Sugar and Amino Acid Transport Across Symbiotic Membranes from Soybean Nodules

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Uptake of a range of sugars and amino acids into isolated peribacteroid units (PBUs) and bacteroids was measured and compared to malate and succinate uptake. Evidence was obtained for the presence of two transport systems on the bacteroid membrane for the transport of D-fructose that can catalyze rapid fructose uptake over a wide concentration range into free bacteroids. Fructose uptake by intact PBUs was slow and showed no evidence of saturation up to 10 mM. Uptake of sucrose, glucose, phenylalanine, methionine, leucine, glycine, and proline into both PBUs and bacteroids was also very slow (less than one tenth of the rate of succinate uptake). Neither sugar nor proline uptake by PBUs showed a tendency toward saturation over a wide concentration range. The peribacteroid membrane apparently lacks carriers for these compounds. Despite slow uptake rates, substantial labeling of bacteroid proteins occurred during a 60-min incubation of intact PBUs with [^{35}S]methionine. Apart from one faint band on the peribacteroid membrane, there was no evidence for secretion of labeled polypeptides from bacteroids. Sugar and amino acid transport was similar in small PBUs from developing nodules; these units also displayed carrier-mediated malate uptake, which occurred at a faster rate per unit protein than that into large units from mature nodules. The polypeptide pattern of the peribacteroid membrane isolated from 4-wk-old nodules was substantially different from that of 8-wk-old nodules, despite their similar permeability to metabolites.

Root nodules of legumes receive sucrose from the shoot to support symbiotic nitrogen fixation (Kouchi and Nakaji 1988). This sucrose is metabolized, largely within uninfected cells of the nodule cortex, to glucose, fructose, and organic acids (Reibach and Streeter 1983; Copeland et al. 1989; Kouchi et al. 1988), at least one of which is subsequently supplied to the bacteroids within the infected cells. Evidence currently available strongly suggests that the dicarboxylates succinate and malate are the major sources of carbon supplied in vivo to support nitrogenase activity (Appleby 1984; Dilworth and Glenn 1984; Udvardi et al. 1988a), although isolated bacteroids are able to exchange a number of other carbon compounds (Streeter and Salminen 1988).

Bacteroids within infected nodule cells are enclosed by a plant-derived membrane known as the peribacteroid membrane (PBM), and any nutrients exchanged between host and endosymbiont must be able to cross this barrier. Recent studies with intact isolated peribacteroid units (PBUs, bacteroids enclosed by the PBM) have shown that the PBM is effectively impermeable to sucrose and glutamate but contains a dicarboxylate carrier capable of transporting malate and succinate at rates sufficient to support measured rates of nitrogenase activity (Price et al. 1987; Udvardi et al. 1988a, 1988b; Day et al. 1989b). However, the nodule contains a wide range of other carbon and nitrogenous compounds that have the potential to be used by the bacteroid, and the permeability of the PBM to such compounds has not been investigated. Given the impor-

tance of carbon supply to the bacteroid in the regulation of nitrogen fixation (Appleby 1984; O'Gara et al. 1988), it is important to determine the permeability of the PBM to those carbon compounds likely to be available in vivo. It is also important to determine its permeability to the products of nitrogen assimilation. In this study, we report the results of an investigation of sugar, dicarboxylate, and amino acid uptake across peribacteroid and bacteroid membranes from soybean at two different stages of nodule development.

MATERIALS AND METHODS

Materials. Seeds of soybean (*Glycine max* (L.) Merr.) cultivar Bragg were inoculated with *Bradyrhizobium japonicum* USDA110 and grown in pots in a naturally illuminated glasshouse as described by Day et al. (1989a). All radiochemicals were purchased from Amersham International (Amersham, England) and are as follows: L-[U-^{14}C]malate (1.85 GBq/mmol), [2,3-^{14}C]succinate (1.55 GBq/mmol), L-[U-^{14}C]glutamate (0.37 GBq/mmol), [U-^{14}C]sucrose (20 GBq/mmol), D-[U-^{14}C]glucose (10 GBq/mmol), D-[U-^{14}C]fructose (10 GBq/mmol), L-[^{35}S]methionine (37 TBq/mmol), ^{3}H$_2$O (185 GBq/ml), L-[4,5-^{3}H]leucine (4.5 GBq/mmol), L-[U-^{14}C]proline (10 GBq/mmol), L-[U-^{14}C]phenylalanine (19 GBq/mmol), L-[U-^{14}C]glutamine (9.25 GBq/ml), and L-[U-^{14}C]glycine (3.7 GBq/mmol). Silicon oil (AR200) was purchased from Wacker Chemical Co. (Munich, Federal Republic of Germany). Other chemicals were from Sigma Chemical Co. (St Louis, MO).

Preparation of PBUs. Intact, large PBUs (8–16 bacteroids enclosed by a single PBM) were isolated from the nodules of 8-wk-old soybean plants using Percoll density

gradient centrifugation as described by Day et al. (1989b). For small PBUs (two to four bacteroids per PBM) from the developing nodules of 4-wk-old plants, the Percoll step gradient was modified to take account of the lighter density of the PBUs. Four, 2-ml steps of 40, 55, 70, and 100% Percoll were used in a 15-ml tube, and the nodule homogenate was centrifuged through these for 15 min at 4,000 × g in a Sorvall HB-4 swing-out rotor at 4° C. The small, intact PBUs were collected from the 55/70% Percoll interface. In both preparations, the bands from the gradients were diluted with wash buffer (0.35 M mannitol, 3 mM $MgSO_4$, and 25 mM MES-BTP buffer, pH 7.0) and pelleted onto a cushion of 80% Percoll by centrifuging at 4,000 × g for 15 sec. PBUs were resuspended in wash buffer and kept on ice. The integrity of the PBUs was routinely checked by light microscopy (Day et al. 1989b). The purity of the preparations was checked by electron microscopy (Price et al. 1987); no plant organelles could be seen in either the large or small PBU fractions. Preparations were routinely checked for mitochondrial contamination by measuring NADH oxidase activity (Day et al. 1986).

Bacteroids and the PBM were isolated from PBUs as described by Day et al. (1989b).

Other techniques. Uptake of ^{14}C-labeled compounds was measured using the silicon oil centrifugal-filtration technique as described by Udvardi et al. (1988b). Internal volumes of PBUs and bacteroids were estimated as the [^{14}C]sucrose-impermeable, ^3H_2O-permeable space (Palmieri and Klingenberg 1979).

Protein was estimated according to Lowry et al. (1951). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was conducted as described by Day et al. (1989b) using a 15% resolving gel, except that 4 M urea was included in the gel and sample buffer.

Proteins of PBUs were radioactively labeled by incubating 2-3 mg of protein with 10 mM succinate (as respiratory

substrate) and 100 μCi of [^{35}S]methionine for 60 min at 22° C (integrity of PBUs was monitored during the course of the incubation). PBUs were then pelleted by centrifuging at 4,000 × g for 5 min. The pellets were rinsed twice with wash buffer (see above) and resuspended in the same. The PBUs were then further fractionated into bacteroid, peribacteroid space (PBS) contents, and the PBM as described by Day et al. (1989b). Proteins were visualized after SDS-PAGE (see above) by silver staining, and the gel was then dried and exposed to X-ray-sensitive film.

RESULTS

Sugar uptake by PBUs. The rates of uptake of sucrose, glucose, and fructose were measured and compared to that of succinate, a known permeant anion of both bacteroid and peribacteroid membranes (Udvardi et al. 1988a). Succinate uptake into PBUs was rapid and rates were sustained over many minutes (Fig. 1A). Rates of uptake of fructose, glucose, and sucrose were extremely slow.

The rate of uptake of all three sugars into PBUs was linear over a wide concentration range (Fig. 2A), suggesting that these sugars cross both the PBM and bacteroid membrane slowly by passive diffusion. The addition of ATP to PBUs did not affect sugar uptake (data not shown).

Sugar uptake by bacteroids. The uptake of sucrose and glucose by bacteroids was also very slow compared to that of succinate (Fig. 1B) and showed no evidence of saturating over a wide concentration range (Fig. 2B); again, we conclude that transport of these substances is via passive diffusion across the bacteroid membrane.

Fructose uptake by bacteroids, on the other hand, showed saturation kinetics over two different concentration ranges (Figs. 3 and 4), indicative of transport systems on the bacteroid membrane. Double-reciprocal analysis of the data yielded two straight lines with different slopes and
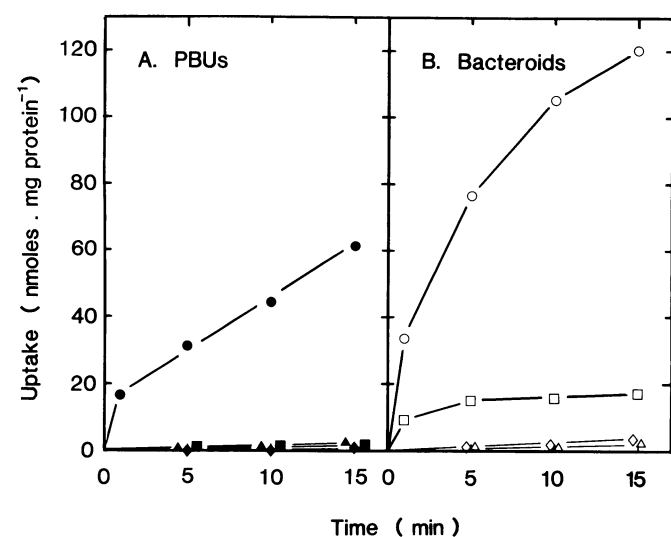

Fig. 1. Time courses for sugar and succinate uptake by isolated peribacteroid units (PBUs) (**A**) and bacteroids (**B**). Uptake of ^{14}C-labeled substrates was measured using centrifugal filtration as described in the text. A final concentration of 1 mM was used for each substrate. Data points are means of duplicate experiments. ●, ○, succinate; ◆, ◇, sucrose; ▲, △, D-glucose; and ■, □, D-fructose.
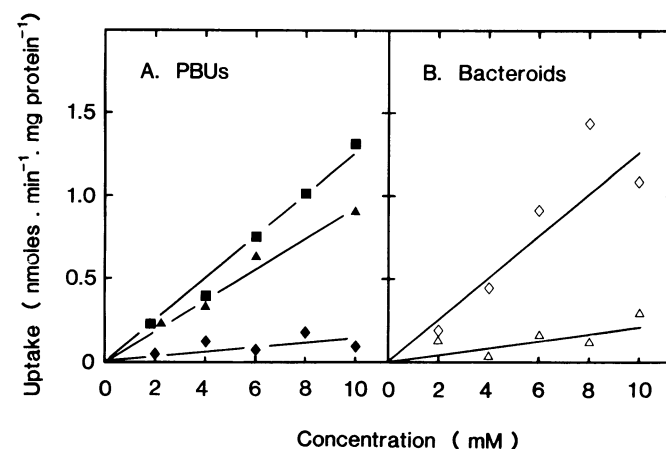

Fig. 2. Concentration dependence of the rate of ^{14}C-labeled sugar uptake by isolated peribacteroid units (PBUs) (**A**) and bacteroids (**B**). Data points represent the slope obtained from linear regression analysis of a time course (duplicates at t = 5, 10, and 15 min) determined at each concentration. ◆, ◇, sucrose; ▲, △, D-glucose; and ■, D-fructose. Note that the scale of uptake rate is much smaller than in Figure 1. Linear regression analysis gave the following correlation coefficients: ◆, r = 0.75; ◇, r = 0.88; ▲, r = 0.87; △, r = 0.70; and ■, r = 0.99.

intercepts (Figs. 3 and 4). Apparent K_m and V_{max} values were calculated from a number of such experiments and are summarized in Table 1. The rate at the higher concentration range is comparable to the rate of uptake of succinate into bacteroids (note that fructose uptake was measured for only 30 sec in the experiments of Figs. 3 and 4). These results suggest that the bacteroid membrane possesses two systems for fructose uptake. When the amount of fructose taken up is expressed as a concentration (using estimates of the internal volume), it can be seen that substantial accumulation of the sugar inside the bacteroid occurred over a large concentration range (Table 2). The uncoupler carbonyl cyanide m-chlorophenylhydrazone inhibited fructose uptake by 80% (data not shown) showing that transport was dependent on a proton motive force across the bacteroid membrane. All of these results are consisent with carrier-mediated uptake of fructose by free bacteroids. However, despite these transport systems on the bacteroid membrane, results with PBUs (Figs. 1A and 2A) indicate that the PBM is only poorly permeable to fructose and would effectively prevent significant uptake by the bacteroids *in vivo*.

Dicarboxylate uptake, on the other hand, showed saturation kinetics with both PBUs and bacteroids (see also Udvardi *et al.* 1988a), was also inhibited by carbonyl cya-

nide m-chlorophenylhydrazone (Udvardi *et al.* 1988a), and was accumulated against a concentration gradient (Udvardi 1989), indicating that it was carrier-mediated.

Amino acid uptake by PBUs. We have shown previously (Udvardi *et al.* 1988b) that L-glutamate uptake by PBUs is very slow while that across the bacteroid membrane is rapid and carrier-mediated. Uptake of a number of other amino acids has now been measured, and the results are shown in Figure 5. In PBUs, uptake of all six amino acids was very slow (no more than one tenth the rate of succinate uptake). From the data in Figure 5 and measurements of internal volume (legend to Fig. 5), the internal concentration of each amino acid can be calculated. For the PBUs, uptake of these amino acids over the first 1–2 min simply resulted in equilibration with the external medium, even when ATP was added to energize the PBM.

Since proline has been suggested as a possible substrate *in vivo* (Kohl *et al.* 1988), we examined its uptake by PBUs over a range of concentrations (Fig. 6). Using 30 sec incubation, between 0.01 and 2 mM, proline uptake showed no tendency toward saturation. Proline uptake was also not inhibited by the inclusion of D-proline or hydroxy-
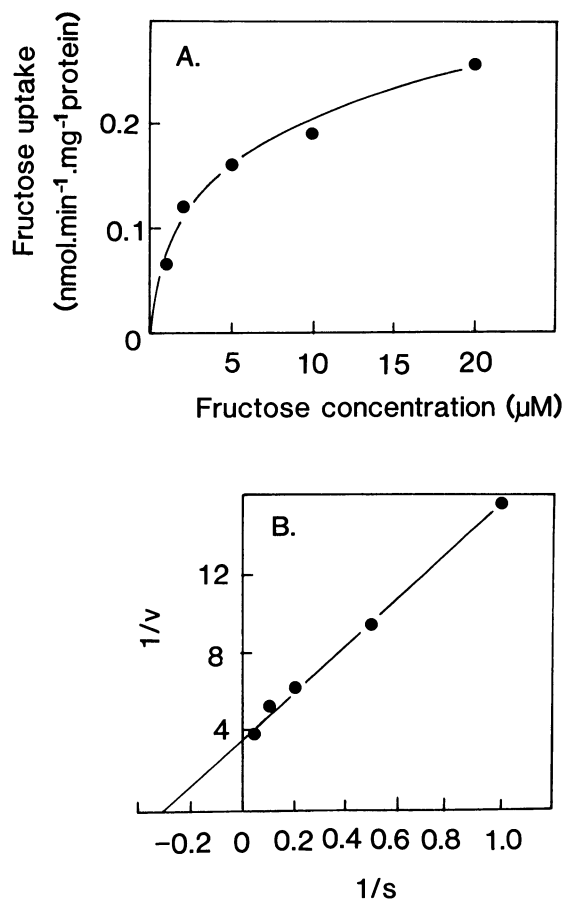

Fig. 3. A, Concentration dependence of the rate of D-[14C]fructose uptake by isolated bacteroids over the range 0–20 μM. Uptake was measured for 30 sec, as described in the text. B, Double-reciprocal plot of the data in A.
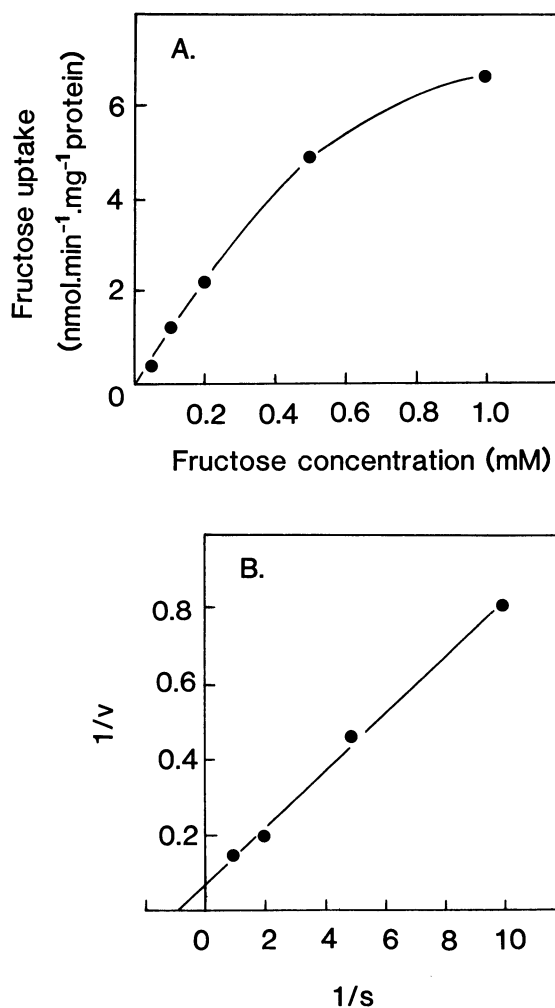

Fig. 4. A, Concentration dependence of the rate of D-[14C]fructose uptake by isolated bacteroids over the range 20–1,000 μM. B, Double-reciprocal plot of the data in A.

proline in the reaction medium, both of which are inhibitors of proline transporters in other membrane systems (Cybulski and Fisher 1977; Day and Wiskich 1984; Yu et al. 1983). Even at a concentration of 2 mM, the rate of proline uptake by PBUs was only 2–3 nmol·min^{-1}·mg^{-1} of protein, which is still substantially slower than succinate uptake (Fig. 1). We conclude that there is no carrier for proline on the PBM.

The ability of these slow rates of amino acid uptake to support protein synthesis was estimated by following the labeling of PBU polypeptides with [^{35}S]methionine, using SDS-PAGE and autoradiography (Fig. 7). The results show clearly that extensive labeling of bacteroid proteins occurred over 60 min. However, apart from a faintly labeled band in the PBM fraction (Fig. 7), no label was detected in the PBS or PBM fractions, in contrast to the results

of Katinakis et al. (1988) with peas, whether small PBUs or large PBUs (see below) were used.

Amino acid uptake by bacteroids. With the exception of glutamate (Udvardi et al. 1988b), uptake of all the amino acids tested was slow (1–4 nmol·min^{-1}·mg^{-1} of protein, Fig. 5); at the same concentration, the rate of glutamate uptake was 10-fold faster (data not shown). Nonetheless, from the calculated internal volume of the bacteroids (legend to Fig. 5), it appears that most of the amino acids were accumulated against a concentration gradient. This accumulation could reflect the presence of transport systems but could also be due to metabolism of the compound taken up.

PBUs from developing nodules. All transport data to date have been obtained with large PBUs from mature nodules (8–10 wk old). Since nutrient exchange between host and bacteroid may change during development of the symbiosis, it is of interest to examine the PBM of develop-

Table 1. D-[^{14}C]Fructose uptake by bacteroids from soybean nodules

Kinetic constants	Concentration range[a]	
	High	Low
K_m (μM)	2.25 ± 0.3	810 ± 120
V_{max} (nmol · min^{-1} · mg^{-1} protein)	0.27 ± 0.04	6.64 ± 1.2

[a] Data shown are the means (± SE) of five separate experiments as described in Figures 3 and 4.

Table 2. Accumulation of D-[^{14}C]fructose by isolated bacteroids[a]

Fructose concentration		
External (C_e)	Internal (C_i)	C_i:C_e
1	25	25
2	33	17
5	55	11
10	71	7
20	98	5
100	790	8
200	1,440	7
500	2,960	6
1,000	3,820	4

[a] Bacteroids were incubated with D-[^{14}C]fructose for 30 sec, and the reaction was terminated by centrifugation through silicon oil. Bacteroid volume was estimated in parallel experiments using [^{14}C]sucrose and ^{3}H$_2$O (Palmieri and Klingenberg 1979).
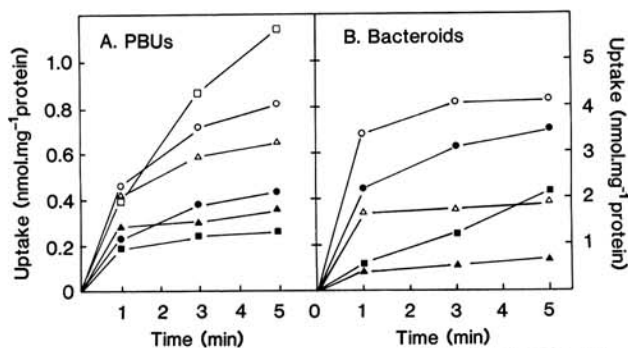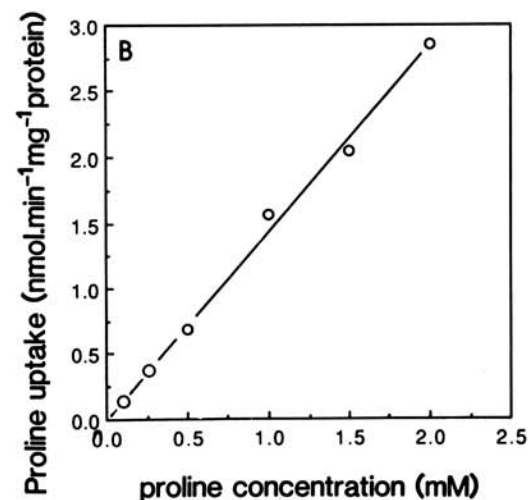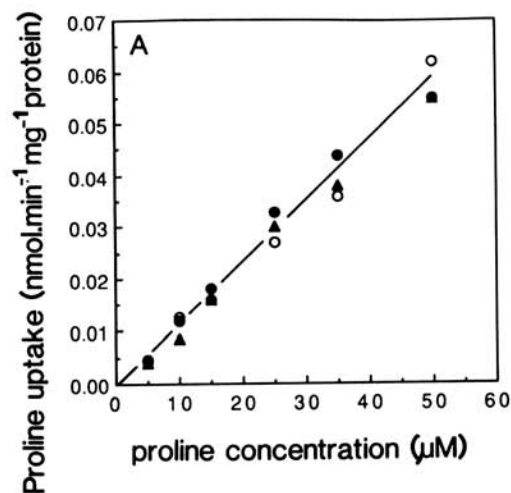

Fig. 5. Uptake of amino acids by peribacteroid units (PBUs) (A) and bacteroids (B). Experiments were performed as described in Figure 1 using 0.1 mM external substrate. O, phenylalanine; ●, methionine; △, leucine; ▲, glycine; □, glutamate; and ■, proline. Note the different scales for uptake in A and B. The internal volume (see Table 1) was 4.7 μl and 1.7 μl per mg of protein for PBUs and bacteroids, respectively.

Fig. 6. Concentration dependence of the rate of L-[^{14}C]proline uptake by PBUs over two concentration ranges. Uptake was measured for 30 sec. O, control, no further additions; ●, 1 mM D-proline included in the reaction mixture; and ▲, 1 mM hydroxyproline included in the reaction mixture.

ing nodules. In the nodules of 4-wk-old plants (under the growth conditions employed), small PBUs containing two to three bacteroids per envelope were found and these could be readily isolated using density gradient centrifugation (Price *et al.* 1987), although we modified our original meth-

Fig. 7. Labeling of peribacteroid unit proteins with [^{35}S]methionine. Peribacteroid units were incubated with [^{35}S]methionine for 60 min and then fractionated into bacteroids (lanes a and d), peribacteroid space (lanes b and e), and peribacteroid membrane (lanes c and f). Lanes a, b, and c are the silver-stained proteins separated on a sodium dodecyl sulfate-polyacrylamide gel; lanes d, e, and f are autoradiographs of the gel. The numbers on the left indicate molecular mass markers in kDa; the arrow on the right indicates a faint band on the autoradiograph of the peribacteroid membrane fraction.

Fig. 8. Polypeptide composition of peribacteroid membranes (PBMs) and peribacteroid space (PBS) from young (4-wk-old; A and C) and mature (8-wk-old; B and D) soybean nodules. Numbers refer to molecular weight standards. The lettered arrows indicate differences between the two fractions. The protein loading was 10 μg for the PBM and 5 μg for the PBS. Detection was by silver staining.

od somewhat to accommodate the lower density of the smaller particles (see Materials and Methods). PBUs from still younger nodules are difficult to separate from free bacteroids with these techniques because of the similarity in densities. We have therefore compared the properties of PBUs from 4- and 8-wk-old nodules.

SDS-PAGE analysis of PBS and PBM polypeptides showed clear differences between PBUs from young and mature nodules, with several qualitative differences being seen in the PBM banding pattern (Fig. 8, A and B). Differences between PBS proteins tended to be more quantitative in nature (Fig. 8, C and D). Despite these changes in PBM protein content, the measured permeability properties of the membrane changed only slightly during the transition from 4 to 8 wk. Sugar uptake by small PBUs was very slow (Fig. 9; similar results were obtained with glucose and fructose), while that of malate was rapid (Fig. 9) and showed saturation kinetics (Fig. 10). Amino acid uptake rates were similar to those observed with large PBUs (results not shown). The apparent K_m for malate uptake by small PBUs was similar to that in large PBUs (Table 3), but malate transport was faster in the smaller units (Fig. 10, Table 3).

DISCUSSION

The results presented show that the PBM of isolated PBUs is poorly permeable to a range of sugars and amino acids and apparently lacks carriers for these compounds. Of all organic compounds tested to date, only a small group of dicarboxylates (the chief ones being malate and succinate) are rapidly taken up by PBUs via a carrier (Price *et al.* 1987; Udvardi *et al.* 1988a; Udvardi 1989). Taken together these results suggest that sugars and amino acids are unlikely to be used as carbon substrates by bacteroids to support nitrogenase at concentrations likely to be found *in vivo*. However, although proline uptake across the PBM was slower than that of malate and seems to occur as passive diffusion, extrapolation of the data in Figure 6 indicates
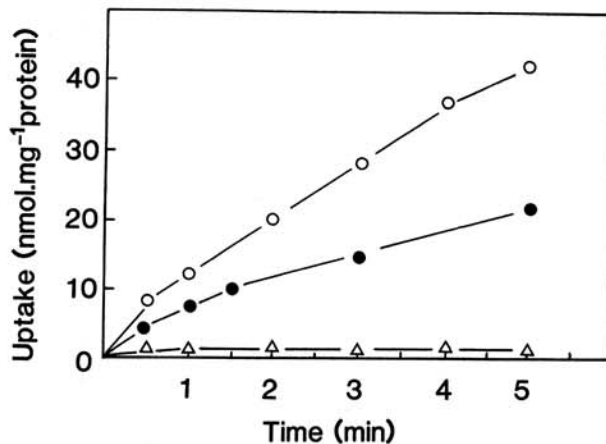

Fig. 9. Time course of sucrose and malate uptake by peribacteroid units (PBUs) from young and mature nodules. ^{14}C-labeled substrates were added to give a final concentration of 1 mM sucrose or 0.3 mM malate, and uptake was terminated after 30 sec. ○, malate uptake by small PBUs from 4-wk-old nodules; ●, malate uptake by large PBUs from 8-wk-old nodules; and △, sucrose uptake by small PBUs.
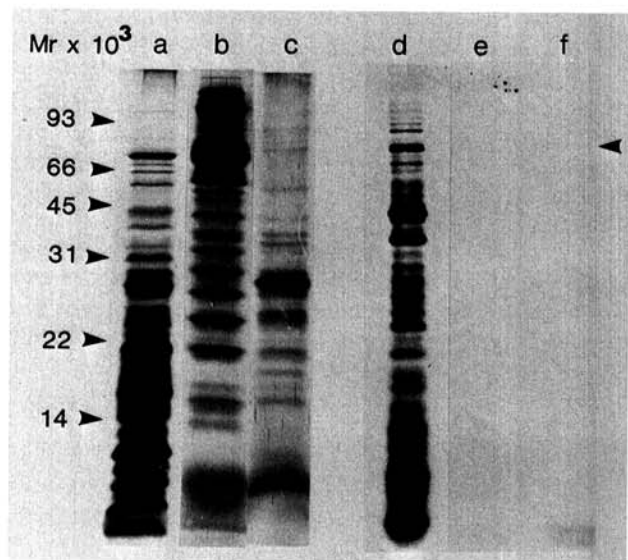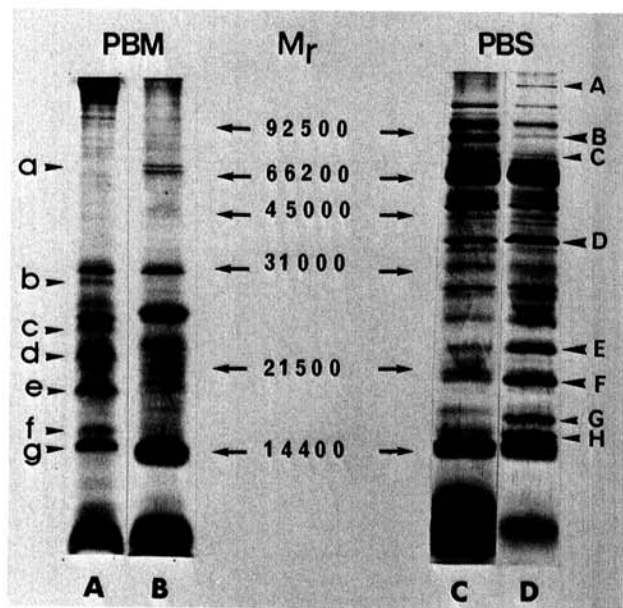
that at high concentrations (10 mM), proline uptake would be fast enough to support nitrogenase. Relatively rapid rates of diffusion of neutral amino acids have been observed in other membranes (Day and Wiskich 1984).

These permeability properties were found in small PBUs from developing nodules as well as in large PBUs from mature nodules, although clearly the polypeptide profile of the PBM changes during nodule maturation (Fig. 7). This implies that the growth in PBUs between 4 and 8 wk after planting is fueled by dicarboxylate uptake, as well as nitrogen fixation. It should be noted that at 4 wk our soybean plants showed rapid acetylene reduction, and it may be that appreciable changes in PBM permeability occur prior to the onset of nitrogen fixation. The faster rates of dicarboxylate uptake by the smaller PBUs are consistent with the additional carbon requirements for bacteroid proliferation but may simply be due to changes in the PBM surface area to bacteroid volume ratio during development. Nonetheless, the results imply that synthesis of the PBM dicarboxylate transporter does not keep pace with bacteroid synthesis during the later stages of symbiotic development.

Although we have obtained no evidence yet for carrier-mediated uptake of amino acids across the PBM, uptake rates were nonetheless rapid enough to support long-term bacteroid protein synthesis (Fig. 7), and provision of amino acids by the plant, for this purpose, may well occur *in vivo*. Passive diffusion across the PBM, coupled to a high-affinity transport system on the bacteroid membrane for
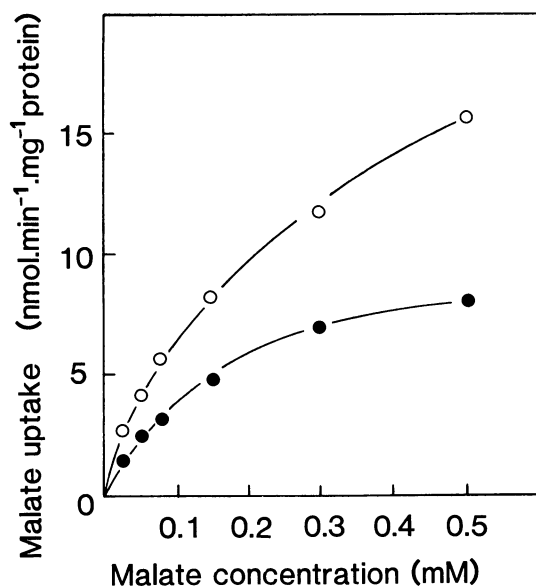

Fig. 10. Concentration dependence of the rate of [^{14}C]malate uptake by peribacteroid units (PBUs). O, small PBUs from 4-wk-old nodules; ●, large PBUs from 8-wk-old nodules. Uptake was measured for 30 sec.

Table 3. Malate uptake by peribacteroid units from young (4-wk-old) and mature (8-wk-old) soybean nodules[a]

Kinetic parameters	Young nodules	Mature nodules
K_m (μM)	139 ± 38	160 ± 42
V_{max} (nmol · min^{-1} · mg^{-1} protein)	18 ± 7	10 ± 2

[a] [^{14}C]Malate uptake was measured as described in Figure 10; data shown are means $\pm$ SE ($n = 3$–6).

at least some amino acids (Udvardi et al. 1988b), may meet the requirements of the bacteroid, and amino acid transport warrants a more thorough investigation. It is noteworthy that we detected little secretion of ^{35}S-labeled proteins into the PBS in our experiments, in contrast to previous results with PBUs of pea (Katinakis et al. 1988) in which large numbers of labeled proteins were detected in the PBS fraction. The results suggest that there are major differences between soybean and peas in this respect. It should also be noted that our experiments were conducted under aerobic conditions, and it could be that PBS or PBM proteins are only synthesized under microaerobic conditions.

The two fructose transport systems detected in isolated bacteroids (Figs. 3 and 4) have not been reported previously and were unexpected. The two systems together are able to catalyze a relatively rapid accumulation of fructose over a wide range of external concentrations. *Bradyrhizobium* species have been shown previously to transport monosaccharides when cultured on such sugars (Stowers and Elkan 1983; San Francisco and Jacobson 1986), but cannot utilize sucrose (Elkan and Kwik 1968). In free-living *B. japonicum*, a dual-affinity system for glucose uptake, similar to that shown here for fructose, has been described (San Francisco and Jacobson 1986), but glucose uptake is repressed by growth on C$_4$-dicarboxylates (Stowers and Elkan 1983; San Francisco and Jacobson 1986) and is not observed in soybean bacteroids (Reibach and Streeter 1984). We also observed very little uptake of glucose in bacteroids gently released from PBUs (Figs. 1 and 2), and lack of a glucose transporter is consistent with the permeability of the PBM to dicarboxylates but not monosaccharides. This situation may not be true in other symbioses, however, since Herrada et al. (1989) have recently reported that French bean bacteroid and peribacteroid membranes are permeable to glucose. However, regulation of fructose uptake clearly differs from that for glucose in *B. japonicum* and warrants further investigation.

Since the PBM is poorly permeable to fructose, the significance of the bacteroid transport systems *in planta* is obscure. Perhaps they become important for bacteroid survival during nodule senescence when the PBM disintegrates and sugars become more available. Alternatively, fructose transport may be important early in the symbiosis, prior to the onset of nitrogen fixation, when the permeability of the PBM may be different. Whatever the significance, it is apparent that the bacteroid maintains certain transport functions (see also Udvardi et al. 1988b) and is a potentially powerful scavenger of carbon substrates. In the absence of the PBM, the bacteroid could compete very efficiently for a wide range of metabolic intermediates in the host cytoplasm, and one of the functions of the PBM may be to restrict the menu of metabolites available to the endophyte.

LITERATURE CITED

Appleby, C. A. 1984. Leghemoglobin and *Rhizobium* respiration. Annu. Rev. Plant Physiol. 35:443-478.

Copeland, L., Vella, J., and Hong, Z. 1989. Enzymes of carbohydrate metabolism in soybean nodules. Phytochemistry 28:57-61.

Cybulski, R. L., and Fisher, R. R. 1977. Mitochondrial neutral amino acid transport: Evidence for a carrier mediated mechanism. Biochemistry 16:5116-5120.

Day, D. A., and Wiskich, J. T. 1984. Transport properties of isolated plant mitochondria. Physiol. Veg. 22:241-261.

Day, D. A., Price, G. D., and Gresshoff, P. M. 1986. Isolation and oxidative properties of bacteroids and mitochondria from soybean nodules. Protoplasma 134:121-129.

Day, D. A., Carroll, B. J., Delves, A. C., and Gresshoff, P. M. 1989a. Relationship between autoregulation and nitrate inhibition of nodulation in soybeans. Physiol. Plant. 75:37-42.

Day, D. A., Price, G. D., and Udvardi, M. K. 1989b. The membrane interface of the *Bradyrhizobium japonicum-Glycine max* symbiosis: Peribacteroid units from soybean nodules. Aust. J. Plant Physiol. 16:69-84.

Dilworth, M., and Glenn, A. 1984. How does a legume nodule work? Trends Biochem. Sci. (Pers. Ed.) 9:519-523.

Elkan, G. H., and Kwik, I. 1968. Nitrogen, energy and vitamine nutrition of *Rhizobium japonicum*. J. Appl. Bacteriol. 31:399-404.

Herrada, G., Puppo, A., and Rigaud, J. 1989. Uptake of metabolites by bacteroid-containing vesicles and by free bacteroids from French bean nodules. J. Gen. Microbiol. 135:3165-3171.

Katinakis, P., Klein-Langhorst, R. M., Louwerse, J., van Kammen, A., and van der Bos, R. C. 1988. Bacteroid encoded proteins are secreted into the peribacteroid space by *Rhizobium leguminosarum*. Plant Mol. Biol. 11:183-190.

Kohl, D. H., Schubert, K. R., Carter, M. B., Hagedorn, C. H., and Shearer, G. 1988. Proline metabolism in N₂-fixing root nodules: Energy transfer and regulation of purine synthesis. Proc. Natl. Acad. Sci. USA 85:2036-2040.

Kouchi, H., and Nakaji, K. 1988. Utilization and metabolism of photoassimilated ¹³C in soybean roots and nodules. Soil Sci. Plant Nutr. (Tokyo) 31:323-334.

Kouchi, H., Fukai, K., Katagiri, H., Minamisawa, K., and Tajima, S. 1988. Isolation and enzymological characterization of infected cell protoplasts from root nodules of *Glycine max*. Physiol. Plant. 73:327-334.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265-275.

O'Gara, F., Birkenhead, K., Wang, Y.-P., Condon, C., and Manian, S. S. 1988. Dicarboxylic acid utilization and nitrogen fixation efficiency in *Rhizobium*-legume symbiosis. Pages 149-157 in: Physiological Limitations and the Genetic Improvement of Symbiotic Nitrogen Fixation. F. O'Gara, ed. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Palmieri, F., and Klingenberg, M. 1979. Direct methods for measuring metabolite transport and distribution in mitochondria. Methods Enzymol. 56:279-301.

Price, G. D., Day, D. A., and Gresshoff, P. M. 1987. Rapid isolation of intact peribacteroid envelopes from soybean nodules and demonstration of selective permeability to metabolites. J. Plant Physiol. 130:157-164.

Reibach, P. H., and Streeter, J. G. 1983. Metabolism of ¹⁴C-labelled photosynthate and distribution of enzymes of glucose metabolism in soybean nodules. Plant Physiol. 72:634-640.

Reibach, P. H., and Streeter, J. G. 1984. Evaluation of active versus passive uptake of metabolites by *Rhizobium japonicum* bacteroids. J. Bacteriol. 159:47-52.

San Francisco, M. J. D., and Jacobson, G. R. 1986. Glucose uptake and phosphorylating activities in two species of slow-growing *Rhizobium*. FEMS Microbiol. Lett. 35:71-74.

Stowers, M. D., and Elkan, G. H. 1983. The transport and metabolism of glucose in cowpea rhizobia. Can. J. Microbiol. 29:398-406.

Streeter, J. G., and Salminen, S. O. 1988. Carbon metabolism and the exchange of metabolites between symbionts in legume nodules. Pages 11-20 in: Physiological Limitations and the Genetic Improvement of Symbiotic Nitrogen Fixation. F. O'Gara, ed. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Udvardi, M. K. 1989. Membrane transport processes and regulation of symbiotic nitrogen fixation. Ph.D. thesis, Australian National University, Canberra.

Udvardi, M. K., Price, G. D., Gresshoff, P. M., and Day, D. A. 1988a. A dicarboxylate transporter on the peribacteroid membrane of soybean nodules. FEBS Lett. 231:36-40.

Udvardi, M. K., Salom, C. L., and Day, D. A. 1988b. Transport of L-glutamate across the bacteroid membrane but not the peribacteroid membrane from soybean root nodules. Mol. Plant-Microbe Interact. 1:250-254.

Yu, C., Claybrook, D. L., and Huang, A. H. C. 1983. Transport of glycine, serine and proline into spinach leaf mitochondria. Arch. Biochem. Biophys. 227:180-187.