Lecture 22: X-Ray Diffraction and Carbohydrates

GOALS:

X-ray Diffraction:
Discuss how molecular structures are obtained using x-ray crystallography
Fit electron density maps

Carbohydrates:
Distinguish between aldose and ketose
Identify the anomic carbon in a carbohydrate
Formation of ring structure
Nomenclature of cyclic sugars
Draw reduced Haworth representation

X-ray Crystallography/Diffraction
Three-dimensional structure of a protein can provide valuable information about:
1) How it functions (e.g. mechanism of action of serine proteases)
2) Protein-ligand interaction (to design allosteric modifiers: e.g. inhibitors for HIV enzymes)
3) How alterations in the structure of a protein can result in disease (e.g. sickle cell anemia is caused due to alterations in amino acid sequence of hemoglobin, which ultimately alters the structure and hence function of hemoglobin in patients)

X-ray crystallography is a very powerful technique used to determine the structure of proteins in atomic detail. Protein structure determination using x-ray crystallography/diffraction require three components: (1) a source of x-ray radiation, (2) purified protein in crystal form, and (3) a detector.

1. When a narrow beam of x-ray is directed a protein crystal, electrons in the atoms scatter/diffract the x-ray. These scattered rays are detected by a x-ray film or an electronic detector. The amplitude of scattering is proportional to the number of electrons present (carbon scatters six times more than a hydrogen atom. Hydrogens are difficult to ‘see’ in crystal structures).
2. In a protein crystal, protein molecules are oriented in a fixed, repetitive arrangement. The unit cell is the basic unit of a crystal. It is repeated over-and-over again to form the macroscopic crystal lattice. A single unit cell can contain multiple protein molecules. (http://www.ruppweb.org/xray/tutorial/Crystal_sym.htm)
3. The scattered rays recombine – interference. The scattered waves cancel if they are out of phase, and they reinforce each other if they are in phase. Interference between X-rays that are scattered from atoms in different locations changes the amplitude and the phase of the scattered X-rays. Therefore, scattered X-rays can be used to determine the position of atoms.
4. Intensities can be measured directly. Phases have to be obtained indirectly. One common method of obtaining phases is called molecular replacement, where a homologous known structure is used to calculate the phases. Other methods include incorporating heavy atoms into the crystal to perturb the scattering.

5. A Fourier transform of the intensity and phases of the scattered X-rays produces an ‘electron density map’, or the number of the number of electrons at each point in space in the crystal \( p(x,y,z) \).

6. The crystallographer must figure out how to place, or "fit", the known primary structure of the protein into this map. This is aided by computational tools.

**CARBOHYDRATES**

1. Most abundant biomolecules on earth
2. Primary source of energy in biosphere
3. Biosynthetic precursors of amino acids and nucleic acids
4. Form structural and mechanical components in cells – cell walls in plants and bacteria, part of cell-cell contacts
5. Help target proteins to different cellular compartments

**Structural Hierarchy:** Carbohydrates can be classified into three major classes:

1) **Monosaccharides:** cannot be hydrolyzed into other simple sugars (= amino acid)
2) **Oligosaccharides:** 'a few' covalently linked monosaccharides (= peptide)
3) **Polysaccharides:** 'many' covalently linked monosaccharides (= protein)

**Monosaccharides:**

Monosaccharides have the general formula \((CH_2O)_n\) – all carbons are ‘hydrated’, and hence the name carbohydrates. Monosaccharides are polyhydroxy aldehydes or polyhydroxy ketones. The backbone of common monosaccharides are unbranched carbon chains in which the carbons are linked by single bonds (open chain form).

The simplest monosaccharides contain three carbons:

- When the C=O group is at the very end it is an **aldehyde** (no chiral center)
- When C=O group is at the second position (C2), it is called a **ketone**

The aldehyde glyceraldehyde has a chiral center, and therefore exist in D- and L-forms, which are mirror images of each other. The D-form is the root form of all naturally existing aldoses, and that is why all naturally occurring sugars are "D", e.g. **D(R) glyceraldehyde**
D-glucose.

**Aldose Series:**
Additional hydrated carbons (HO-C-H) are added just below the aldehyde group. Therefore, the chiral center of D-glyceraldehyde is preserved. The added carbon generates a new chiral center. The two different molecules generated by the addition of another carbon are called **epimers** because they differ in only one chiral center. For example, erythrose and threose are epimers.

**Ketose Series:**
These are formed in the same way, the addition of a hydrated carbon below the keto group, with each addition forming a new chiral center. Dihydroxyacetone has no chiral center, but ketoses with 4 or more carbon contain chiral centers.

**Carbon numbering:** Begin at the end closest to the C=O group.
- Aldoses – C1 is the aldehyde carbon
- Ketoses – C2 is the ketone carbon

**Important sugars to remember:**
- Glyceraldehyde (C3 aldose)
- Ribose (C5 aldose)
- Glucose (C6 aldose)
- Fructose (C6 ketose)
Ring Formation:
In general, alcohols can attack the C=O group in sugars to form hemiacetals. Since sugars have OH groups, they can form hemiacetals by an intramolecular reaction, forming closed rings.

Only long (>C4) saccharides can form internal hemiacetals, giving closed rings (Includes ribose, glucose, fructose). No atoms are lost or gained in this reaction!

Ring Formation in Glucose:
1. Six membered ring created by forming a bond between C1 and O5 (most stable ring size).
2. This form is called pyranose, i.e. glucopyranose after the organic compound, pyran.
3. The C1 carbon becomes chiral and is called the anomeric carbon
4. The new OH group (on C1) can exist in either the α or β form.
5. The α and β forms can readily inter-convert via the linear intermediate.

Ring Formation in C5 Aldose, Ribose:
1. Formation of a 5 membered ring can occur by forming a bond between C1 and O4.
2. The cyclic form is called a furanose (i.e. ribofuranose) after the organic compound, furan.
Ring Formation in C6 Ketose: Fructose

Although this is a 6-carbon sugar, because it is a ketose a five membered ring is formed.

This is also called a furanose (i.e. fructo-furanose).

Representation of Structures:
Fischer, Haworth, Reduced Haworth Representations.

How to go from chemical structure to Haworth:
(i) tip clockwise, (ii) move –O- to back. (right = down, left = up)

How to identify the anomic carbon:
Bound to 2 oxygen atoms

Example: Galactose is an epimer of glucose at C4, draw β-galactopyranose, in the reduced Haworth representation:

In next lecture.

Expectations:
- Aldose versus ketose
- Structure of glyceraldehyde, ribose, glucose, fructose
- Ring formation - pyranose versus furanose, identification of anomic carbon
- Conversion of linear (Fischer) to ring representation, with correct chirality.