Databases of complex carbohydrates

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The current general interest in complex carbohydrates[1–6] ranges from the physical properties of polysaccharides, to the involvement of glycoconjugates in biological recognition. The latter feature is extremely important since the carbohydrate parts of glycoconjugates such as glycoproteins and glycolipids play key roles as recognition determinants in protein targeting, cell–cell interaction, and as cell-surface receptors. For the biotechnological production of recombinant glycoproteins in heterologous cell types, it is important to understand the influence that sugar chains have on the characteristics of these compounds. The carbohydrate moieties of glycoproteins are determined by the origin and type of the cell in which the proteins are expressed and, to some extent, by the structure of the protein.

The enormous structural diversity of oligosaccharides arises from the wide variety of component monosaccharide residues, each of which, in turn, can have different anomeric configurations and ring forms. These residues are connected via inter-glycosidic linkages at different positions, and can also contain non-carbohydrate substituents. The primary structures of thousands of naturally occurring carbohydrate chains (isolated from many different sources) have been reported together with information on their biological and/or physical properties (e.g. their ability to bind to lectins or to antibodies, and conformational data).

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A database of complex carbohydrate structures

The Complex Carbohydrate Structure Database (CCSD) was created[7] (Complex Carbohydrate Research Center, Univ. Georgia, Athens, GA 30602, USA) to facilitate access to the enormous number of published carbohydrate structures. The CCSD aims to include the primary structures of all naturally occurring carbohydrates that are larger than disaccharides, together with references to the papers where these structures were originally reported. The format is that of a flat-file database (i.e. each CCSD record contains the primary structure of one carbohydrate chain, one bibliographic citation, and associated text; Fig. 1). The CCSD now contains nearly 8000 records, and ~5400 unique carbohydrate chains, but is not yet up-to-date in representing all the currently available information, and is still being compiled. The corresponding database-management program 'CarbBank' runs on IBM-compatible microcomputers under the MS–DOS operating system. Program and data together require 14 megabytes (Mb) of disk space and

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updates are released every six months. (See Box 1 for contact addresses for further information about the computer databases mentioned in this article.)

Carbohydrate structures entered into the CCSD are acquired from literature by specialist curators, and by co-operation with Chemical Abstract Services (CAS). Data obtained from CAS have to be rigorously processed before being entered into the CCSD. This processing is done, in the first instance, by a computer program that translates and merges the CAS bibliographic data and structural data to create CCSD records. The output is then verified by the curators, who compare the records against the original literature. The large number of corrections that are made by the curators demonstrates that this verification is essential in producing a high quality database.

The CarbBank program is specifically designed to enable fast searches for branched carbohydrate structures to be made, taking into account that it would be extremely difficult to build a satisfying search-profile from scratch (Fig. 2). To enter a carbohydrate structure that is a reliable search profile for CarbBank requires experience and a thorough understanding of the carbohydrate nomenclature. Furthermore, typing errors are easily made when a branched structure is entered: the D or L configuration, the anomeric configuration, the ring-form, and the non-carbohydrate substituents must be correct and complete.

```
; start of record
; Database= CCSD5.C22
; Record #= 2866
CC: CCSD:5170
AU: Conradt HS; Ausmeier M; Dittmar KEJ; Hauser H; Lindenmaier W
TI: Secretion of glycosylated human interleukin-2 by recombinant
mammalian cell lines
JL: Carbohydr. Res. (1986) 149: 443-450
FC: 132efc31
NT: lymphokines and cytokines
BS: Human Interleukin-2, IL-2 N2
BS: recombinant Human Interleukin-2, IL-2 N2
BS: mammalian cells
KW: glycosidation
KW: Interleukin-2, IL-2 N2
VS: verified; Paulsen H
DA: 27-11-1990
CA: 105:22816j
PR: PIR1:ICHU2
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structure:

                α-D-Neup5Ac-(2-6)┐
                                 │
                              D-GalNAc
                                 │
 α-D-Neup5Ac-(2-3)-ß-D-Galp-(1-3)┘
================end of record
```

Figure 1

An example of a single CCSD record. Each record contains a carbohydrate structure, bibliographic data, and additional information.

The residue-complex concept

To make it feasible for inexperienced users to search for branched carbohydrate structures, CarbBank uses the 'residue-complex' concept. A residue-complex is a structural element that comprises a monosaccharide residue, together with all residues that are attached to it. With this residue-complex, the program searches for all carbohydrate structures that contain this element. Ths user is guided step-by-step in the building of the residue-complex. For each step, the user is prompted to choose from a list of relevant items, which is derived from all data in the database. In this way a search-profile is built from only those items that really exist in the CCSD. The first selection has to be made from a general class of monosaccharides, e.g. Man. Next, a complete residue has to be selected from a second list, e.g. β-D-Man*p* (for carbohydrate nomenclature abbreviations, see Box 2). Subsequently, this residue is extended with linkages, from a list of linkage patterns (e.g. 1,3-linked), and finally a residue-complex can be selected (e.g. α-D-Man*p*-[1-3]-β-D-Man*p*-[1-4]-β-D-Glc*p*N [a simple non-branched example]). With this residue-complex, CarbBank produces a list that holds all the records from the CCSD that contain the selected structural element ('hitlist'). If the hitlist holds only a few structures, the easiest way to find the desired structure is to browse through it, but if the number of hits is too large, supplementary searches can be made that narrow the number of branches or monosaccharide residues, or that put other constraints by using different search profiles. If a structure is selected from the hitlist, a homology-search can give all the citations that contain exactly that structure or structures that are closely related, e.g. structures that have other non-carbohydrate substituents. In a similar way, guided searches can be made for other items (such as authors' names, journal names, year of publication, words in the title of an article, words in all text fields, non-carbohydrate substituents, molecular formulae, and nominal molecular mass).

As well as the carbohydrate structure and the bibliographical data, CCSD records also contain two other categories of information. The first category summarizes additional details that can be present in the article, such as analytical methods, binding of the carbohydrate chain to lectins or antibodies, position of attachment to the protein, the biological source and the biological activity. The second category provides linkages to other databases by giving patent numbers, and accession numbers, e.g. to CAS, International Protein Sequence Database (PIR) or the Protein Data Bank (PDB).

The CCSD is growing rapidly. In a few years it will contain at least 22000 records and will require 45 Mb of disk space. Distribution on CD-ROM (US National Library of Medicine/National Center for Biotechnology Information) is under consideration for the near future.

A NMR-spectroscopic database

The CCSD, as a database of primary structures of carbohydrate chains, can be used to build other databases that permit access to extra information to these structures. One can imagine carbohydrate databases, including fast-atom bombardment mass spectrometry (FAB-MS) or nuclear magnetic resonance (NMR) data, or databases containing three-dimensional information obtained from X-ray crystallography, NMR–NOE (nuclear Overhauser effect) measurements or molecular dynamics calculations. Indeed, a database that combines the structures of carbohydrates with NMR spectroscopic data has already been

Contact addresses, access and costs for computer databases

and CCSD

CarbBank
114 W. Magnolia St, Suite 305,
Bellingham, WA 98225, USA.

Was distributed on diskette, and will be distributed on CD-ROM
Currently unknown, due to the transition to CD-ROM

atabase and program

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Distributed on diskette
Dfl 300.00

In Denmark, Finland, Ireland, The Netherlands, Norway, Sweden, and the UK:
The Royal Society of Chemistry,
The University,
Nottingham,
UK NG7 2RD.

In Austria, Switzerland and Germany:
Fachinformationszentrum Chemie GmbH,
Postfach 12 60 50,
Steinplatz 2,
D-1000 Berlin 12,
Germany.

In Japan:
The Japan Association for International Chemical Information,
Gakkai Center Building,
2-4-16 Yayoi, Bunkyo-ku,
Tokyo 113, Japan.

In France:
Centre National de l'Information Chimique,
La Maison de la Chimie,
28 ter rue Saint Dominique,
75007 Paris, France.

In all other countries:
Chemical Abstracts Service,
Marketing Dept 30586,
2540 Olentangy River Road,
PO Box 3012,
Columbus, OH 43210, USA.

On-line
Depends on connection time

constructed (Bijvoet Center, Department of Bio-Organic Chemistry, Univ. Utrecht, NL-3584 CH, The Netherlands).

To determine the primary structure of complex carbohydrate chains, tables of ^{1}H-NMR and ^{13}C-NMR chemical shifts are used extensively. These tables are usually acquired from the literature[8–10]. Although review articles provide easy access to the data, they usually cover only a selected part of all the NMR data available, are neither corrected nor updated, and have to be surveyed manually. These are good reasons to store NMR tables in a computer database, and to develop a program for easy manipulation of the data. The database which has emerged uses a relational model to combine complex carbohydrate structures, bibliographic data, ^{1}H-NMR tables and ^{13}C-NMR tables. The carbohydrate structures and the bibliographic data are taken predominantly from the CCSD, whereas the NMR tables are collected from the literature. The database-management program runs on IBM-compatible personal computers under MS–DOS, and database and program together currently require 3 Mb of diskspace. At present the NMR data set consists of 508 tables of ^{1}H-NMR

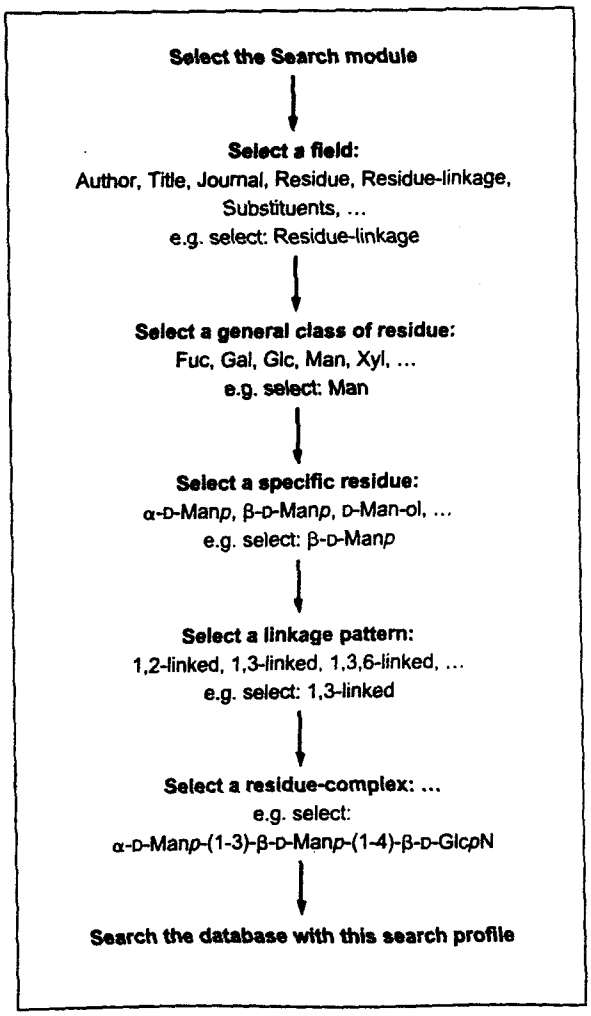


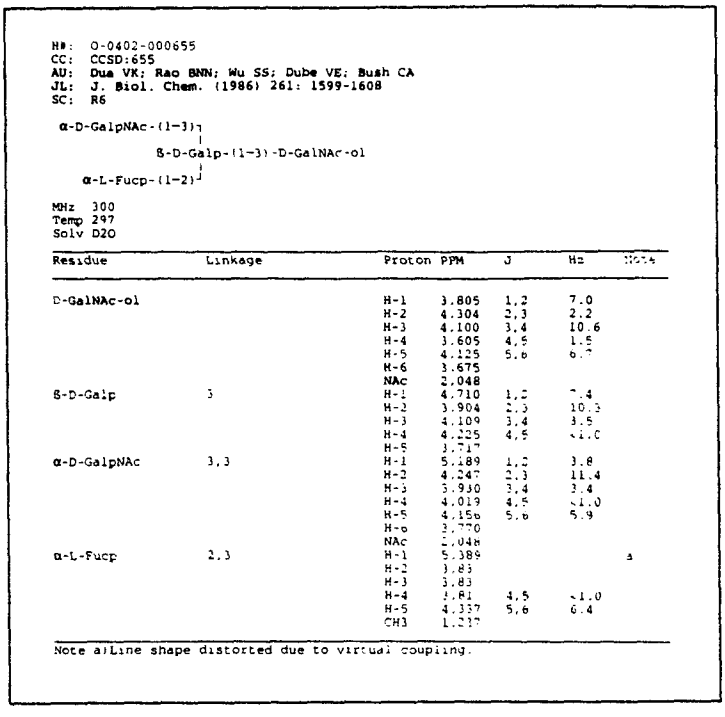
Figure 2
The steps required to build a CarbBank search profile.

chemical-shifts (Fig. 3) (essentially corresponding to oligosaccharides derived from glycoproteins), and of 237 tables of ^{13}C-NMR chemical-shifts (in general, associated with fragments of polysaccharide).

To search for the primary structure of a carbohydrate chain with the NMR database program, the construction of a search-profile is required. This profile consists of a list of chemical-shift values and an optional list of monosaccharide residues, that can be used to specify the 'background' of the chemical-shift values that produce a hit. The structures in the database are organized by carbohydrate-chain type (e.g. *N*-linked, *O*-linked, polysaccharides; see Box 2) into different sections, which can be searched separately. In addition, the minimum percentage of matching chemical-shift values per structure that will result in a hit, and the tolerated variation of matching chemical-shift values, can be defined.

After a search, the hitlist of structures and corresponding NMR tables are simultaneously displayed, such that the matching monosaccharide residues in the structures and the chemical-shift values in the tables, are highlighted. This presentation clearly demonstrates which part of the carbohydrate structure displayed is recognized by the search-profile, and which chemical-shift values are involved in this recognition. In this way, searches for known carbohydrate structures can be performed easily. A novel aspect of the program which is a major advantage is that it can also assist in the structure-determination of unknown carbohydrate chains by suggesting structural elements that may be part of these unknown chains.

As the number of published tables of NMR chemical-shift values is growing exponentially, a computerized approach of NMR data storage and structure retrieval is essential. The growing size of the NMR database inevitably leads to porting the database to other platforms, e.g. UNIX.



```
H#:  O-0402-000655
CC:  CCSD:655
AU:  Dua VK; Rao BNN; Wu SS; Dube VE; Bush CA
JL:  J. Biol. Chem. (1986) 261: 1599-1608
SC:  R6

 α-D-GalpNAc-(1-3)┐
                  │
            β-D-Galp-(1-3)-D-GalNAc-ol
                  │
    α-L-Fucp-(1-2)┘

MHz  300
Temp 297
Solv D2O
```

Residue	Linkage	Proton	PPM	J	Hz	Note
D-GalNAc-ol		H-1	3.805	1,2	7.0	
		H-2	4.304	2,3	2.2	
		H-3	4.100	3,4	10.6	
		H-4	3.605	4,5	1.5	
		H-5	4.125	5,6	6.7	
		H-6	3.675			
		NAc	2.048			
β-D-Galp	3	H-1	4.710	1,2	7.4	
		H-2	3.904	2,3	10.3	
		H-3	4.109	3,4	3.5	
		H-4	4.225	4,5	<1.0	
		H-5	3.717			
α-D-GalpNAc	3,3	H-1	5.189	1,2	3.8	
		H-2	4.247	2,3	11.4	
		H-3	3.930	3,4	3.4	
		H-4	4.019	4,5	<1.0	
		H-5	4.156	5,6	5.9	
		H-6	3.770			
		NAc	2.048			
α-L-Fucp	2,3	H-1	5.389			a
		H-2	3.83			
		H-3	3.83			
		H-4	3.81	4,5	<1.0	
		H-5	4.337	5,6	6.4	
		CH3	1.237			

Note a)Line shape distorted due to virtual coupling.

Figure 3

An example of a single ^{1}H NMR record from the NMR database. Each record contains a carbohydrate structure, bibliographic information, and a ^{1}H NMR table. In the header of the table, PPM marks the column with the chemical-shift values of the protons (or carbons), J indicates which protons are coupled, and Hz represents the values of the coupling constants.

Box 2. Abbreviations for carbohydrate nomenclature

Fuc – fucose
Gal – galactose
GalNAc – *N*-acetyl-galactosamine
Glc – glucose
GlcNAc – *N*-acetyl-glucosamine
Man – mannose
NeuAc – *N*-acetyl-neuraminic acid
Xyl – xylose

α-L-Fucp – α-L-fucopyranose
β-D-Galp – β-D-galactopyranose
β-D-GalpNAc – 2-*N*-acetyl-β-D-galactopyranose
β-D-Glcp – β-D-glucopyranose
β-D-GlcpNAc – 2-*N*-acetyl-β-D-glucopyranose
α-D-Manp – α-D-mannopyranose
α-D-Neup5Ac – 5-*N*-acetyl-α-D-neuraminic acid
β-D-Xylp – β-D-xylopyranose

***N*-linked** – Carbohydrate chain attached to a protein via the HN of Asn
***O*-linked** – Carbohydrate chain attached to a protein via the HO of Ser or Thr

Future prospects for carbohydrate databases

In the future, we expect to see the creation of new carbohydrate databases, e.g. a database of three-dimensional structures of carbohydrate chains. All databases together will provide the carbohydrate chemist with the necessary tools that are needed to keep access to the growing amount of information.

Acknowledgements

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