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LEGHEMOGLOBIN AND *RHIZOBIUM* RESPIRATION

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INTRODUCTION

It is misleading to describe the principal function of leghemoglobin (Lb)[1] as a barrier or scavenger which, by binding O_2, prevents free O_2 from reaching nitrogenase within the N_2-fixing *Rhizobium* bacteroids of legume root nodules (39, 50). By 1974, after the first comprehensive reviews of Lb structure and properties were published (4, 58), its function was generally accepted as the facilitation of O_2 flux to the vigorously respiring, phosphorylating, N_2-fixing *Rhizobium* bacteroids, albeit at a stabilized O_2 tension (~ 10 nM in soybean nodules) presumed to be too low to damage the O_2-intolerant nitrogenase enzyme complex. As late as 1980, however, the reported absence of Lb from the N_2-fixing symbiosis between *Rhizobium* and a nonleguminous plant *Parasponia* (originally misnamed *Trema*) (45), and the demonstration of nitrogenase activity in certain free living rhizobia grown under microaerobic conditions, led Robson and Postgate (130) to the reasonable conclusion that "leghemoglobin is a sophistication rather than essential." Since then the perspective has changed dramatically. A hemoglobin (Hb) which resembles Lb in some (but not all) of its properties has been purified from *Parasponia* nodules (12, 13), Hb has been extracted from *Casuarina* nodules (145; A. I. Fleming and C. A. Appleby, unpublished observations), and Hb spectra have been observed in slices of actinorhizal nodules from other plant families (145).

A principal objective of this review will be the reexamination of Lb properties and function, particularly in relation to an efficient phase of *Rhizobium* bacteroid respiration which undoubtedly occurs in legume root nodules at extremely low free O_2 tension, and also in relation to the apparently uncoupled

[1]Abbreviations and definitions: The term leghemoglobin, and occasionally the abbreviations Lb, LbO_2 (oxyferrous Lb), LbCO (carboxyferrous Lb), are used only to describe the O_2-carrying hemoprotein found in legume root nodules. Myoglobin (Mb) refers only to the monomeric protein found in animal muscle tissue. The term hemoglobin (Hb), with descriptive prefix, e.g. *Parasponia* Hb, is used to describe the proteins found in all other animal and plant tissues. "Oxygenation" describes the reversible combination of ferrous Lb, Mb, or Hb with O_2; "oxidation" describes the conversion of ferrous Lb, Mb, or Hb to the corresponding ferric species, thereby rendering these proteins incapable of oxygenation until they have been re-reduced to the ferrous species. "Bacteroid" is used to describe the N_2-fixing rhizobia found in both legume and *Parasponia* nodules. CCCP is used to describe the uncoupling agent carbonyl cyanide *m*-chlorophenylhydrazone. With some reluctance, the author uses the recently popularized term "peribacteroid membrane" (129) instead of the classical term "membrane envelope" (25) to describe the plant membrane which surrounds each bacteroid or group of bacteroids in N_2-fixing legume root nodules; hence the term "peribacteroid space" rather than "envelope space" is used to describe the space between the peribacteroid membrane and its contained bacteroid(s). The term "periplasmic space" is used to describe the space between the bacteroid inner (plasma) membrane and outer membrane (cell wall). It is critically important to distinguish between the "periplasmic" and "peribacteroid" spaces; they may have very different functions (see below). In describing membrane energetic phenomena, $\Delta\Psi$ represents the electrical potential (or membrane potential), $\Delta\mu H^+$ the electrochemical potential (or proton motive force), and ΔpH the pH difference, all between the bulk phases on either side of a membrane; at 25°C, $\Delta\mu H^+ \approx \Delta\Psi - 60\ \Delta pH$.

(protective?) respiration which is demonstrable in certain bacteroids at artificially induced high O_2 tension. The genetic origin of Lb, the mechanism and control of its biosynthesis, and relationships among Lb and other plant hemoglobins will also be considered. If Hb does indeed seem to be a necessary part of all natural symbioses involving rhizobia, then knowledge of the genetic origin and control of biosynthesis of present plant hemoglobins may be critical for the success of new genetically engineered symbioses. On the other hand, if there exist natural or mutated *Rhizobium* strains which can be shown to fix N_2 vigorously in aerated cultures in the absence of an O_2 carrier, will these be the organisms favored for the establishment of new, simpler symbioses? Or will the otherwise advantageous containment of endophyte at high local density in a nodule or similar structure (69) cause such constraint on O_2 supply as to require the presence of an O_2 carrier similar to Lb of legume nodules?

Almost all early studies on the physiology of symbiotic N_2 fixation, including those on Lb and *Rhizobium* respiration, were made with the experimentally convenient soybean nodule which, with hindsight, appears to be one of the most demanding symbioses, at least with respect to O_2 supply (see below). It is probable that some recent controversies, notably those on Lb localization (25, 109, 129, 161) and the nature of the efficient and inefficient phases of nodule respiration (22, 158), may have arisen because of the unrecognized but greater O_2 tolerance of other symbioses such as that between the pea and *R. leguminosarum*. Except where it would cause historical injustice, the overall plan of this review will be to discuss the soybean symbiosis first, then consider in turn what seem to be increasingly O_2-tolerant systems. This review will assume knowledge of the Lb literature to 1974, summarized elsewhere (4, 58). Because of space limitations, some topics will be covered only by a single recent reference from each laboratory active in the area. As far as possible these will be to papers in which authors have summarized their own work in relation to that of others. All cited Russian papers are available in English translations.

Leghemoglobin, with very high O_2 affinity resulting from an unusual combination of extremely fast O_2 association rate and rather slow O_2 dissociation rate (4), has been much investigated by those wishing to answer fundamental questions about Hb structure-function relationships. This subject, of necessity only superficially treated in the present review, will be adequately covered elsewhere (P. E. Wright, manuscript in preparation). General references consulted during the preparation of this review are (4, 6, 23, 52, 57, 58, 65, 69, 79, 113a, 114, 125, 130, 159, 162, 169).

LEGHEMOGLOBIN

Occurrence, Purification, Sequence Analysis

It is well documented (e.g. 4) that the Lb content of most legume root nodules is correlated with their N_2-fixing ability. Although some mutant strains of rhizo-