Haemoglobin Revisited

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Introduction

A theoretical study of catalysis by soft materials, such as protein, has shown the relationship between the degree of catalysis and local transient rigidity [1]. Since the intrinsic rigidity of protein is quite low, a theoretical source of local rigidity has been found in the effects of surface tension on multi-subunit proteins [1]. This concept has already been used to generate new quaternary structures for the neuraminidases and galactose oxidase [2]. It was assumed that crystallisation of the proteins in preparation for x-ray crystallography had left their tertiary structures essentially the same as in solution, but their quaternary (multi-subunit) structures were possibly very different.

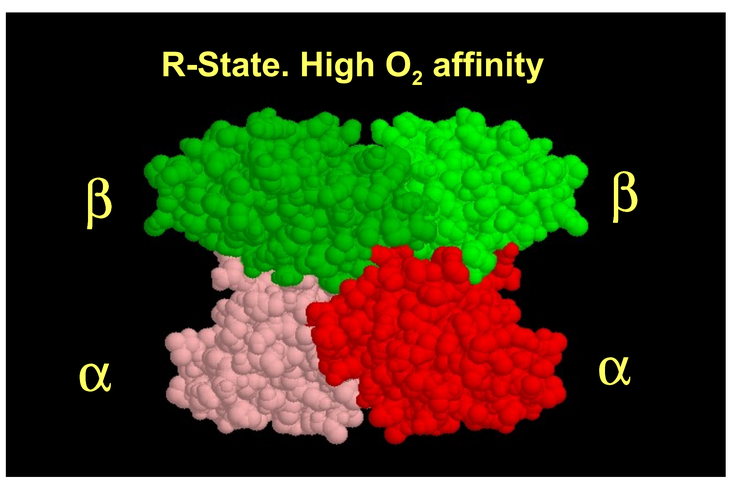
This poster shows a new model structure for haemoglobin based on the same concepts.

Natural haemoglobin occurs as an $\alpha_2\beta_2$ tetramer (α and β being different forms of haemoglobin) that shows cooperative binding of O_2. There are also other interactions between the O_2 binding, the pH and the concentrations of certain other chemicals. These interactions require that a binding site at one location in the tetramer does work on binding sites elsewhere in the tetramer. Some degree of rigidity would be required to allow specific transmission of this energy to the correct location.

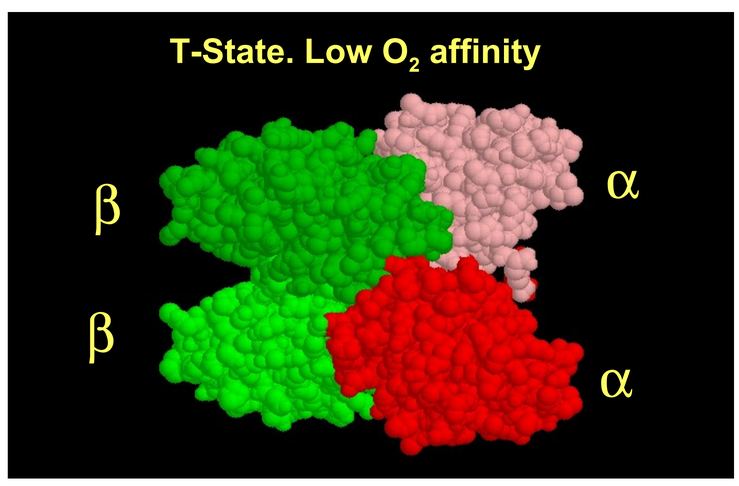
The widely accepted structure of haemoglobin is based on x-ray crystallography, initially by Muirhead and Perutz in 1963. (Some recent NMR studies suggest that the solution structure is similar to the crystal, but they have problems with assumptions and verifiability.) In 1965 Monod, Wyman and Changeux proposed a new mechanism to explain cooperative O_2 binding, as it was previously thought that the hemes interacted directly [3]. In Perutz's structure the hemes could not directly interact and an intricate mechanical and chemical mechanism was proposed. As the complexity of haemoglobin chemistry has emerged since then, much ingenuity has been applied to modifying the models to fit the data. However, serious inconsistencies remain [4, 5].

The New Structure

The new quaternary structure shown in this poster differs markedly from Perutz's structure. The new structure is consistent with the complex chemical properties of haemoglobin and is supported by cross-linking studies. It provides an explanation for the apparent necessity that highly cooperative O_2 binding involves $\alpha_2\beta_2$ tetramers rather than homotetramers. The "switch" between high and low affinity forms involves isomeric quaternary structures only achievable with an $\alpha_2\beta_2$ tetramer. The interactions between sites are mediated by delocalised π-electrons connected through the rigid central core of the tetramer. This mechanism is reminiscent of the mechanism proposed by Pauling before the x-ray crystal structure became known [3].



Two subunits change places. Residues with NMR signals that change with isomerisation [6] are located at the changed interfaces.



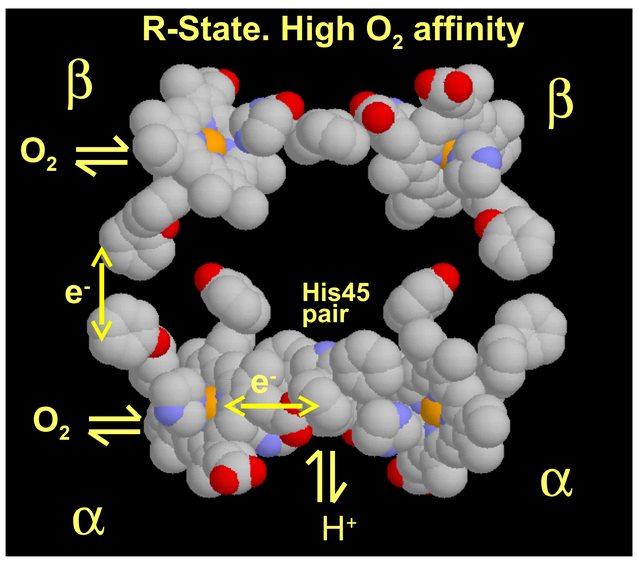
In the T-State, the surface amine groups are located in places consistent with cross-linking studies. In particular it is clear why the β N-terminal amine groups cannot be linked to each other [7]. ~~The crystal structure cannot account for this.~~

The New Mechanism

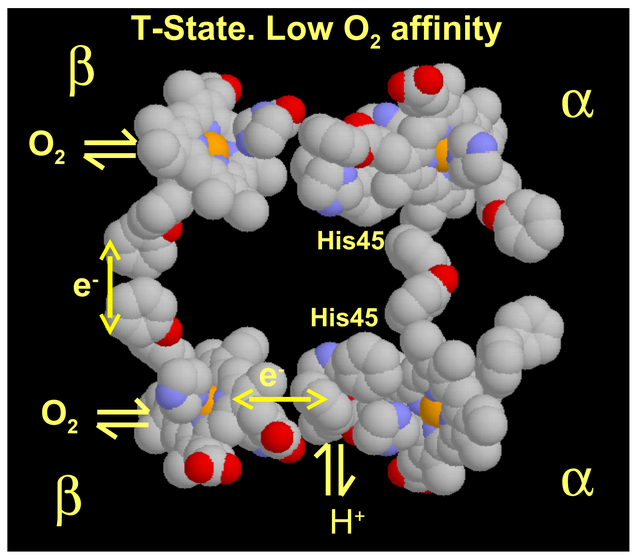
Embedded in the rigid central core are the four hemes linked by aromatic amino acid side-chains. It is proposed that this extended π-electron system transmits electron availability between the hemes and αHis45s, which lie in very different electronic environments in the two isomers (see below). Thus oxygen at the heme and hydrogen ions at αHis45 compete for electrons. The state of this equilibrium affects the charge on αHis45 and thus the relative populations of the R- and T- states. These coupled equilibria are the basis of the Bohr Effect and cooperative oxygen binding. The Acid Bohr Effect can be attributed to the carboxylate groups of hemes being part of the extended π-electron system.

Heme groups and linking aromatic rings in the centres of the isomeric tetramers.

Note that there is no central cavity in these proposed structures. The spaces shown are occupied by non-aromatic atoms that have been rendered invisible to show the π-electron system more clearly.

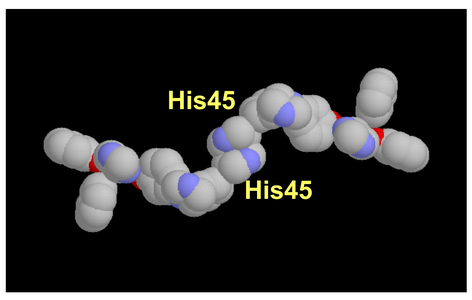


It is proposed that protonation of His45s increases as O_2 is released or pH decreases. Repulsion between positive charges in the paired conformation shifts the isomerisation equilibrium towards the T-state, as observed experimentally.



The His45s will be more protonated in the T-state because there is no charge repulsion. This will tend to withdraw electrons from the system, contributing to the lower O_2 affinity.

Side-on view of the His45 pair sandwich in the R-state, with linking aromatic groups. Phe46 and heme have been deleted to improve visibility.



References
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