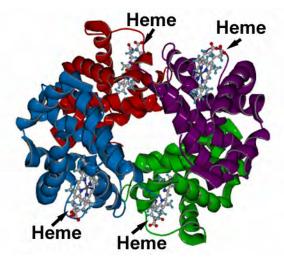
## Hemoglobin

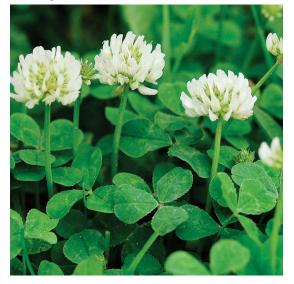
Hemoglobin is the substance that makes blood red and carries oxygen from gills or lungs to peripheral cells. It has been one of the most intensively studied biomolecules since biochemists first sought to understand the



structural basis of physiological function on a molecular level. Because of its characteristic absorption of light and resultant red color, its presence can be recognized on sight. Hemoglobin is contained in erythrocytes, which fill the circulatory vessels of all vertebrates. Visual assessment of the oxygen content of hemoglobin is used to monitor the health of individuals or of their tissues: pink is good and well-oxygenated, blue (cyanotic) is bad and poorly oxygenated. The color difference is due to a shift in the frequencies of light absorbed by hemoglobin with high-oxygen (bright red) and low-oxygen (dull red) content.

← Hemoglobin molecule, showing four separate protein chains (2 alpha and 2 beta), each with an associated oxygen-binding heme

Out in the yard and in the fields, there's a lot of hemoglobin, too:



Hemoglobin-containing root nodules of clover

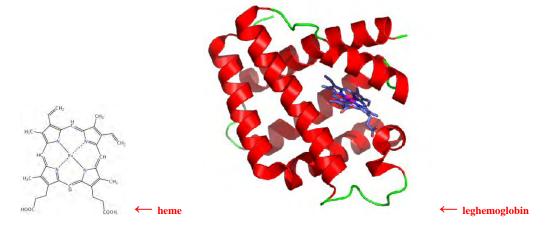


It's pink and serving a similar purpose (binding oxygen) as it does in your bloodstream ... but it's in plants. Certain common plants of the legume family – clover, for example – have root nodules that are loaded with hemoglobin. This can be simply observed by pulling up a bit of clover and rinsing off the roots. You'll see numerous rounded nodules – smaller than rice grains – all along the root fibers. Slicing through one of these and holding it up to the light reveals a slight pinkish color – that's hemoglobin.

One of the limiting factors for plant growth in many areas is nitrogen – a curious fact when you consider that 80% of the volume of each breath you take is nitrogen. The planet is bathed in the stuff. The problem is that atmospheric nitrogen is in diatomic molecular form –  $N_2$  – the nitrogen atoms strongly bound together with covalent triple-bonds. In this form, nitrogen is unavailable for metabolic use to make amino acids for proteins or nucleic acids for ATP and DNA and RNA. Animals and plants are incapable of breaking these bonds, so  $N_2$  remains an unreactive, inert gas for this large sector of the biosphere.

In legumes and certain other plants, however, an arrangement has developed between the roots of the plant and specific types of soil bacteria (rhizobacteria) that are capable of splitting  $N_2$  and forming  $NH_4^+$ , which may then be incorporated into the amino acid glutamine and thus enter metabolic pathways. These bacteria contain the enzyme nitrogenase, which is able to catalyze this reaction, though at a significant energy cost (16 ATP consumed per N<sub>2</sub> split). There is an additional problem, however, in that the nitrogenase complex is rapidly inactivated upon exposure to oxygen and can only function in a nearly anaerobic environment.

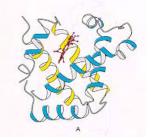
By a complex interaction between root hairs of these plants and the surrounding rhizobacteria, the bacteria enter into the roots and stimulate growth to form rounded nodules. Once inside these nodules, bacteria are supplied with abundant carbohydrate as an energy source by the plant. Nodule formation stimulates plant root cells to activate specific DNA sequences that are transcribed to generate mRNA that is then translated into 150 amino acid-long polypeptide chains. These newly-made polypeptides associate with heme moieties (produced by the rhizobacteria) to form hemoglobin. This hemoglobin binds oxygen - providing a local nearly-anaerobic environment inside the nodule for bacterial nitrogenase activity - and gives the nodules a pink color.

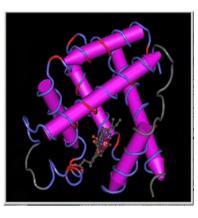


Concentrations of this plant-produced hemoglobin (called *leg*hemoglobin due to its occurrence in legumes) approaches 0.3 mM in nodules, which is similar to the concentration of myoglobin (another related  $O_2$  - binding protein) in mammalian heart muscle cells. The affinity of leghemoglobin for  $O_2$  is so high that mean  $O_2$  pressures in the nodules are maintained at 0.01 mmHg (compared with atmospheric  $O_2$  pressure of 159 mmHg).

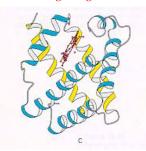


alpha chain of human hemoglobin





leghemoglobin



Interestingly, leghemoglobin is significantly homologous in its amino acid structure and 3dimensional conformation when compared with vertebrate – including human – hemoglobin.