EPR spectroscopy as an electronic and structural tool to study photosynthetic cytochrome c550

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Cytochrome C_{550} (Cc_{550}) is associated with photosystem II (PSII) in some photosynthetic organisms such as cyanobacteria, diatoms and red algae. The heme group in this protein has the characteristic covalent link to the protein chain and is coordinated to two histidine side chains. Although its function is not determined, a redox role has been proposed based on the redox potential measured when the protein is bound to the PSII. In addition, the redox potential of Cc_{550} decreases substantially when it is measured in solution in the soluble Cc_{550} form. The parameters governing this redox potential variation are not known. A change in the electronic levels of iron due to a rearrangement of the axial ligands has been hypothesized. Also, a change in the hydrophobicity of heme environment has been postulated.

Here we use Electron Paramagnetic Resonance (EPR) techniques to study two variants of Cc_{550} from the cyanobacteria *Synechocystis sp.* and the diatom *Phaeodactylum tricornutum* to determine the energy distribution of iron electronic levels. Our findings show that variations in relative energy between bound and unbound states and among the different variants are discrete and of comparable magnitude. The comparison of crystal structures, EPR spectra and the sequence rules out the hypothesis of a major rearrangement of axial ligands and rather indicates a different distribution of charges in the heme. The study of nuclear energy levels of iron using 2D pulse EPR techniques of both variants reveals more details about the geometry of the heme site, such as the effect of tilting of one of the axial imidazole rings or its rotation with respect to each other or to the heme molecular frame.