Physiological limitations in BNF

THE INFLUENCE OF HOST PLANT ENERGY SUPPLY ON NITROGEN FIXATION ABSTRACT

In white clover plants dependent on N2 fixation and acclimated to a uniform, artificial environment, N2 fixation potential in the photoperiod is closely linked to current photosynthesis, while in the night it is limited by assimilate generated in the previous photoperiod. Darkening or defoliating plants reduces N? fixation potential in a manner which is quantitatively linked to tne diurnal supply of assimilate. The effects of such treatments are apparent within 30-60 min. Enhancing photosynthesis above the level to which the plants are acclimated increases N2 fixation potential, but the effect is relatively small.

INTRODUCTION

Rapid biological nitrogen fixation in legumes requires a substantial supply of respiratory substrates, provided directly or indirectly by photosynthesis, to provide the energy for the metabolic train associated with nitrogenase activity in the root nocules. In the forage legumes, where leaves and shoots provide the harvestable product the flux of photosynthetic products for growth and N2 fixation is disrupted, to a greater or lesser extent, depending on the intensity and frequency of defoliation. The accurate measurement of the effects of defoliation on day-to-day rates of N2 fixation presents insurmountable difficulties in the field. In such venues, measurements are laborious, expensive and provide only a general account of long-term N2 fixation trends.

An earlier paper provided an account of some short-term changes in N2 fixation potential in white clover when photosynthesis was disrupted by darkening and defoliation in controlled environment experimentation (Ryle et al., 1985). This paper extends these observations and provides a more detailed account of the relationships between nitrogenase activity in root nodules and the photosynthetic activity of shoot tissue.

CARBON METABOLISM AND THE EXCHANGE OF METABOLITES BETWEEN SYMBIONTS IN LEGUME NODULES

Two examples of exchange of carbon between host and bacteroids are presented. In the first example, a,a-trehalose which accumulates in nodules is synthesized in bacteroids, but substantial quantities of trehalose are released to the host cytoplasm where it is "recycled" to glucose. In comparisons across Bradyrhizobium japonicum strains, trehalose concentrations in nodules are negatively correlated with acetylene reduction activity. We still have no explanations for these unusual relationships 'involving trehalose.

In the second example we have preliminary evidence for the operation of a malate/aspartate shuttle in B. japonicum bacteroids. Malate is taken up and, after oxidation and transamination, is returned to medium as aspartate. Glutamate which may be taken up or synthesized in bacteroids, is transaminated to a-ketoglutarate, which is exported to the medium.

INTRODUCTION

Current concepts of carbon metabolism in legume nodules begin with sucrose which is clearly the major source of reduced carbon entering all legume nodules which have been studied. Enzymes of glycolysis, which are very active in host cytoplasm, convert sugars to organic acids. Organic acids, malate and succinate in particular, are thought to pass through the peribacteroid membrane and serve as the main sources of reduced carbon for bacteroids. It is generally indicated that acids are oxidized to CO 2 by bacteroids with the concomitant "extraction" of electrons which, in turn, are used for vital bacteroid functions including the operation of nitrogenase. The above concepts are supported by numerous lines of evidence, including the following:

• Organic acids are rapidly absorbed by an active uptake mechanism, whereas carbohydrates are only slowly absorbed by bacteroids (Reibach and Streeter, 1984).

• Organic acids support higher rates of respiration and nitrogenase activity by isolated bacteroids than are supported by sugars (Saroso, et al., 1984).

• Rhizobia which lack the ability to absorb dicarboxylic acids form Fix nodules. This has been observed independently by several different groups (Ronson, et al., 1981; Finan, et al., 1983; Arwas, et al., 1985) .

• Bacteroids have high activity of enzymes for organic acid metabolism, but low activity of key enzymes of glycolysis, the pentose phosphate and Entner-Doudoroff pathways (Saroso, et al., 1986; Salminen and Streeter, 1986b).

Although the concepts described in the first paragraph are widely accepted, our thesis here is that a concept of carbon exchange between symbionts should be considered. Two examples of carbon exchange are described. The first, involving a,a-trehalose is well documented, but we presently have no knowledge of its importance. The second involves the possible operation of a malate/aspartate shuttle in nodules. Our evidence for this is very preliminary, but if correct, the concept could be highly important in our attempts to understand nodule function. TREHALOSE This glucose-glucose disaccharide is the blood sugar of insects and is probably present in all fungi, the organisms where it has been most thoroughly studied. Recently, nitrogen fixing organisms including cyanobacteria, actinomycetes, and Rhizobium have been found to accumulate trehalose (Streeter, 1985). The sugar is not synthesized in higher plants where, in fact, it may be toxic unless the hydrolytic enzyme trehalase is present . Trehalose accumulates in all legume nodules we have examined and the concentration present depends on the Rhizobium strain. In soybean (Glycine max) nodules formed by indigenous B. japonicum and harvested weekly, the seasonal mean (72 observations) concentration of nodule sugars included sucrose (2.8 mg/g fresh wt of nodule), glucose (1.4), trehalose (1.3), maltose (0.41), and fructose (0.31). Several lines of evidence have proven that trehalose in nodules is synthesized in bacteroids; for example, the key enzyme of trehalose synthesis could be found only in bacteroids (Salminen and Streeter, 1986a). Trehalose comprised 65% to as

BACTERIAL CATABOLISM OF NITROGEN CONTAINING COMPOUNDS IN SYMBIOTIC NITROGEN FIXATION

ABSTRACT

Recent work indicating that there may be a flow of fixed nitrogen to Rhizobium bacteroids is discussed in light of a hypothesis that such a flow may be important to nodule function. Evidence that nitrogen is transported to the microsymbiont includes analysis of auxotrophic mutants, consideration of proline metabolism in nodules, and the finding of unusual amino compounds within nodules that can specifically support bacteria growth. We describe experiments that implicate an NAD dependent glutamate dehydrogenase in glutamate catabolism by free living bacteria.

INTRODUCTION

In the symbiosis between leguminous plants and Rhizobium or Bradyrhizobium bacteria, the plant provides the bacteria with carbon and energy and in return it obtains ammonia produced by bacterial nitrogen fixation. Which specific compounds are provided to the bacteria has been a major question of research since it has generally been felt that one method of increasing yields due to symbiotic nitrogen fixation would be to increase the amount of energy available to the bacteria. This depends both on knowing how chemical energy is transferred to the bacteroid and how the bacteroid uses this energy to fix nitrogen. The carbon compounds delivered to the nodule are primarily monosaccharides and disaccharides, chiefly glucose and sucrose (Emerich et al., 1983). However, strong arguments have been presented that these compounds are not the primary carbon sources for the bacteroids (Dilworth and Glenn, 1985). These sugars are therefore likely to be converted by the plant into other compounds which are delivered to the bacteroid through the peribacteroid membrane. Dicarboxylic acids are thought by many to be the class of compounds most likely to serve as primary carbon source (reviewed in Ronson and Astwood, 1985). Mutants of various bacterial species that are blocked in carboxyl acid transport are Fix-. In addition, succinate supports the highest levels of nitrogen fixation by isolated bacteroids and other dicarboxylic acids are also excellent substrates in this system. Kahn et al. (1985) have proposed that a carbon compound that contains nitrogen might also be important as an energy or carbon source for the bacteroids. The model had two major components:

1. Bacteroids do not appear to be nitrogen stressed, they have low levels of nitrogen assimilating enzymes and export ammonia. If they are not nitrogen stressed, it is peculiar that the bacteroids continue to reduce nitrogen, a process that requires significant energy.

2. If the bacteroides carbon supply was dependent on the plantas nitrogen status, there would be a reason for them to fix nitrogen despite the abundance of ammonia available. Kahn ~ al. proposed that, by coupling the metabolism of the symbionts through the exchange of compounds that contained nitrogen, a more evolutionary stable relationship between the plant and its symbiont would be produced. Although the use of amino acids by the bacteroid had been considered previously, the general view seemed to be that the flow of nitrogen was unidirectional and away from the bacteroid. However, because of the reversibility of many of the enzymes used in amino acid metabolism, most of the data in the literature are consistent with either an anabolic or catabolic role for amino acids. In this paper some recent evidence concerning nitrogen flow and catabolism will be reviewed. DOES NITROGEN FLOW TO THE BACTEROID? Two sorts of data have previously supported the view that at least some nitrogen containing compounds are available to the endosymbiont. The first is that root exudates contain relatively high concentrations of amino acids, especially glutamate. Although it can be argued that compartmentalization within the nodule prevents these compounds from reaching the bacteroids, the additional observation that amino acid auxotrophs are generally Fix+ suggests that sufficient quantities of amino acids are available within the infected cells. In!.:. meliloti, this argument is especially strong with respect to the amino acid glutamate. Because glutamate and glutamine are used as amino group donors in a number of biosynthetic reactions and glutamate is

ENERGETICS OF SYMBIOTIC NITROGEN FIXATION: THE RELATIONSHIP BETWEEN OXYGEN ,MALATE AND HYDROGEN

The energetics of symbiotic nitrogen fixation was Investigated by measuring on intact soybean plant, with an open fiow device, the Influence of the root medium oxygen and malate content on the nodule nitrogenase acetylen reducing activity. It was shown that the maximum nodule activity required more oxygen than In air and was higher In presence of malate, but the photosynthesis modification by light Intensity manipulation had no effect on nodule activity, although it affected the total mass of nodule per plant. The presence of an hydrogenase, which oxidized the hydrogen produced by the nitrogenase, had no beneficial effect on nitrogen fixation and growth of soybean, probably because of the oxygen limitation of nodule activity. In the absence of hydrogenase, the hydrogen production was higher than the minimum one measured on the purified enzYme; it varied with the environment and the host-plant cultlvar.

INTRODUCTION

The reduction of atmosphere nitrogen into ammonium by the nitrogenase enzyme requires a lot of energy in reducing power and ATP (Salsac et aI., 1984). This high energetic cost of nitrogen fixation Is increased by the nitrogenase synthesiS of hydrogen, a gas which is generally evolved by root legume nodules except with the few strains of Rhizobium that possess an active hydrogenase (Eisbrener and Evans, 1983). Other major expenditures associated with the symbiotic N2 flxatlon are the requirements for the nodule development and malntalnance, and the carbon skeletons, reducing power and ATP necessary for the ammonium assimilation. Consequently, the root-nodule operation requires important fiuxes of carbon substrates for reducing power and oxygen for ATP generation. These fluxes are mediated respectively by the host-plant supply of sucrose from its photosynthesis and a facilitated ~ diffusion inside the nodule (Sheehy et aI., 1985). This paper presents the results of in situ assays of nodule acetylen reduction in presence of various oxygen partial pressures. The infiuence of the carbon flux was studied by comparing plants grown under various illuminations or by supplying carbon substrates in the root medium. The energetic Influence of the hydrogenase and the magnitude of the hydrogen production by the nitrogenase is also addressed.

PERI BACTEROID MEMBRANE STABILITY AND PHYTOALEXIN PRODUCTION IN LEGUME NODULES A.

The biogenesis and functions of the peribacteroid membrane (PBM) are summarised. Examples for the structural variability of the PBM and the peribacteroid space in Vicia faba are given. In order to use phytoalexin production in nodules-a5 a biochemical marker for istabilities of the PBM in Vicia faba , the development of radio-immune-assays for the detection of wyerone and wyeronic acid is described. Thin layer chromatography and HPLC techniques were used for the purification of these phytoalexins. The nitrogenase activity of the nodules studied was determined from field grown plants as well as from plants infected with nod+ fix+ defined strains of Rhizobium leguminosarum.

INTRODUCTION

The faba bean (Vicia faba), a major seed legume with a world annual production of 4.3 million t and an EEC production of 0.44 million t (FAO data from 1981), has a stagnating or decreasing area of production. The low yield stability which is also accompanied by a large variation in nitrogen fixation is one of the major handicaps of the cultivars of this crop. Reported data of nitrogen fixation vary between 40 and 500 kg N per hectare per year. On the other hand the potential of this old European crop is rather high. Nodulation and nitrogen fixation in Vicia faba is distinguished by - a high dominance of the endogenous strain of Rhizobium leguminosarum against inoculating strains; - the large number of often very small nodules per plant (up to 1000); - vacuolated host cells after bacteroid differentiation; - a sometimes low responsiveness to shading experiments; - an often high responsiveness to water conditions and - a low responsiveness to low and medium N- fertilisation.