GlycoMapsDB: a database of the accessible conformational space of glycosidic linkages

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INTRODUCTION

Ramachandran plots (1) of protein backbone torsions $\phi/\psi$ are frequently used to validate 3D structures of proteins (2,3). The quality of a protein structure is considered to be ‘good’ when (preferably all) amino acids have $\phi/\psi$ values located in ‘allowed’ regions of the plot. For carbohydrates the glycosidic torsions $\phi/\psi$ are the main determinants of the 3D structure and it is straightforward to validate the quality of an experimentally determined carbohydrate structure in a similar way to protein structures. In contrast to proteins, however, the ‘allowed’ regions on a conformational map for a given glycosidic linkage not only depend on the linked monosaccharide types, but also on the linkage type and—a feature completely different from proteins—the degree of branching of the glycan. The number of available high quality crystal structures of carbohydrates is too limited to serve as a basis to determine the ‘allowed’ regions for all linkage types so the accessible conformational space of carbohydrate linkages has therefore to be estimated using computational methods. Consequently, over the last 30 years a considerable effort has been put into the development of force fields that are able to predict accurately the local minima and flexibility of glycosidic torsions (4). The force field most frequently used to calculate conformational maps of glycosidic linkages is MM3 (5).

CALCULATION OF CONFORMATIONAL MAPS

A variety of methods exist to calculate the energy of a carbohydrate as a function of the glycosidic torsions $\phi/\psi$ (or $\phi/\psi/\omega$ for 1–6 linkages). Traditionally systematic search methods are applied to disaccharides (6). A ‘relaxed’ map is obtained by systematically changing $\phi$ and $\psi$ in small intervals (normally $10^\circ$) and minimizing all degrees of freedom while restraining $\phi/\psi$ using an external force. Relaxed conformational maps depend on the orientation of the exocyclic torsions in the starting conformation of the carbohydrate and therefore the calculation of an ‘adiabatic’ map is advisable. To generate a fully adiabatic map the calculation of $3^{16}$ relaxed maps would be required for a simple disaccharide (7). In routine calculations the computational cost is reduced by taking into account only $gg$ and $gt$ (or $gt$ and $tg$ for monosaccharides that have the OH4 group in an axial orientation) conformations for the hydroxymethyl groups and a $clock$- and $anticlockwise$ orientation for the hydroxyl groups. This reduces the number of relaxed maps to be calculated to eight resulting in a total of 10 368 conformations to minimize for each ‘pseudo’ adiabatic map. It is obvious that this approach is limited to disaccharides since the number of
conformations to be minimized for a trisaccharide would already be \( \sim 3 \times 10^9 \) (assuming the second linkage is searched in intervals of 30°), far too many for a large-scale project where thousands of maps need to be calculated.

High temperature molecular dynamics simulation is a robust and efficient method to explore the accessible conformational space of carbohydrates (8). Conformational free energy maps can be derived from population analysis by applying the Boltzmann equation. This approach has several advantages compared to the systematic search methods:

(i) it is directly applicable to branched oligosaccharides, so that the same method can be used for disaccharides and larger oligosaccharides,
(ii) the low energy conformational space only is explored and no computational time is wasted to calculate unrealistic high energy conformations,
(iii) the required computational cost increases therefore only moderately with the number of atoms of the oligosaccharide,
(iv) the data-flow can be easily optimised in such a way that the conformational maps are generated automatically and only minimal human interaction is required.

It was therefore decided that free energy maps derived from MD simulations were a good first set of conformational maps with which to populate the GlycoMaps database. An in-house library of carbohydrate fragments (up to pentasaccharides) derived from structures described in the CARBBANK (9) and built using the SWEET-II program (10) served as input. The MM3 force field as implemented in the TINKER suite (dasher.wustl.edu/tinker/) [for a comparison with the original MM3 implementation see (11)] was used to calculate the trajectories at 1000 K. The length of the MD simulation was 10 ns for disaccharides and 30 ns for larger oligosaccharides. The carbohydrate rings were restrained to a chair conformation. The Conformational Analysis Tools (CAT) software (www.md-simulations.de/CAT/) was used for data processing and analysis.

ACCESSING AND ANALYSING CONFORMATIONAL MAPS

Conformational maps of carbohydrate linkages can be retrieved from the database by entering the disaccharide fragment in IUPAC form into the search database web interface of GlycoMapsDB (Figure 1). Wildcards are supported, which allows searching, e.g. Glcp and Glcp2NAc residues simultaneously. Maps found in the database are displayed as a list, with each entry showing a preview picture and the full structure of the carbohydrate for which the map has been calculated in extended IUPAC form (Figure 2). Difference maps can be calculated e.g. to evaluate the influence of branching on the accessible conformational space of a linkage (Figure 3). Individual maps can be explored in more detail by clicking on the preview picture. A 3D structure of the carbohydrate—generated by Sweet II (10)—can be displayed using Jmol (jmol.sourceforge.net). If experimental data for the disaccharide fragment is available in the Protein Data Bank (PDB) (12), a link is displayed, which leads to a page where \( \phi/\psi \) torsion values retrieved from PDB data [using the GlyTorsion tool (13)] are overlaid onto the calculated conformational map (Figure 4). Some statistical data regarding the fit of the experimental data to the calculated data is also displayed. The PDB structures evaluated can be accessed following the ‘show details’ link. If further information such as literature references or NMR data for the carbohydrate is available in GlycosciencesDB (14) a link to the corresponding entry in that database is displayed.

IMPLEMENTATION

GlycoMapsDB is running on a Linux PC with Apache web server software. Interaction with the user is mediated through PHP interfaces. The datasets are stored in a mySQL database. Visualization of carbohydrate structures is performed using the java applet Jmol or the plugin Chime (www.mdlchime.com). Diagrams and plots are generated in scalable vector
Currently, the GlycoMapsDB contains ~2500 conformational maps of carbohydrate fragments originally described in the CARBBANK. Conformational maps for most fragments found in glycoproteins are available in the database. The direct crosslink between calculated maps and PDB data opens a very efficient route to crosscheck the quality of experimental structures as well as the quality of the maps. In general the amount of available high quality experimental data currently available for carbohydrates in the PDB database is rather limited compared to that for proteins and for some linkages there is no experimental data available at all, so there is a clear lack of experimental reference data. In this respect, the conformational maps contained in GlycoMapsDB might help crystallographers to crosscheck their data before submission similar to the Ramachandran plot analysis for proteins. For this purpose, maps from the GlycoMapsDB are also accessed by the carp (Carbohydrate Ramachandran Plot) software (13), where users can upload a structure in PDB file format and retrieve plots comparing the torsions present in the structure with the conformational maps.

GlycoMapsDB indirectly offers an interface for ‘data mining’ in the PDB database, e.g. to find carbohydrate entries with unusual glycosidic torsion values. For β-D-GlcNAc-(1-4)-β-D-GlcNAc, a frequent fragment contained in N-glycans, the agreement between experimental and calculated data is remarkably good (Figure 4). More than 80% of the crystal structures have values in low energy areas of the conformational map. But there are also some outliers that are located in ‘not allowed’ areas of the maps. Of special interest are also those structures that have φ or ψ values of ~180° (anti conformation). The ‘show details’ link would help to find the corresponding entries in the PDB.

In the near future we will provide also conformational maps calculated with force fields other than MM3, so that the influence of different parameter sets can be investigated. An upload feature for maps will be added so that users can upload and compare their own calculated maps with the maps stored in the database. In addition, a functionality that will allow users to calculate maps not already contained in the database is planned.

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