of samples being analyzed can be compared more readily to the spectra in the databases.

The workshop participants noted that dividing the spectra according to type of complex carbohydrate reduces the number of entries in each data collection. For example, the CCSD has a total of ~42,000 records, but contains only ~3500 records of *N*-linked chains. Furthermore, because many structures are listed more than once, the CCSD contains only ~1100 unique records of *N*-linked chains. The data collections will strive toward acquiring at least one high-quality spectrum of each complex carbohydrate that has been published (for example, a spectrum of each of the 1015 unique *N*-linked chains). An attempt will be made to further enhance the value of the data collections by attempting to include in the collections several independently acquired, high-quality spectra of each carbohydrate, for this would reduce errors in assigning structures and would improve the quality of the databases to be developed from the collections.

Acquiring all the requisite spectra for any type of complex carbohydrate is likely to be achieved only rarely. However, it is a relatively straightforward task to acquire sufficient spectra to account for the majority of each type of complex carbohydrate that is analyzed. For example, the spectra of the 61 most frequently characterized *N*-linked chains accounted for 50% of the 3162 records of *N*-linked chains in the CCSD in August 1995, and the spectra of the 322 most frequently characterized *N*-linked chains accounted for 78% of the 3162 records. In another example, the CCSD had 5692 records of ceramides of which only 788 were unique. A collection of just 19 spectra of the most frequently characterized ceramides accounted for 50% of the 5692 ceramide records, and 105 spectra accounted for 80% of the records. Thus, even databases with limited numbers of well-selected, high-quality spectra would greatly reduce the effort wasted by carbohydrate chemists when they structurally characterize known carbohydrates.

Collections of mass spectra. Mass spectra of large complex carbohydrates are valuable but less definitive than the spectra of smaller carbohydrates because slight variations in experimental conditions can cause large variations in the spectra of larger molecules. Furthermore, the structures of large molecular weight carbohydrates are often deduced from the mass spectra of oligosaccharide fragments of the parent molecule. Whenever possible, all of these mass spectra should be included in the collections. Even the mass spectra of mixtures can be valuable, such as the array of molecular and fragment ions generated from the mixture of oligosaccharides attached to one site on a glycoprotein. As the number of available mass spectra of large carbohydrates increases, much useful information will be obtained. Since it is not yet possible to define protocols for acquiring mass spectra of larger complex carbohydrates, in order for their spectra to be of value the details of the instrument settings and experimental conditions used to acquire each spectrum must accompany it. Guidelines for the information needed in these "data sheets" will be published by the curators in letters to the editors of leading carbohydrate journals and in the CHO-DATA listserv (see below). The text information accompanying a spectrum will include the CCSD accession number to provide access to the structure of the molecule, its calculated molecular weight, and appropriate literature citations.

Collections of NMR spectra. NMR spectra will include, when available, both proton (^1H) and carbon-13 (^{13}C) spectra. The ^1H -NMR spectra will be acquired at a field strength of 500, 600, or 750 MHz. The identity and chemical shift (off-set) of the reference compound used to acquire the ^{13}C - and ^1H -NMR spectra must be included. The temperature, solvent, pH, and instrument settings used to acquire a spectrum will also accompany the spectrum, and these conditions will be standardized as much as possible. The most useful conditions *vis à vis* the data collections for obtaining one-dimensional ^1H -NMR spectra will be agreed upon and published. The parameters will be based as closely as possible on those specified by BioMagResBank of the University of Wisconsin to acquire its database-compatible NMR spectra of proteins. However, spectra obtained using other conditions will also be accepted in the collections, but spectra of new complex carbohydrates will be entered into the collections only following acceptance for publication of a refereed paper describing the structure.

Accessing archived spectra currently requires a major effort. A great majority of the records in the CCSD contain structures that were deciphered with the assistance of mass spectra and NMR spectra. These spectra, if they still exist, were generally stored on poorly documented magnetic tapes often by workers who have since left a particular laboratory. It would be a considerable undertaking to find and confirm such archived spectra. Furthermore, the spectra were often acquired under instrument settings or solvent conditions that would not be suitable for comparison with spectra in a searchable database. For example, many of the spectra analyzed successfully by experts came from impure preparations that would reduce or negate their value for inclusion in databases with standardized parameters that will be searched electronically. It would often be easier, and in many cases essential, to isolate and characterize the complex carbohydrate again rather than to utilize the data of previously characterized spectra in the databases. The workshop participants agreed that it is important to initiate electronic data collection sites so that well-documented, high-quality spectra acquired in the future will no longer be lost.

Collections of carbohydrate spectra have already been initiated. The Utrecht group has developed Sugabase, a large, useful database centered on the structural-reporter-group concept of well-defined chemical shifts of ^1H - and ^{13}C -NMR signals obtained from the spectra of various types of complex carbohydrates. Sugabase contains, for example, the defined chemical shifts of the ^1H -NMR spectra of ~550 *N*-linked carbohydrate chains, representing roughly half of the *N*-linked chains in the CCSD. Sugabase also contains the corresponding data for >300 *O*-linked chains and the ^{13}C chemical shifts of several hundred oligosaccharide repeating units of polysaccharides.

The chemical shifts listed in Sugabase are sufficient in many instances to identify the complex carbohydrate. Sugabase can be downloaded from the Internet and then queried by presenting it with a list of the chemical shifts of an unidentified complex carbohydrate to ascertain whether Sugabase contains information to identify a complex carbohydrate with the same chemical shifts. Each complex carbohydrate in Sugabase has the CarbBank accession number attached to it so that the structure and associated text file of a complex carbohydrate matching a query is readily available to the investigator. Furthermore, Sugabase will provide the predicted chemical shifts for all records in the CCSD for which Sugabase contains chemical shift data. Although most NMR spectra will be exchanged and transmitted as free induction decays (FIDs) in a to-be-agreed-upon digital format, the workshop participants agreed that all available NMR spectra of complex carbohydrates should be submitted to Sugabase as lists of the chemical shifts of identified signals.

Several other collections of carbohydrate spectra have been started. The Stockholm and Ottawa groups have an extensive collection of ^1H - and ^{13}C -NMR spectra of bacterial polysaccharides, the Athens and the London groups have a collection of the ^1H -NMR and mass spectra of *N*- and *O*-linked carbohydrates, and the Athens group has collections of the electron-impact mass spectra of partially methylated alditol acetates and of partially

methylated anhydro alditol acetates. The Bonn and Heidelberg groups have an extensive collection of NMR and mass spectra of glycolipids, and similar spectra are probably available in Gothenburg. The Dundee group has a collection of the spectra of glycoposphoinositols, and the Japanese have large numbers of saponin spectra. The workshop participants constructed a set of recommendations designed to "grow" these and yet-to-be-formed data collections into useful databases.

Recommendations.

1. An Internet listserv will be organized to facilitate e-mail communications between those wishing to establish and utilize collections of carbohydrate spectra. William York has already established the CHO-DATA listserv. The membership of the listserv includes those in attendance at the workshop who provided their e-mail addresses for this purpose. Other scientists can join the listserv by sending an e-mail message to:

listserv@uga.cc.uga.edu

with the body of the message containing the command:

sub CHO-DATA <first-name> <last-name>

The initial CHO-DATA listserv members and their e-mail addresses are:

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 William York

24

2. Carbohydrate spectra data collections will be organized by volunteer curators at FTP (File Transfer Protocol) sites on the Internet. The sites will be connected electronically to form an interactive distributed collection of carbohydrate spectra. Limitations on the quality and usefulness of the collections will result primarily from the quality (purity) of the complex carbohydrates from which spectra are obtained.

3. All carbohydrate spectra will be made freely available to anyone who wishes to download them from the FTP site. Each time a spectrum is used to identify a carbohydrate and the result is published, the author should cite both the collection that provided the spectrum and the original publication in which the spectrum was characterized.

4. Carbohydrate scientists will be urged to submit their published spectra to the appropriate FTP sites using standardized protocols available at each FTP site. The protocols will also be sent to all subscribers to the CHO-DATA listserv. The existence of the FTP sites will be further advertised by letters to the editors of major carbohydrate journals.

5. The spectral collections will be associated with the CCSD by attaching to each spectrum the appropriate CCSD substance identifier number.

6. The collections of spectra will form the foundation for the formation of databases with easy-to-use search engines that utilize appropriate semantics and syntax. It is hoped that one or more databases will be formed from each collection of spectra. The databases may be formed by academic or commercial groups.

7. Those who develop databases from the collections of spectra may, if they wish, charge a fee for use of the databases.

8. An international Board of Overseers will be organized to stimulate the organization of collections of spectra by attracting and coordinating curators, recommending standards, and encouraging the conversion of the data collections into databases. The Board of Overseers will also publicize the data collections and any resulting databases and will encourage continuation of the international cooperation exhibited by the workshop participants. The Board will ordinarily meet in conjunction with an annual international carbohydrate symposium, coordinated with the meeting of the CCSD Board of Overseers (the two boards have overlapping interests and members).

9. The Chair of the Board of Overseers will conduct the affairs of the Board with the assistance of a small, executive committee, which the Chair will select from members of the Board of Overseers. Hans Vliegthart, who has agreed to chair the Board of Overseers, will select its members. Candidates for the Board include: Albersheim, Bock, Brisson, Bundle, Costello, Dell, Doubet, Jansson, Valafar, Van Halbeek, and York. Japanese representatives will be recommended by Kobata.

FTP sites for spectra collections. The workshop participants agreed to initiate FTP sites containing collections of spectra as described in the following table.

Type of Complex Carbohydrate	Type of Spectra	Curator	Comments
Various	NMR	Vliegenthart, Van Kuik	Reside in Sugabase, an established chemical shift database
N-linked chains,	NMR	Van Halbeek	
Xyloglucan	Mass, NMR	York	
Methylated alditol acetates	Mass, NMR	Valafar	
O-linked chains	NMR	Hounsell (?)	
Larger carbohydrates	Mass	Costello, Dell	Electronic spectra with extensive data sheet
Glycophosphoinositols	Mass, NMR	Homans, Ferguson (?)	
Glycosphingolipids	Mass, NMR	Peter-Katalinic	
Bacterial polysaccharides	Mass, NMR	Jansson, Brisson	
Saponins	Mass, NMR	Takeda (Tokyo)	
Glycosaminoglycans	Mass, NMR	To be selected	

Candidates for the Board of Overseers (to be selected by Vliegenthart):

Closing comments. The databases to be developed as a result of this workshop will accelerate progress in glycoscience by making knowledge of the structural biology of complex carbohydrates more accessible and by opening up the structural characterization of complex carbohydrates to analysts with little formal training in carbohydrate chemistry. These advancements will benefit the pharmaceutical and agrochemical industries as they seek to develop an enhanced ability to control the quality of carbohydrate-based products, improve the design of mimetics, and better define the epitopes of antibodies, lectins, and receptors, which will be used for therapeutics, diagnostics, delivery of drugs to specific cells, and control of abnormal regulatory phenomena. Improvements in the structural analyses of complex carbohydrates will improve technologies applicable to the development of renewable energy sources, dietary fiber, and the viscous molecules used for such diverse functions as enhanced oil recovery, food thickeners, and paint. Indeed, more rapid progress in glycoscience will stimulate the rate of progress in many areas of biotechnology.

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