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Tyr(b10) Prevents Stabilization of Bound Oxygen in Soybean Leghemoglobin

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Interpretive Summary: Root nodules are complex structures that occur on roots of many agronomically important plants such as soybeans. Root nodules are a symbiosis between the plant and soil-borne bacteria. Once formed, and functional, root nodules can fix atmospheric nitrogen and provide the plant with a pollution-free source of nitrogen for seed production. A basic component of functional and efficient root nodules are the heme proteins called leghemoglobins. Leghemoglobins can bind to, and transport molecular oxygen and thereby maintain a low oxygen environment within the root nodule, permitting the microaerobic bacteria to convert atmospheric dinitrogen into ammonia, which is then assimilated by the plant for growth. Thus understanding the oxygen-binding characteristics of leghemoglobin are of major importance from both a basic and applied point of view. In this paper we have shown that specific amino acid residues are critical for allowing the orderly binding and transfer of oxygen from leghemoglobins. Although several hypotheses have been advanced to account for the biochemical properties of leghemoglobins, this work is the first that clearly demonstrates the molecular consequences of amino acids that surround the heme-pocket. Using engineered mutant proteins and spectroscopy, the role of Tyr (B10) has been investigated in detail for several vertebrate and plant hemoglobins.

Technical Abstract: Leghemoglobin performs two functions simultaneously in the root nodules of legumenous plants; oxygen scavenging and facilitated diffusion to nitrogen fixing bacteria. Myoglobin, with a similar structure, serves only the latter function in muscle tissue. For this reason, oxygen affinity in leghemoglobin is much greater than myoglobin, and recent investigations have suggested that leghemoglobin has evolved a mechanism strikingly different from that of myoglobin for regulating oxygen binding. However, the molecular details of this mechanism are not well understood, and a test of this hypothesis requires structural or spectroscopic investigation. The present FTIR spectroscopic investigations of iron-carbonyl stretching frequencies in soybean leghemoglobin, sperm whale myoglobin, and many of

their mutant proteins, support this novel mechanism in leghemoglobin. Instead of using a hydrogen bond to stabilize bound oxygen, soybean leghemoglobin has evolved a distal pocket that destabilizes the ligand. For this purpose, soybean leghemoglobin uses the uncommon combination of TyrB10 and HisE7 to manipulate the electrostatic environment in the distal pocket thus weakening hydrogen bonding with oxygen, and ensuring the requisite kinetic rate constants for high affinity binding. The general role of the amino acid at position B10 is also investigated by examining FTIR iron-carbonyl stretching frequencies in several other leghemoglobins and in nonsymbiotic plant hemoglobins.

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