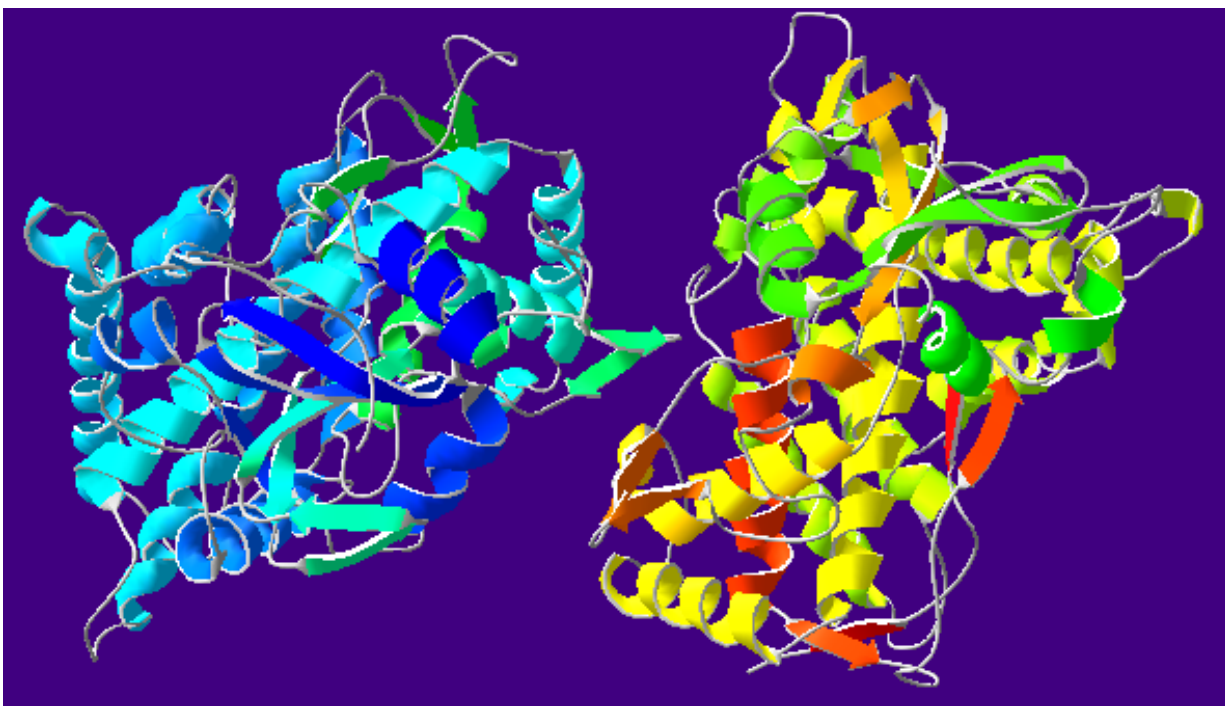


Cytochrome P450 synthetic analogs are temperature-dependent valence tautomers

June 24 2016, by Heather Zeiger



Cytochrome P450. Credit: Wikipedia

Cytochrome P450 is a well-known family of enzymes that are involved in drug metabolism. The active site contains a heme chemical structure in which an iron atom is bound to the heme cofactor within the enzyme. The oxidation state of the iron plays a key role in catalysis, notably in oxidizing organic compounds using O_2 , and an iron-sulfur bond is a key

component to the enzyme's function.

Something that has puzzled chemists for many years is why synthetic analogs of cytochrome P450 are much more sensitive to degradation from O_2 than its enzyme form. In an effort to better understand this discrepancy Pradip Kumar Das, Subhra Samanta, Ashley B. McQuarters, Nicolai Lehnert, and Abhishek Dey of the Indian Association for the Cultivation of Science and the University of Michigan examined the electronic structure of four different iron porphyrin complexes using resonance Raman, [electron paramagnetic resonance](#), and 1H NMR spectroscopy. They found that the iron porphyrins are temperature dependent valence tautomers, ferric thiolate and ferrous thiyl, which may play a key role in O_2 sensitivity. Their research appears in the *Proceedings of the National Academy of Sciences*.

The stable form of the cytochrome P450 active site contains Fe^{3+} coordinated to a thiolate. It will only react with O_2 when Fe^{3+} is reduced to Fe^{2+} . However, in synthetic analogues the Fe^{3+} porphyrin complex degrades in the presence of O_2 . Oxygen will oxidize the thiolate ligand unless it is sterically protected.

The difference between the enzyme and the synthetic analogs is likely due the electronic structure. To understand the electron structure of these iron porphyrins, Das, et al. tested four known compounds with variances in the thiolate ligand. The first compound contains an alkyl thiolate ligand and is unstable in air. The second compound has a bulky aliphatic complex that sterically protects the thiolate group and is stable in air. The third complex has a benzylic group attached to the thiolate and is stable in air. The fourth compound has an aromatic thiolate and is unstable in air.

Each of these compounds was tested with resonance Raman (rR), electron paramagnetic resonance (EPR), and 1H NMR spectroscopy.

Resonance Raman for all four compounds indicated that there are two different species present at room temperature, but only one present at low temperatures. The two species have frequencies suggestive of a high- and low-spin Fe^{3+} state and a high-spin Fe^{2+} state. Low temperatures seem to only contain compounds with Fe^{3+} .

These results were corroborated with EPR spectroscopy which indicated the presence of a radical species at room temperature. This radical species is likely the $\text{Fe}^{2+}\text{—RS}\cdot$ radical, or the thiyl radical, a valence tautomer to the $\text{Fe}^{3+}\text{—RS}^-$ complex. Proton NMR studies, while perhaps indicative of two species present at room temperature, were less conclusive.

Variable temperature resonance Raman and variable temperature EPR studies showed that as the temperature decreases, less Fe^{2+} was present. However, Das, et al. found that the signal is reversible. As temperature increased, the radical signal returned. In all four compounds there seemed to be a valence tautomerism between Fe^{3+} thiolate and Fe^{2+} thiyl radical. Analysis of the temperature studies indicated that the Fe^{3+} thiolate tautomer is energetically favored while the Fe^{2+} thiyl radical is entropically favored.

Solid state studies confirmed that there is apparently a change in geometry in the solution state that does not occur in the solid state, again, indicating a tautomer.

Finally, the aliphatic and benzylic complexes were immobilized on a silver surface to test the effects of hydrogen bonding on the stability of the iron porphyrins. In an organic solvent, such as THF, the complex degrades in the presence of O_2 . However, when only 0.5% water, by volume, was added, then the complex is stable in the presence of O_2 . Hydrogen bonding must play a role in stabilizing the Fe^{3+} thiolate complex.

This research provides essential insight to a decades-long mystery regarding synthetic analogs of cytochrome P450. Iron porphyrin analogs exist as a valence tautomer at room temperature, which likely enhances the compound's sensitivity to O₂. Furthermore, hydrogen bonding likely plays an essential role in stabilizing the enzyme's ground state.

More information: Pradip Kumar Das et al. Valence tautomerism in synthetic models of cytochrome P450, *Proceedings of the National Academy of Sciences* (2016). [DOI: 10.1073/pnas.1600525113](https://doi.org/10.1073/pnas.1600525113)

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Citation: Cytochrome P450 synthetic analogs are temperature-dependent valence tautomers (2016, June 24) retrieved 9 April 2024 from <https://phys.org/news/2016-06-cytochrome-p450-synthetic-analogs-temperature-dependent.html>

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