HOST AND ENVIRONMENTAL FACTORS AFFECTING HYDROGEN EVOLUTION AND UPTAKE

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Hydrogen production is an integral part of nitrogen fixation. Considerable attention has been given to the recovery of the energy "lost" in producing this H_2, primarily through the activity of uptake hydrogenases (Hup) found in many rhizobia and other diazotrophs (1). This paper summarises efforts to determine whether Hup activity benefits symiotic nitrogen fixation in legumes, and a study of the factors influencing both H_2 production and the development of Hup in nodules.

Materials and Methods

Various legumes, nodulated by appropriate effective strains of rhizobia, were grown on agar slopes in test tubes, or in vermiculite/perlite medium, under optimal conditions in controlled environment cabinets (2). Decapitated, nodulated roots were assayed for H_2 production, H_2 uptake, C_2H_2 reduction, and/or $^{15}N_2$ fixation in vessels of adequate volume to maintain pO_2 at 0.2 atm. throughout the 30 min. assays. Reciprocal grafts were made between four Vigna species at the seedling stage, and assays made three weeks after inoculation.

Results and Discussion

1. The host controlled development of Hup in nodules formed by Rhizobium sp. strains CB756 and 32H1. On Vigna radiata, the nodules were Hup^-, but with V. unguiculata, V. mungo and two other Vigna species, the nodules were Hup^+.
2. Providing 1% H_2 in the gas phase had no effect on the rate of $^{15}N_2$ fixation by Hup^+ nodules, even though 1-1.5 µmole H_2 were taken up for each µmole N_2 fixed. Perhaps of greater significance was the failure of 1% H_2 to significantly promote C_2H_2 reduction; C_2H_2 would block normal H_2 production and thereby deprive the Hup^+ nodules of any energy they would normally regain through recycling. Under these circumstances, providing H_2 would be expected to have a promoting effect. There was some indication that H_2 uptake was less under $C_2H_2/O_2/Ar$ (10:20:70) under air.
3. Based on the determination of Relative Efficiency (RE = 1 - H_2 evolved/C_2H_4 produced) of reciprocally-grafted Vigna species, the root genotype, as opposed to the shoot genotype, was the principal factor in determining Hup activity in CB756- and 32H1- nodules.
4. With soybeans nodulated by a number of R. japonicum strains, some forming Hup^+ nodules and the remainder Hup^- nodules, no advantage (dry weight, total N) was found in the plants with Hup^+ nodules up to 7 weeks after sowing. The correlation between RE and symbiotic effectiveness (either total N or plant dry weight) was not significant. Two strains of "exceptional efficiency" (3) were tested; both evolved H_2 at a high rate.
5. With Trifolium subterraneum nodulated by each of 10 strains of R. trifolii, there was an inverse relationship between RE and symbiotic effectiveness ($r = 0.65$, $P < 0.05$), i.e., the more effective the strain, the higher the proportion of electrons passing through nitrogenase that were lost as H_2. With this species, H_2 evolution utilised approx. 50% of the electron flow, and initial estimates of the $C_2H_4:N_2$ ratio varied between 5.5 and 7.0. Correction for H_2 evolution restored the ratio to 2.9-3.2 over the six strains examined in detail.
6. With lupins, the H_2 evolved : N_2 fixed ratio was 4-5, while with peas it was circa. 2. These observations, plus those with cowpea (2:1), soybeans (1.5:1), and sub clover (above) indicate that more H_2 is evolved than would be expected from some theoretical estimates, and recorded values, of 1:1.
7. The RE with sub clover was raised by lowering the light intensity, by providing 3 mM KNO_3 or making the estimations during the dark period, all conditions under which photosynthate supply to the nodules would be reduced. However, it was only be defoliating the plants that the RE rose above 0.75 (actually 0.95-1.0), indicating Hup activity. This happened with 6 of 9 plants examined 24 h after defoliation (2/3 plants inoculated with each of 3 strains), but the effect was transitory as the RE values declined to 0.6 as nitrogenase activity rose again.
8. Based on measured rates of H_2 evolution, nitrogen and dry weight increase in the plants, and the assumptions that the oxidation of 1 mole of glucose in the nodules yields 36 ATP and that 4-5 moles of ATP are used for each mole H_2 produced, it can be calculated that soybeans will use only 2.7% of their daily dry weight increase in producing H_2. Furthermore, if only 1-2 ATPs are recovered by the activity of the Hup system, the overall effect of Hup would be to increase the energy supply to the Hup^+ plants by 0.9% (cf. Hup^-).
9. The conclusions are that the host has a marked effection both H_2 production and on Hup activity, and that closer definition of the physiological processes involved is required. A greater energy saving than that possibly effected by Hup would be achieved if the level of H_2 production could be lowered to the $1H_2:1N_2$ level.

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(3) Dobereiner, J. et al., 1970. Pesq. Agropec. Bras. 5:155-161.
(4) Dixon, R.O.D., 1972. Arch. Microbiol. 85: 95-103.