

1. X-ray crystallography is a technique whereby a crystal of a pure protein (or DNA or RNA) is subjected to beams of x-rays from every conceivable angle. The movement of x-rays through the crystal is affected by their interaction with electron clouds, causing the x-rays to be bent or deflected. By studying the angles of deflection (on an x-ray diffraction pattern), one can, using sophisticated computer analysis determine the electron density of the material the x-rays passed through. The electron density map give 3D coordinates of every atom in the crystal to within a few Angstroms.
2. Nuclear magnetic resonance (NMR) is a technique that uses the inherent spin of certain nuclei, such as protons. The energy it takes to change the spin state of nuclei varies with the electronic environment that the nucleus is in. Methyl groups, for example, will have different energies than hydroxyl groups. One dimensional NMR gives information about the types of nuclei present (methyl, for example). Two dimensional NMR gives information about nuclei that interact with each other and this can be very valuable for determining which regions of a protein interact when the protein folds.

Highlights Hemoglobin

1. Myoglobin and hemoglobin are related proteins involved in (respectively) storing and carrying oxygen in the body.
2. Myoglobin is a single subunit protein with high affinity for oxygen. It holds oxygen tighter than hemoglobin and serves in a battery-like capacity in tissues to release oxygen when tissue oxygen concentration is very low. Myoglobin can take oxygen from hemoglobin.
3. Hemoglobin is a four-subunit protein complex (two alpha subunits and two beta subunits) that serves to carry oxygen from the lungs to the tissues where it is needed. Hemoglobin is genetically related to myoglobin and is evolutionarily derived from it.
4. Myoglobin and hemoglobin both have porphyrin rings (like in chlorophyll) to hold ferrous (Fe^{++}) iron. The specific porphyrin in these proteins is Protoporphyrin IX. Ferrous iron is the form of iron involved in carrying oxygen. Ferric iron (Fe^{+++}), which is the oxidized form of iron will not carry oxygen. Heme is a term used to describe the protoporphyrin IX complexed with iron.
5. The iron in hemoglobin and myoglobin is held in place by five molecules - four nitrogens of the protoporphyrin IX ring and a histidine (called the proximal histidine). Oxygen is carried between the iron and an additional histidine (called the distal histidine) not involved in holding the iron.
6. If one plots the percentage of oxygen sites bound versus partial pressure for myoglobin, a hyperbolic curve is generated, consistent with a molecule with a single binding site and a high affinity for oxygen. The P_{50} , which is the partial pressure of oxygen necessary to fill 50% of the myoglobins with oxygen, is very low for myoglobin, consistent with high affinity. Because myoglobin has high affinity for oxygen, it doesn't release much oxygen until it is in an environment with very low oxygen pressure. For this reason, it would be a poor oxygen transport protein.
7. Hemoglobin is much better designed to meet an organism's physiological needs for carrying oxygen than myoglobin. This is due to its four-subunit organization (one heme per subunit and one oxygen carried per subunit) which behaves in a cooperative fashion in binding oxygen.
8. Binding of oxygen by the iron atom causes it to be pulled 'up' slightly. This, in turn, causes the histidine

attached to it to change position slightly, which causes all the other amino acids in the subunit to change slightly. The changes in shape (different 'states') result in the protein gaining affinity for oxygen as more oxygen is bound. The phenomenon is referred to as cooperativity.

9. Hemoglobin can exist in a "tight" state, called "T", which exhibits low oxygen binding affinity. Hemoglobin in the T state will tend to release oxygen.

10. A second state of hemoglobin is the "relaxed" or R state, which exhibits increased oxygen binding affinity. Binding of oxygen by hemoglobin helps it to "flip" from the T to the R state and release of oxygen by hemoglobin helps it to flip from R to T.

11. A molecule called 2,3-bisphosphoglycerate (2,3 BPG) is produced by actively respiring tissues. It can bind in the gap in the center of the hemoglobin molecule and in doing so, stabilize the T state and favor the release of oxygen. Thus, tissues that are actively respiring get more oxygen. 2,3 BPG is noteworthy because the blood of smokers has a higher concentration of the molecule than the blood of non-smokers.

12. Fetal hemoglobin differs from adult hemoglobin in having two gamma subunits in place of the two beta subunits that adults have. This changes the hemoglobin molecule such that 2,3BPG can't bind, so fetal hemoglobin exists more in the R state and thus has a higher affinity for oxygen than adult hemoglobin. Thus, the fetus can take oxygen from the mother's hemoglobin, due to this property. The fetus won't release oxygen as readily as the adult, but it doesn't have a high oxygen need, due to not exercising heavily.