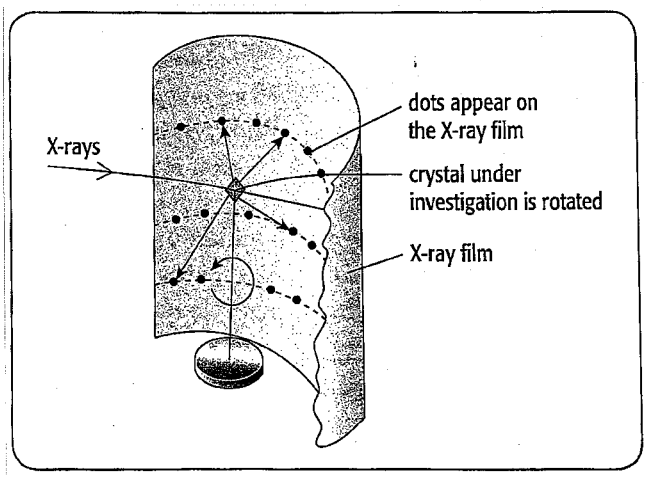
X-ray Crystallography (A2)

X-rays are very short-wavelength electromagnetic rays. Their wavelength of about 0.1nm is comparable to the interatomic distances in solids.

If a beam of monochromatic X-rays (rays of a single wavelength) is passed through a crystal, some X-rays will be diffrated by the electrons that surround the atoms in the crystal. The electron clouds interact with the electric field of the X-rays.

The single incoming beam produces many diffrated beams.



The apparatus used in X-ray crystallography.

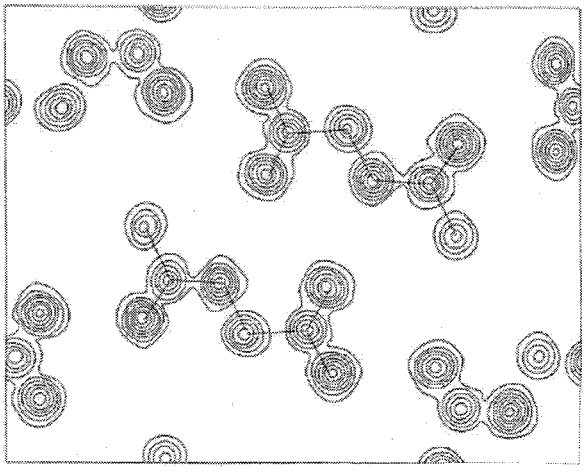
The larger the atom, the more electrons it contains and the more intense the spot it produces in its diffraction pattern. All atoms except hydrogen contain enough electrons to diffract the X-rays.

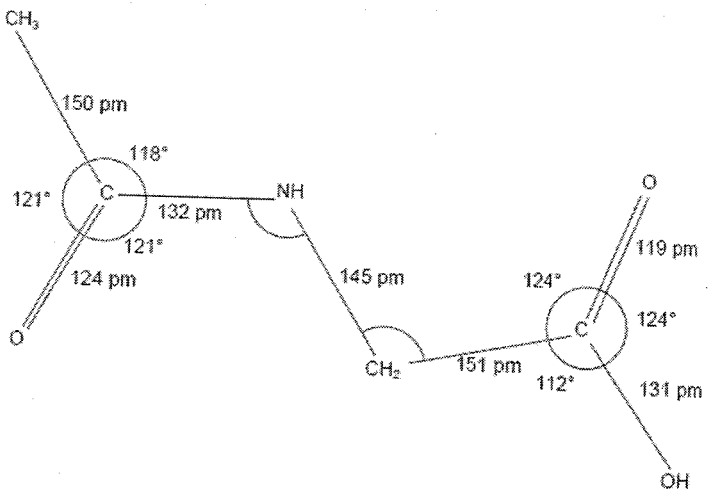
By measuring the angles between the incoming beam and the diffrated beams, and the relative intensities of those beams, a picture of the electron density at all points in the unit cell can then be pieced together.

This process requires an enormous number of calculations to be done, requiring many hours of high speed computer time.

The best results of X-ray crystallography can only be obtained from a very pure crystal of the sample. Fortunately, many large biological molecules can be crystallised from solution.

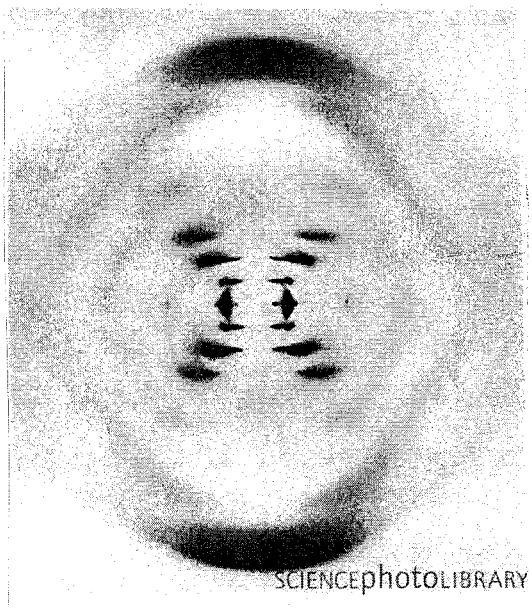
Example: The electron density map of N-ethanoylaminoethanoic acid.



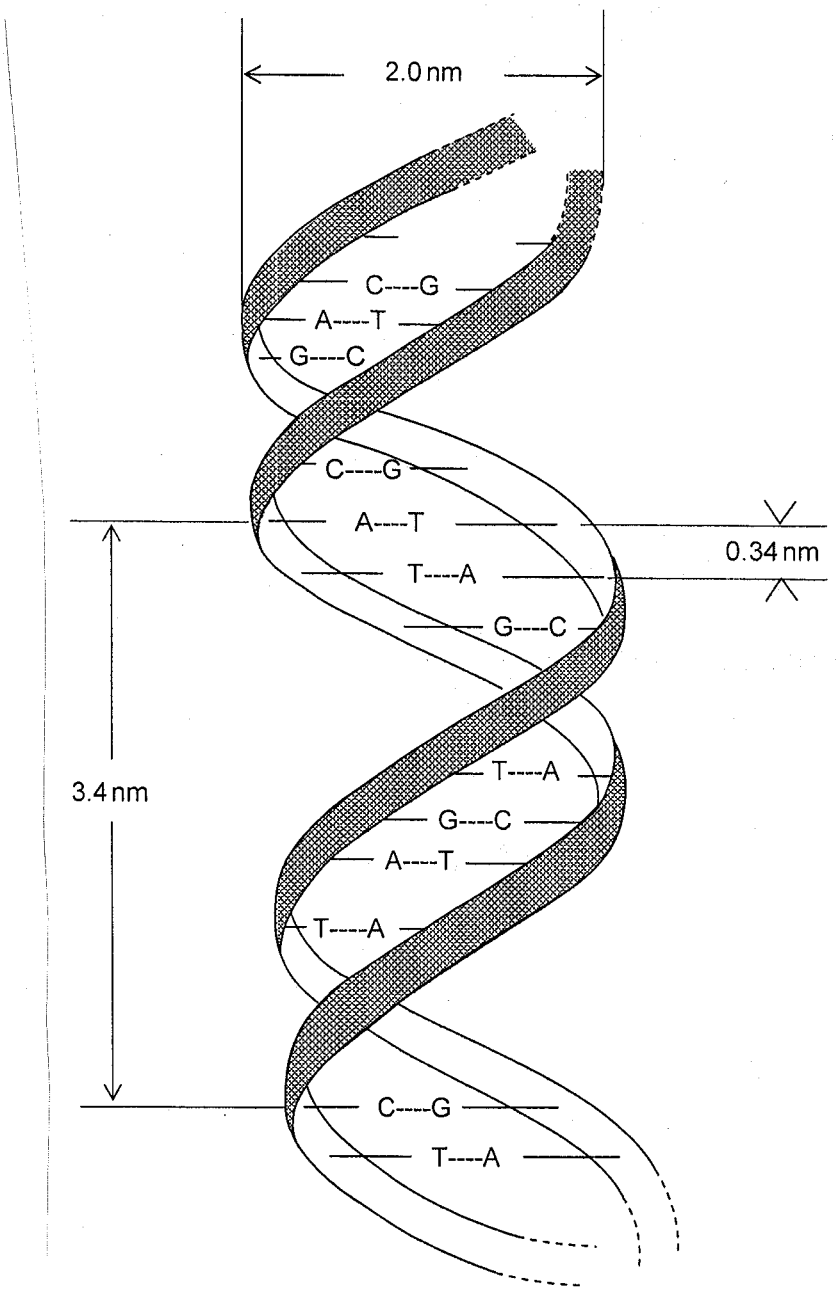


The structure of DNA.

The role of X-ray crystallography in the discovery of the structure of DNA by Watson and Crick in 1953 is well known.
The DNA molecule has a good deal of symmetry, with the planar base-pairs taking up positions parallel to each other.
The relatively simple X-ray diffration photograph of a hydrated DNA fibre shows the central spots arranged in a cross pattern - typical of a helical structure.



Key dimensions obtained for DNA by X-ray crystallography:



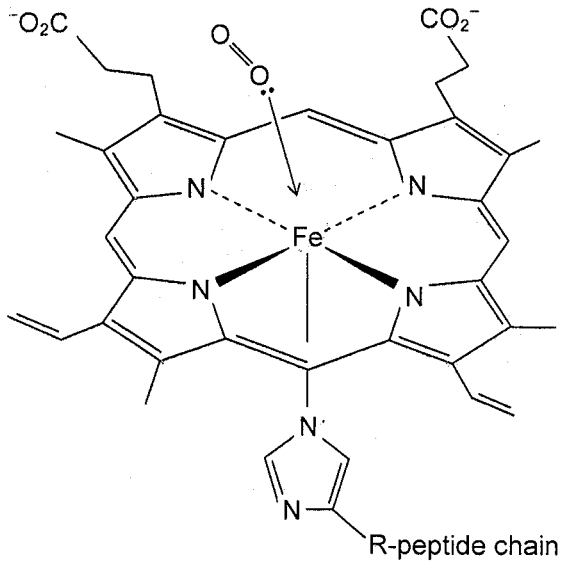
The structure and function of myoglobin

Myoglobin is an oxygen transporting agent and oxygen reservoir found in the muscular tissues of vertebrates.

It achieves its function by the use of a haem group. This consists of a Fe^{2+} ion surrounded by a protoporphyrin ring.

The iron is bound to the polypeptide chain by an Fe-N covalent bond to a histidine residue.

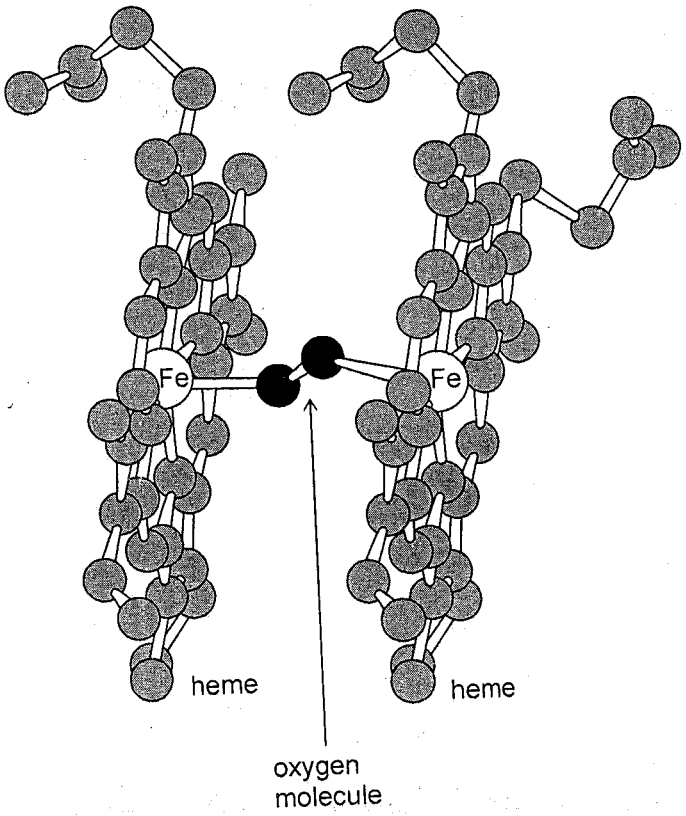
The oxygen molecule is transported by forming a dative bond to the Fe^{2+} ion.



iron protoporphyrin with O_2 and histidine.

When myoglobin present in the muscular tissues, the Fe^{2+} ion does not become oxidised to Fe^{3+} with the presence of oxygen.

A key intermediate in this oxidation is a complex of an oxygen molecule sandwiched between two haem groups.



two iron protoporphyrins with O_2 sandwiched in between.

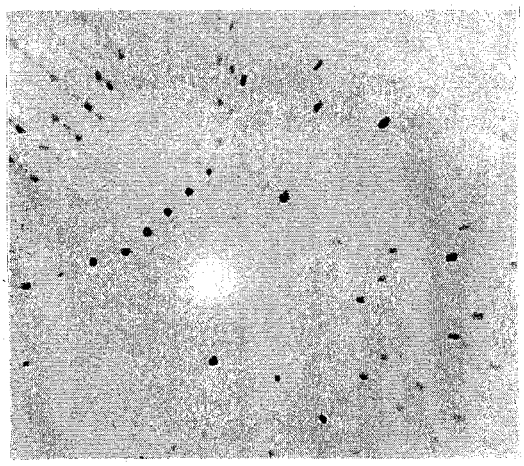
(note: If iron(II) protoporphyrin is dissolved in water, and oxygen is bubbled through the solution, and almost immediate oxidation to Fe^{3+} occurs.)

The affinity of carbon monoxide by myoglobin

Like many iron complexes, haem has a large affinity for carbon monoxide. This displacement of O_2 by CO is why carbon monoxide is so poisonous.

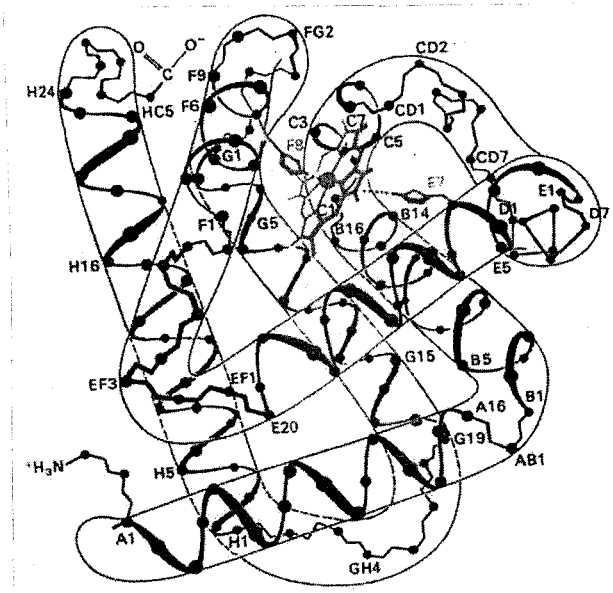
But another curious property of the haem groups in both myoglobin and its blood counterpart, haemoglobin, is that their affinities for carbon monoxide physiologically, although large, are very much smaller than that of an isolated haem group in solution.

Both these observations were readily explainable once the three-dimensional structure of myoglobin had been determined.



X-ray diffraction photograph of myoglobin

The myoglobin molecule is extremely compact, with large percentage of its amino acids joined in the α-helical configuration.
This produced several stable, stiff "rods", which are joined by small lengths of more flexible parts of the amino acid chain.

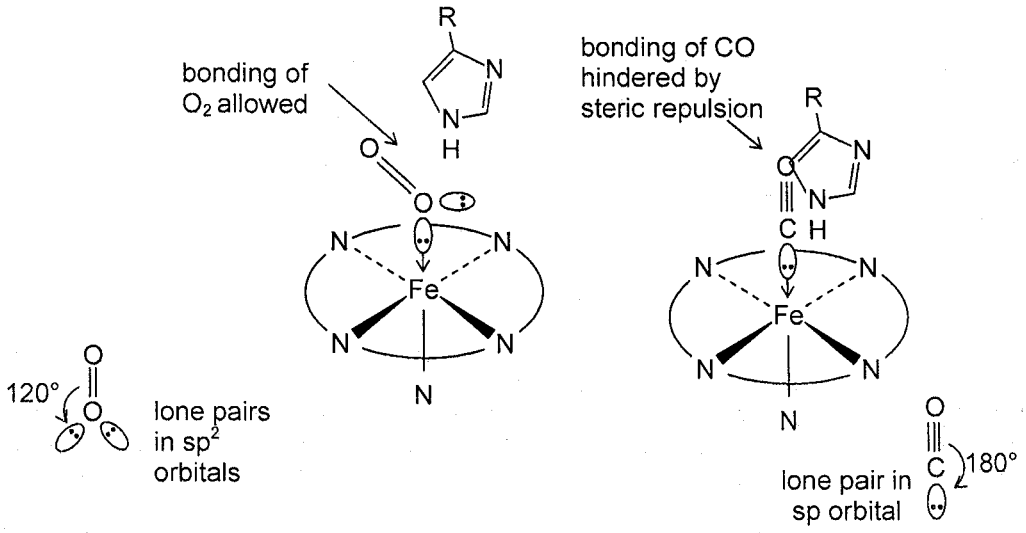


structure of myoglobin molecule.

The oxygen-binding site is very hindered by the amino acids surrounding it, and the haem group is on the inside of the molecule.
There is a second histidine group positioned just over (but slight to one side of) the 6th coordination position of the Fe^{2+} ion, where the O_2 molecule sits.

The fact that the haem group is on the inside of the molecule makes it impossible for two such groups to come together with an oxygen molecule bridge between them.

In addition, the second histidine group, above the Fe^{2+} ion, forces the complexing molecule to bond with the Fe^{2+} ion at an angle. This favours O_2, but hinders the bonding of CO.



arrangement of molecules within the myoglobin complex.

Methods of determining the structures of molecules.

1) Mass spectrometry

2) Nuclear magnetic resonance (NMR) spectroscopy.

3) X-ray crystallography.

Mass spectrometry

- can determine the number of carbon atoms in a molecule (from the M : M+1 ratio)
- the molecular formula (from an accurate determination of the Mr of the molecular ion)
- some preliminary knowledge about its structure.

Nuclear magnetic spectroscopy

- informs on the number of hydrogen atoms in each chemical environment in a molecule (from the integration trace and the chemical shift, δ values).
- the number of hydrogen atoms of their nearest neighbours (from the splitting patterns). (If a particular type of proton has n nearest neighbours, its peak is split into (n+1) lines).

X-ray crystallography

- tells the arrangement of the atoms in a crystal of the compound.
- determine the positions of all atoms except hydrogen.