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Physical and Morphological Constraints on Transport in Nodules

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ABSTRACT

For active nodule nitrogen fixation, O_2, N_2, and carbohydrate must be transported throughout the nodule. No quantitative analysis of these transport processes in the nodules has been presented. By invoking several simplifying assumptions, a second-order differential equation for the various gradients and concentrations in the nodule was solved. Even though the nodule can only be approximated in this analysis, it indicates clearly that intercellular gas spaces must exist in nodules for adequate O_2 distribution. To preserve low O_2 concentrations and protect the nitrogenase, these gas spaces cannot be in direct contact with the ambient atmosphere. It is hypothesized that a gas barrier exists in the cortical region of the nodule to limit O_2 diffusion. This barrier would not substantially inhibit N_2 transport. Carbohydrate transport from the vascular tissue via diffusion in the liquid phase can adequately accommodate the requirements within the nodule.

To sustain symbiotic N fixation, there are a number of physical processes involved in transporting materials about an intact nodule. Each of them may place a limit on high N-fixation rates. One process which has been given some attention is the transport of O_2 in the intact nodule.

The significance of O_2, of course, is the requirement that bacteroids have for O_2 to sustain N_2 fixation. Nitrogenase is readily inactivated by O_2 so that very low concentrations must be preserved around the enzyme. This paradoxical situation appears to be resolved by the presence of leghemoglobin which has a very high affinity for O_2 and can apparently make O_2 available to the bacteroids even at very low O_2 concentrations (3).

Tjepkema and Yocum (15) measured O_2 concentration in soybean nodules with microelectrodes. They found a substantial drop in O_2 concentration at the inner layer of the nodule cortex and a uniformly low O_2 level in the bacteroidal volume of the nodule. Sprent (14) and Bergersen and Goodchild (2) found, in anatomical studies of soybean nodules, that there were gas-filled, intercellular spaces which were apparently continuous from the nodule surface to the central volume. On the nodule surface, Pankhurst and Sprent (9) found lenticals, which they concluded allowed gas to readily enter the nodule under turgid conditions.

No quantitative analysis of the O_2 transport process in nodules has been made to elucidate the significance of these observations. Of course, the complex geometry and biochemistry of nodules make an exact mathematical analysis impossible. Here, we solve a second-order differential equation to approximate the O_2 gradients and concentrations which may exist in nodules. Even though several simplifying assumptions are required, this analysis confirms that the intercellular air spaces are essential for gas transport in nodules and that a barrier to O_2 diffusion apparently must exist in the nodules.

Further, the solution of the second-order diffusion equation also allows an analysis of the problem of carbohydrate transport in nodules. Since vascular tissue forms a spherical shell around the bacteroidal volume, carbohydrate as a substrate must diffuse from the nodule perimeter throughout the nodule. The diffusion of carbohydrate may or may not limit N_2 fixation.

MATERIALS AND METHODS

Estimation of Flows. We will approach the quantification of the flows from a realistic agronomic goal of a nitrogen fixation rate of 2 kg N ha^{-1} day^{-1} (13). We will consider a soybean crop with a plant population of 2×10^5 plants ha^{-1} and with 100 nodules/plant. Each nodule then will be assumed to fix nitrogen at a rate of about 1×10^{-9} g N s^{-1}. This number will be used to estimate first the required flow of glucose and then of O_2.

Glucose is required in the nitrogen fixation process as a source of energy and carbon skeletons and as the substrate for sustaining maintenance respiration in the nodule. On the basis of the theoretical considerations of Penning de Vires (10, 11), it is possible to construct the following reaction for the fixation of 1 g N:

$$15.5 \text{ g glucose} + 6 \text{ g } O_2 + 1 \text{ g } N_2 \rightarrow 7 \text{ g amino acids} + 10.7 \text{ g } CO_2 + 4.8 \text{ g } H_2O$$

Experimentally, Ryle *et al.* (12) measured the CO_2 loss from nodules of soybean, cowpea, and clover. Expressing this loss/g nitrogen fixed, full-grown, nonsenesced nodules were found to lose 3 to 4 g C/g N fixed. Converting this ratio to CO_2 loss yields $4 \times 44/12 = 14.7$ g CO_2 evolved, or 4.0 g more than derived above. This excess CO_2 loss can be attributed to maintenance respiration and represents an additional glucose requirement of about 2.7 g. Approximately 18 g glucose is used by the nodule during its fixation of 1 g N or, to meet the agronomic goals, about 18×10^{-9} g glucose s^{-1} needs to be imported by a nodule.

A similar approach can be used to estimate the O_2 requirement. From the reaction above, 6 g O_2 is required in fixation itself and maintenance respiration requires 2.9 g O_2. Consequently, approximately 8.9 g O_2 is required during the fixation of 1 g N, and the agronomic goal requires 8.9×10^{-9} g O_2 s^{-1} to be obtained by a nodule from the surrounding atmosphere.

Model. A second-order differential equation can be used to define material transport in the nodule after invoking several simplifying assumptions. It is assumed that the nodule is a uniform, homogeneous sphere because this geometry reasonably approximates many nodules and it allows an analytic solution. The biochemical events of N_2 fixation are assumed to be reasonably

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represented by a Michaelis-Menten expression. The differential equation is

$$\frac{D}{r^2}\frac{d}{dr}\left(r^2\frac{dS}{dr}\right) = \frac{V_m S}{S + K_m} \quad (1)$$

where D = diffusion coefficient (cm^2/s), r = distance from center of sphere (cm), S = substrate concentration (g/cm^3), V_m = maximum biochemical velocity ($g/cm^3 \cdot s$), and K_m = Michaelis-Menten constant (g/cm^3). To solve equation 1, the additional assumption will be made that the substrate concentration everywhere in the nodule is much greater than K_m ($S \gg K_m$). Therefore, equation 1 reduces to

$$\frac{D}{r^2}\frac{d}{dr}\left(r^2\frac{dS}{dr}\right) = V_m \quad (2)$$

Equation 2 is readily solved for substrate concentration by defining the boundary conditions. At the surface of the sphere ($r = R$), the substrate concentration is defined (S_0). The second boundary condition is obtained by assuming an inner, concentric sphere containing senesced material ($r = R_x$). Since no fixation is occurring in the inner sphere at its surface there is no net movement of substrate, $(dS/dr)_{r=R_x} = 0$. Solving for S,

$$S = S_0 - \frac{V_m}{6D}\left[(R^2 - r^2) + 2R_x{}^3\left(\frac{1}{R} - \frac{1}{r}\right)\right] \quad (3)$$

Since the flux/unit surface area (ψ) is given by $D(dS/dr)_{r=R}$

$$\psi = \frac{V_m R}{3}\left(1 - \frac{R_x{}^3}{R^3}\right) \quad (4)$$

Finally, by multiplying ψ by the sphere surface area ($4\pi R^2$), the total flux (ϕ) is

$$\phi = \frac{4}{3}\pi R^3 V_m\left(1 - \frac{R_x{}^3}{R^3}\right) \quad (5)$$

Equation 5, of course, confirms that the total flux is equivalent to the volumetric consumption rate (V_m) multiplied by the total volume

$$\left[\frac{4}{3}\pi R^3\left(1 - \frac{R_x{}^3}{R^3}\right)\right].$$

Of course, if the nodule contains no senesced material, then $R_x \rightarrow 0$ and equation 3, 4, and 5 are further simplified. It is also evident from these equations that R_x can be a substantial fraction of R before any appreciable reduction in flux will result. Due to the third power ratio of R_x/R, which reflects the volumetric ratio, R_x must approach nearly half the radius of the nodule before the flux is reduced 10%. For convenience in the following discussion, R_x will be assumed to be negligible.

RESULTS AND DISCUSSION

O_2 Transport. A basic assumption employed in the solution of equation 1 is that the substrate concentration, in this case O_2, is much greater than the Michaelis-Menten constant. For O_2, leghemoglobin is assumed to initially react with O_2, and its K_m for O_2 has been found to be very low (16). Therefore, under many conditions of diffusion the assumption of $S \gg K_m$ would be met.

If simple diffusion of O_2 through liquid is the main mode of transport, the distance into the nodule at which anaerobic conditions exist can be estimated from equation 3 by solving for r where $S = 0$. To make this calculation estimates of R, D, S_0, and V_m are required. The nodule diameter will be assumed to be 0.3 cm, so R = 0.15 cm. The value of the diffusion coefficient (D) is assumed equal to that of O_2 in H_2O or 1.8×10^{-5} cm^2/s. The estimate of S_0 will be that for O_2 in equilibrium between the atmosphere and the outer shell of H_2O. Therefore, S_0 is assumed to be the atmospheric concentration of O_2 (0.27×10^{-3} g/cm^3) multiplied by its solubility (0.033 at 20 C), or about 9×10^{-6} g/cm^3. The O_2 flow/nodule was previously estimated as 8.9×10^{-9} g s^{-1}. Using equation 5, V_m is estimated to be 6.3×10^{-7} g cm^{-3} s^{-1}.

Solving equation 3 for the distance into the nodule where conditions become anaerobic, only an outer shell of 52 μm thickness would be aerated. This, of course, would be an unacceptable assumption because it would mean that the entire central volume of the nodule was anaerobic and N fixation would be impossible. To obtain a more satisfactory solution, the variables would have to be changed by orders of magnitude. The estimates of R, D, and S_0 are reasonably good and have limited flexibility. The estimate of V_m would have to be decreased by a factor 15 to permit O_2 to reach the center of the nodule, but then only 0.13 kg N ha^{-1} day^{-1} can be fixed.

The only resolution of the above dilemma seems to be a revision in the assumption that O_2 diffuses only through liquid. Since Pankhurst and Sprent (8, 9) observed lenticels on the nodule surface and Sprent (14) and Bergersen and Goodchild (2) observed continuous air spaces inside nodules, these air passages may allow considerable gaseous diffusion. Bergersen and Goodchild estimated that the air spaces occupied 2.5 to 5% of the nodule volume. The value of D for O_2 in air is 4 orders of magnitude greater than in H_2O, or 0.18 cm^2/s. Assuming an "effective" D roughly proportional to the fraction of air spaces, we estimate D for a nodule with air spaces to be about 5×10^{-3} cm^2/s. If the air spaces are continuous within the nodule, this new estimate of D results in a decrease of O_2 concentration in air from the nodule surface to its center of about 5×10^{-7} g/cm^3, which is a negligible portion of the atmospheric concentration of 0.27×10^{-3} g/cm^3. Therefore, the air spaces seem adequate and crucial to having O_2 transported throughout the bacteroidal volume of the nodule.

Since the air spaces provide adequate "ventilation" for O_2, equation 3 can next be used to solve for the O_2 concentration in the bacteroid-containing cells. In this case, it is clear that D must be for O_2 in liquid. Assuming the radius of the bacteroid-containing cell is 20 μm, the O_2 concentration at the center of the cell is calculated to be about 99.5% of that at the cell surface. Consequently, this situation would lead to possible O_2 inactivation of nitrogenase.

A barrier to O_2 diffusion seems to be required to minimize the O_2 concentration in the bacteroid-containing volume. Fraser (6) observed a common endodermis in the cortex of nodules which she suggested would restrict gaseous diffusion. Tjepkema and Yocum (15) suggested, from their measurements of O_2 concentration in the nodule, the existence of a barrier to O_2 transport in the inner layer of the nodule cortex. Analysis of nodule gas-exchange data also led Pankhurst and Sprent (8) to hypothesize at the diffusion barrier. Simply imposing a H_2O shell around the bacteroid-containing volume would effectively provide such a barrier. Assuming that most of the O_2 concentration drop occurs at this barrier and the flux density of O_2 (ψ) is 3.1×10^{-8} $g/cm^2 \cdot s$, then the permeability coefficient to gaseous O_2 for this barrier would need to be about 1.3×10^{-4} cm/s. The thickness of a H_2O-layer barrier then must be about 45 μm. This thickness for a barrier is consistent with the concept of a continuous shell of cells completely surrounding the central volume of the nodule.

Another consideration in relation to gas transport is the potential inhibition of N_2 transport imposed by this barrier. The concentration of N_2 in the ambient atmosphere is 4 times greater than that of O_2, but its solubility in H_2O is only half of O_2. However, the flux density of N_2 is expected to be less than 15% of O_2. For the same thickness of H_2O shell, the concentration of N_2 inside the barrier would be more than 90% ambient. Consequently, this barrier would impose no serious limitation to N_2 transport.

It seems that anatomically nodules are very well designed to

accommodate the dual requirement of low, but uniform, O_2 requirement throughout the bacteroidal volume. The proposed barrier at the inner cortex apparently would need to be only one or two cells thick to result in a low O_2 concentration. The intercellular air spaces would allow O_2 at the lowered concentration to be transported rapidly inside the nodule. Increases in ambient O_2 concentration up to 0.4 atm would raise the O_2 levels in the bacteroidal volume and allow greater N_2 fixation rates. The data of Bergersen (1) suggest that, under this elevated O_2 concentration, the concentration of O_2 in the central volume of the nodule may be 0.02 to 0.03 atm. Further increases in O_2 concentration potentially inhibit the activity of nitrogenase. These increases in O_2 concentration would, however, also stimulate the respiratory activity of the mitochondria in the central volume of the nodule. Consequently, a rather complicated response to changes in O_2 concentration by intact nodule systems may result (1, 5).

Carbohydrate Transport. Since there is no vascular tissue permeating the interior of the nodule, imported carbohydrate must diffuse into the nodule from the vascular shell. Therefore, this process can be examined with the same set of equations as used for O_2 transport. In this case, however, the value of S_0 is not known and it is the variable for which equation 3 is solved. If the diffusion of carbohydrate into the nodule requires a high carbohydrate concentration in the vascular shell, then either turgor flow in the phloem has to be reconsidered or there needs to be a carbohydrate concentrating process in this region outside the phloem.

To make these calculations, estimates for several variables are required. The diffusivity of sucrose in H_2O is about 5×10^{-6} cm^2/s (7). The values of ϕ and, consequently, V_m are obtained from the previous assumptions of N_2 fixation rate. That is, ϕ equals about 18×10^{-9} g glucose/cm^3·s.

From equation 3, it is clear that S_0 must be greater than $V_m R^2/6D$ or 1×10^{-3} g/cm^3 (or 3 mM for sucrose). Encouragingly, this estimated minimum S_0 is very small and represents an osmotic potential of only about 0.07 bars. This small gradient certainly allows room for an increase in the background concentration of sucrose so that it is sufficiently high for $S \gg K_m$. We conclude that the requirements for diffusional transport of carbohydrate in the nodule probably induce little negative feedback on turgor-driven phloem flow to the nodule.

CONCLUSIONS

This analysis indicates that the physical constraints to carbohydrate transport involved in maintaining a functioning nodule are not significant. Only a relatively small gradient in sucrose expressed as osmotic potential between the vascular shell of the nodule and its interior is required for adequate transport by diffusional processes.

On the other hand, the analysis of gaseous transport in nodules points to several important conclusions. First, it seems impossible for N_2 fixation to be sustained at high rates if N_2 and O_2 must be transported only by diffusion through the liquid. We conclude that the air spaces observed in soybean nodules by Sprent (14) and by Bergersen and Goodchild (2) are critical for adequate "ventilation" of nodules fixing N_2 at a relatively high rate. The continuity and abundance of the observed air spaces are adequate to allow diffusional transport of these gases. We hypothesize that such air spaces exist in other species that exhibit high N_2 fixation rates. Second, to maintain near-anaerobic conditions around the bacteroid-containing cells, a barrier to O_2 diffusion is seemingly required in the nodule. The barrier at the inner cortex proposed by Fraser (6), Tjepkema and Yocum (15), and Pankhurst and Sprent (8) could result in the required low O_2 concentrations in the bacteroidal volume. A continuous shell of H_2O only about 45 μm thick would apparently be adequate as a barrier to O_2. The resulting low O_2 concentrations around the bacteroids would, of course, demand the presence of an O_2 scavenger, such as leghemoglobin, to provide the O_2 required for high N_2 fixation rates (4).

LITERATURE CITED

1. Bergersen FJ 1962 The effects of partial pressure of oxygen upon respiration and nitrogen fixation by soybean root nodules. J Gen Microbiol 29: 113–125
2. Bergersen FJ, DJ Goodchild 1973 Aeration pathways in soybean root nodules. Aust J Biol Sci 26: 729–470
3. Bergersen FJ, GL Turner 1975 Leghemoglobin and the supply of O_2 to nitrogen-fixing root nodule bacteroids: studies of an experimental system with no gas phase. J Gen Microbiol 89: 31–47
4. Bergersen FJ, GL Turner, CA Appleby 1973 Studies of the physiological role of leghemoglobin in soybean root nodules. Biochim Biophys Acta 292: 271–282
5. Criswell JG, UD Havelka, B Quebedeaux, RWF Hardy 1976 Adaptation of nitrogen fixation by intact soybean nodules to altered rhizosphere pO_2. Plant Physiol 58: 622–625
6. Fraser HL 1942 The occurrence of endodermis in leguminous root nodules and its effect upon nodule function. Proc R Soc Edinb Sect B 61: 328–343
7. Nobel PS 1973 Introduction to Biophysical Plant Physiology. W. H. Freeman and Company, San Francisco, p 17
8. Pankhurst CE, JI Sprent 1975 Effects of water stress on the respiratory and nitrogen-fixing activity of soybean root nodules. J Exp Bot 91: 287–304
9. Pankhurst CE, JI Sprent 1975 Surface features of soybean root nodules. Protoplasma 85: 58–98
10. Penning de Vries FWT 1975 Use of assimilates in higher plants. *In* JP Cooper, ed, Photosynthesis and Productivity in Different Environments. Cambridge University Press, Cambridge, England
11. Penning de Vries FWT 1975 The cost of maintenance processes in plant cells. Ann Bot (Lond) 39: 77–92
12. Ryle GJA, CE Powell, AJ Gordon 1979 The respiratory costs of nitrogen fixation in soybean, cowpea and white clover. I. Nitrogen fixation and the respiration of the nodulated root. J Exp Bot 30: 135–144
13. Sinclair TR, CT De Wit 1976 Analysis of the carbon and nitrogen limitations to soybean yield. Agron J 68: 319–324
14. Sprent JI 1972 The effects of water stress on nitrogen-fixing root nodules. II. Effects on the fine structure of detached soybean nudules. New Phytol 71: 443–450
15. Tjepkema JD, CS Yocum 1974 Measurement of oxygen partial pressure within soybean nodules by oxygen microelectrodes. Planta 119: 351–360
16. Wittenberg JB, CA Appleby, FJ Bergersen, GL Turner 1975 Leghemoglobin: the role of hemoglobin in the nitrogen-fixing legume root nodule. Ann NY Acad Sci 244: 28–34