Protoplasma (1986) 134: 121–129

PROTOPLASMA

Isolation and Oxidative Properties of Mitochondria and Bacteroids from Soybean Root Nodules

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Received March 13, 1986
Accepted May 15, 1986

Summary

A method for the separation and purification of bacteroids and mitochondria from nodules of soybean roots is described. Cross contamination between these two oxidative fractions was easily assessible by using NADH oxidase and β-hydroxybutyrate dehydrogenase respectively as specific mitochondrial and bacteroid markers. Bacteroid respiration was characterized by substantial endogenous respiration which could be reduced by keeping plants in the dark prior to isolation, and stimulated by uncoupler or organic acids. Nodule mitochondria readily oxidized external NADH and a range of tricarboxylic acid cycle intermediates, with good respiratory control. A major difference between nodule and root mitochondria was the former's high sensitivity to the inhibitors rotenone and cyanide. This indicates a reduced capacity for non-phosphorylating electron transport in nodule mitochondria, which may be related to the large energy demand during ammonia assimilation in nodule cells.

Keywords: Bacteroids; Mitochondria; Respiration; Root nodules; Soybean.

1. Introduction

Nitrogen fixation in the nodules of legume roots requires a complex metabolic interaction between the plant host cytoplasm and the *Rhizobium* bacteroids contained within that cytoplasm. The bacteroids receive carbon substrate from the plant and in return export ammonia which is assimilated in the cytoplasm of the infected cells. The initial reactions of the NH_3 assimilation pathway are catalysed by the glutamine synthetase-glutamate synthase complex and, in soybeans, the resulting amino acids are further metabolised to ureides which are exported to other parts of the plant (BOLAND *et al.* 1982). These reactions are very energy demanding and the ATP required is thought to be provided by mitochondria of infected and interstitial cells. It is also possible that the mitochondria play a role in the production of α-ketoglutarate as amine acceptor in the NH_3 assimilation reaction sequence.

Carbon supply to the bacteroids is most probably in the form of organic acids, with succinate and malate as the prime candidates (APPLEBY 1984, DILWORTH and GLENN 1984). The mitochondria of nodule cells are also likely to participate in the production of these organic acids.

To date, there have been very few attemps to isolate mitochondria from nitrogen fixing nodules and very little is known about their metabolism. MUECKE and WISKICH (1969) demonstrated that functional mitochondria could be isolated from soybean nodules and more recently, RAWSTHORNE and LARUE (1985) isolated mitochondria from cowpea nodules and examined their oxygen use efficiency. In this paper we describe a method for the purification of mitochondria from soybean nodules and compare their oxidative properties to those of bacteroids and root mitochondria.

2. Materials and Methods

2.1. Plants and Chemicals

Soybeans (*Glycine max* L. Merr) cv Bragg, inoculated with *Bradyrhizobium japonicum* strain CB 1809 (= USDA 136), were grown in large (20 cm diameter) pots of vermiculite in a naturally illuminated

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glasshouse. Plants were watered daily with tap water and three-times a week with a nitrogen-free nutrient solution (HERRIDGE 1982). Roots were harvested seven days, and nodules forty days, after planting. Chemicals used in the nutrient solution were purchased from Ajax Chemicals (Aust.) abd other reagents from Sigma Chemical Co. (Missouri, USA) and Calbiochem (Sydney, Aust.).

2.2. Bacteroid and Mitochondria Purification

The procedure outlined in Fig. 1 was followed. Thirty to 50 g of nodules were harvested, washed in distilled water and stored on ice until utilized. Storage for up to 24 hours did not affect the activity of isolated mitochondria or bacteroids. Nodules were homogenized with a Phillips kitchen grinder (Model HR 1194) in 300 ml of ice-cold grinding medium which contained 0.4 M sorbitol, 50 mM TES buffer, 2 mM EDTA, 10 mM KH_2PO_4, 30 mM ascorbate, 2% (w/v) PVP-40 and 1% (w/v) BSA. The final pH was adjusted to 7.6 with KOH. The homogenate was filtered through 4 layers of miracloth and centrifuged at 4,000 g for 5 minutes in a Sorvall RC-5 B (SS 34 rotor; Dupont Instruments) centrifuge.

The pellet, containing bacteroids and cell debris, was resuspended in wash medium (0.4 M sorbitol, 10 mM TES buffer pH 7.2 and 0.1% BSA) and layered over 30 ml of wash medium containing 70% Percoll (v/v), in a Sorvall SS-34 tube, and centrifuged at 40,000 g for 30 minutes. The purified bacteroids were located in a broad band near the bottom of the tube (Fig. 1). This band was removed by suction, diluted at least 5-fold in wash medium and the bacteroids pelleted by centrifuging at 10,000 g for 10 minutes. The bacteroids were resuspended in wash medium at a concentration of approximately 20 mg protein ml^{-1} and kept on ice until assayed.

The 4,000 g supernatant (above) was carefully decanted and recentrifuged at 10,000 g for 15 minutes. The pellet, which contained mitochondria, plastids, bacteroids and membrane fragments, was resuspended in about 10 ml of wash medium, layered over 30 ml of wash medium containing 45% (v/v) Percoll, in a Sorvall SS-34 tube, and centrifuged at 40,000 g for 30 minutes. The mitochondria were located in a tight brown band near the top of the tube, together with plastids, peroxisomes and membrane fragments (determined by electron microscopy). This band was removed by suction, diluted at least 5-fold with wash medium and concentrated by centrifuging at 15,000 g for 10 minutes. The loose pellet was resuspended in about 5 ml of wash medium and applied to the top of 30 ml of wash medium containing 28% (v/v) Percoll and a linear gradient of 0–10% (w/v) PVP-25 (see DAY et al. 1985), in a Sorvall SS-34 tube, and centrifuged at 40,000 g for 30 minutes. The mitochondria were found in a pale brown band near the bottom of the tube (Fig. 1) and were washed and concentrated as described above for bacteroids. The mitochondria were finally resuspended in 1–2 ml of wash medium, at a protein concentration of about 10 mg · ml^{-1}. The whole procedure could be completed in 4–5 hours.

Mitochondria were purified from roots by the method of NEUBURGER et al. (1982), after homogenization as described above for nodules.

2.3. Oxygen Consumption

O_2 uptake was measured at 25 °C with a Clark-type O_2 electrode purchased from Rank Bros (Cambridge, U.K.). The standard reaction medium contained 0.4 M sorbitol, 5 mM $MgCl_2$, 10 mM phosphate buffer, 10 mM TES buffer and 0.1% (w/v) BSA. The pH was 7.2. The concentration of O_2 in air-saturated medium was taken to be 250 µM.

2.4. Enzyme Assays

The following enzymes were assayed according to published procedures. Catalase (DAY et al. 1979), lipoxygenase (SIEDOW and GIRVIN 1980), cytochrome oxidase (NEUBURGER et al. 1982) and β-hydroxybutyrate dehydrogenase (SCHULLER et al. 1986).

2.5. Other Assays

Mitochondrial integrity was measured using cytochrome c dependent O_2 uptake as described by NEUBURGER et al. (1982) and protein by the method of LOWRY et al. (1951). Carotenoids were extracted and measured by the procedure of NEUBURGER et al. (1982).

2.6. Electron Microscopy

Washed mitochondrial pellets were fixed for electron microscopy by a method similar to that detailed by NEUBURGER et al. (1982). Pellets were fixed overnight at 2 °C with 0.25% glutaraldehyde in wash buffer (minus BSA). The pellets were then washed in wash buffer 3 times (15 minutes each) prior to postfixation with 1% OsO_4 in wash buffer (2 hours at 4 °C). After washing in 0.1 M cacodylate buffer (pH 7.6) for 1 hour (4 changes) the pellets were dehydrated in a graded ethanol series and embedded in either an acrylic resin (London White resin) or an epoxy resin (Spurr's resin). Pellets embedded in Spurr's resin were washed for 2 hours in propylene oxide between the dehydration and resin infiltration stages. Use of Spurr's resin has the advantage of enhancing the contrast of membranes whilst London White resin retains the smooth spherical shape of the mitochondria and gives the matrix space high electron density (compare Figs. 3 *a* and *b*).

Isolated bacteroid pellets and soybean nodule slices were fixed by the procedure detailed by PRICE et al. (1984) and embedded in Spurr's resin. Ultra-thin sections were stained in aqueous uranyl acetate and lead citrate (Reynold's) prior to viewing in a Jeol 100 CX transmission electron microscope.

3. Results

3.1. Purification and Structure of Mitochondria and Bacteroids

The method outlined in Fig. 1 yielded mitochondria largely free from contamination by other membrane components, despite the very large number of other membranes in the infected tissue of soybean nodules (Fig. 2). The crude mitochondrial fraction from the 45% Percoll gradient (see Fig. 1) was heavily contaminated by membrane fragments, plastids, peroxisomes and some bacteroids, as judged by electron microscopy (not shown). The enzyme activities associated with this fraction (Tab. 1) confirmed this contamination. On the other hand, the fraction from the bottom of the final 28% Percoll gradient (Fig. 1) was essentially composed of mitochondria, although some membrane fragments were still observed (Fig. 3 *a*). Enzyme analysis (Tab. 1) showed that intact bacteroids had been completely removed, as judged by the absence of β-hydroxybutyrate dehydrogenase (a

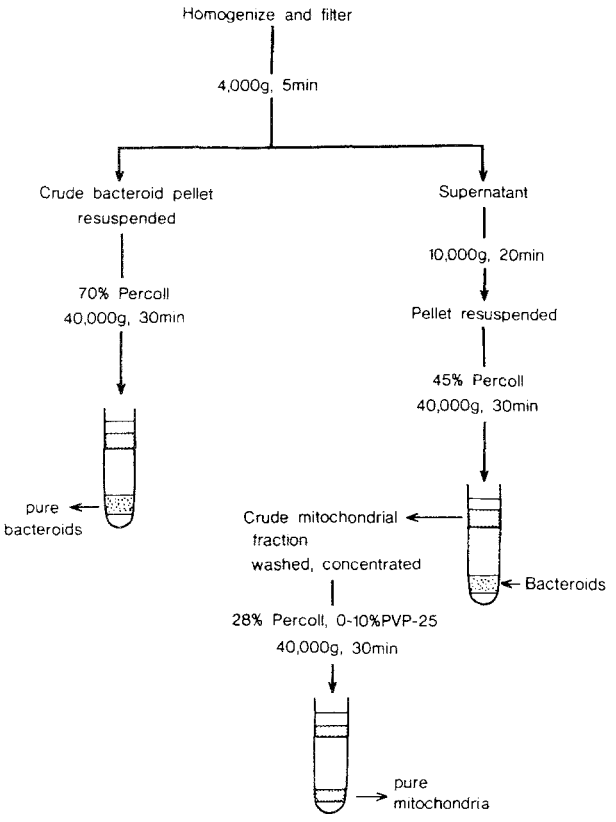


Fig. 1. Schematic outline of the procedure used to purify bacteroids and mitochondria from soybean nodules. Details are provided in Materials and Methods

Table 1. *Enzyme activity of mitochondrial and bacteroid fractions*

Enzyme	Crude mitochondria (10,000 g pellet)	Purified mitochondria	Purified bacteroids
β-hydroxybutyrate [1] dehydrogenase	11.5	n.d. [3]	65.7
Catalase [2]	2.571	0.938	1.210
Lipoxygenase [1]	327	40	n.d.
Cyt. c oxidase [1]	171	625	165

[1] nmol · min^{-1} · mg^{-1} protein.
[2] μmol · min^{-1} · mg^{-1} protein.
[3] n.d., not detectable.

specific marker for the bacteroid cytoplasm, SCHULLER *et al.* 1986). Catalase (a peroxisomal marker) was reduced by more than 50%, but the remaining activity suggests some peroxisomal contamination or adsorption of the enzyme to mitochondrial membranes (DAY *et al.* 1979). Occasional peroxisomes were seen under the electron microscope (not shown). Lipoxygenase activity was dramatically decreased in the final mitochondrial fraction, while cytochrome oxidase activity showed that a 3.7-fold purification (on a protein basis) had been achieved by the final gradient (Tab. 1). The inclusion of PVP-25 in the 28% Percoll gradient enhanced the separation of mitochondria from the lighter membrane fractions, as noted previously with green leaf membranes (DAY *et al.* 1985). The reason for this effect of PVP has not been determined, but it seems that the technique may be applicable to a wide range of tissues.

Electron microscopy showed that the mitochondria isolated from nodules had a well-defined matrix and largely intact membranes (Fig. 3 *b*). The use of cyt *c*, which cannot penetrate the intact outer mitochondrial membrane, in an O_2 electrode assay (NEUBURGER *et al.* 1982) confirmed the integrity of the mitochondria; by this method, integrity ranged from 85–90%. It is noteworthy that neither isolated (Figs. 3 *a* and *b*) or *in situ* (Fig. 2 and unpublished results) mitochondria from nodules show the matrix disorganization associated with anaerobiosis in other root tissue (ALDRICH *et al.* 1985, VARTAPETIAN *et al.* 1985), despite the very low estimated O_2 tension in nodules. The free O_2 concentration within infected cells of nodules has been estimated to be about 10 nM, increasing to about 20–26 nM at the cell periphery (SHEEHY *et al.* 1985). The mitochondria in mature infected cells are localized at the periphery (Fig. 2, BERGERSEN and GOODCHILD 1973, NEWCOMB *et al.* 1985), presumably to take advantage of the higher O_2 concentration (RAWSTHORNE *et al.* 1985). Apparently O_2 concentrations in this range are sufficient to maintain functional mitochondrial integrity. It is also noteworthy that the mitochondria in infected cells are often associated with plastids (Fig. 2, BERGERSEN and GOODCHILD 1973).

Examination of the bacteroid fraction by electron microscopy (Fig. 3 *c*) showed virtually no contamination by organelles or membrane fragments and that the cells were intact. However, not all of the bacteroids pelleted at 4,000 g in 5 minutes, suggesting some variation in density within the total bacteroid population, and this necessitated the use of two Percoll gradients to purify the mitochondria (Fig. 1). The purified bacteroids displayed high rates of β-hydroxybutyrate dehydrogenase and catalase but lipoxygenase was undetectable (Tab. 1). There was no indication of peroxisomal contamination of bacteroids (Fig. 3 *c*) suggesting that catalase is a true bacteroid enzyme which might be part of an O_2 protection system designed to prevent damage to nitrogenase. This idea is supported

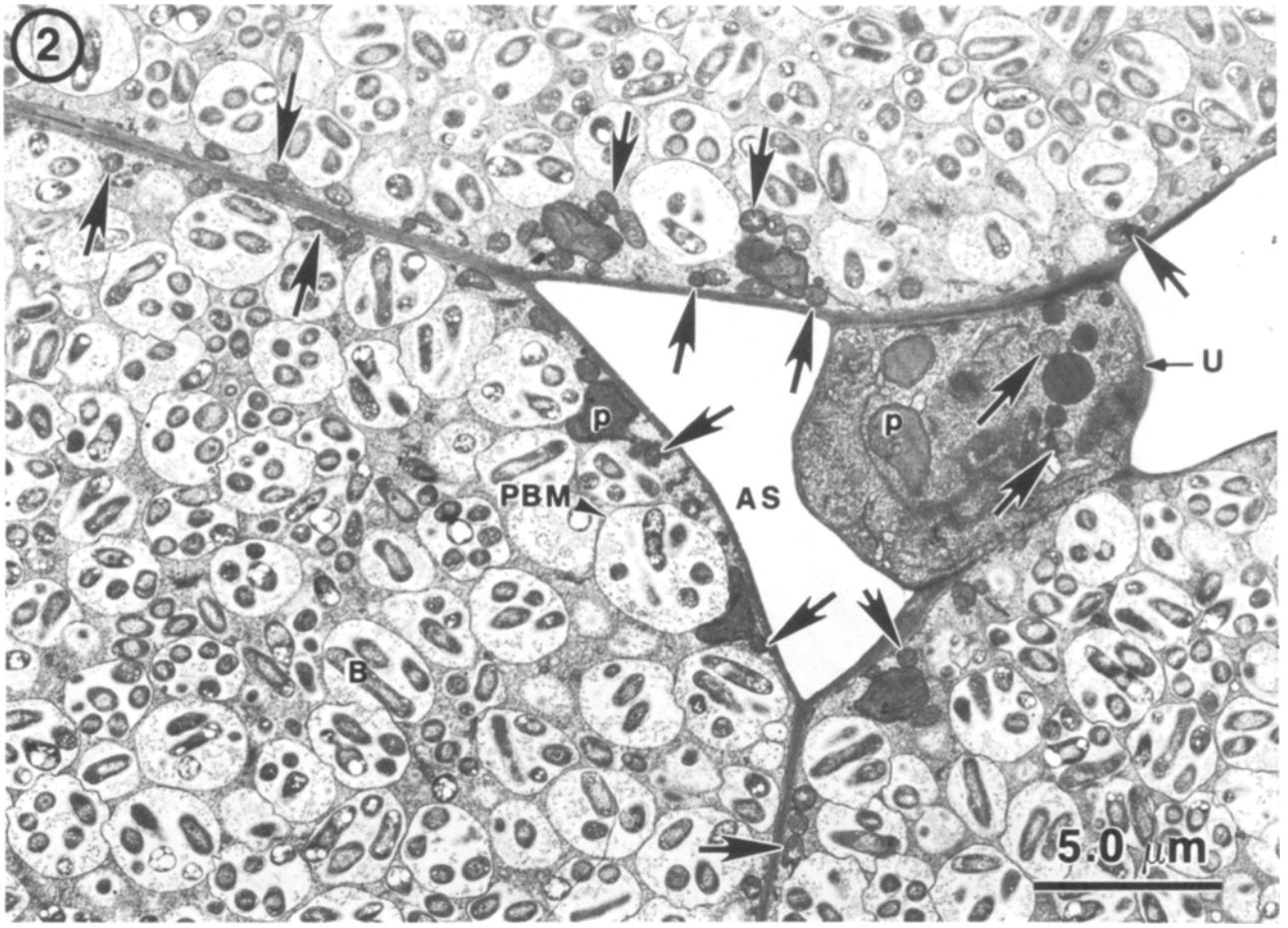


Fig. 2. Electron micrograph of the infected zone of a soybean nodule. Shown are portions of three infected cells and a glancing view of an uninfected cell (*U*). Note that the mitochondria (examples are arrowed) are located at the cell periphery, especially near intercellular airspaces (*AS*) and that the density of mitochondria is low compared to that of the bacteroids (*B*). *PBM* peribacteroid membrane, *P* plastid

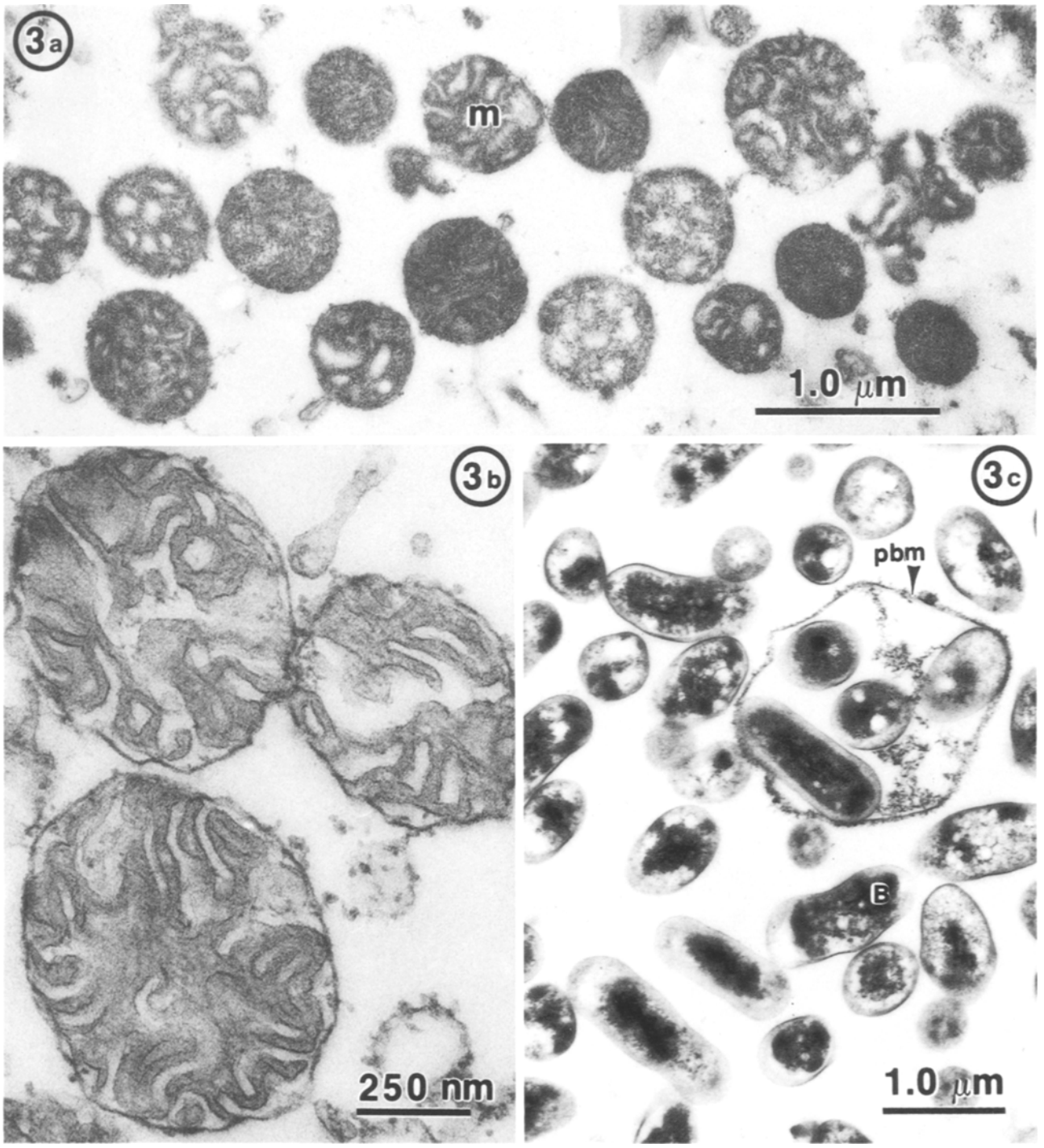
by the observation that, although free-living *Rhizobium* contain some catalase, this activity is significantly greater in bacteroids from effective nodules, but not those from ineffective (non-nitrogen fixing) nodules (THYGESEN, DAY and GRESSHOFF, unpublished results).

3.2. Bacteroid Respiration

Typical O_2 electrode traces obtained with bacteroids are shown in Fig. 4. Bacteroid respiration was characterized by a substantial endogenous rate of O_2 uptake which could be stimulated by addition of organic acids such as malate (Fig. 4 *A*). The endogenous rate could be decreased by placing soybean plants in the dark for 2–3 days prior to bacteroid isolation (Figs. 4 *B* and *C*, CARROLL 1985), presumably because endogenous substrates became depleted when photosynthate supply to the nodule ceased. O_2 uptake was restored by addition of malate (Fig. 4 *B*). Addition of succinate stimulated O_2 uptake to approximately the same extent, while pyruvate and arabinose stimulated about 50% less; sucrose, glucose and fructose failed to stimulate bacteroid respiration (results not shown). These results are similar to those reported by numerous other authors (see APPLEBY 1984 for a review).

Addition of uncoupler (CCCP) stimulated O_2 uptake further when added after malate (Fig. 4 *B*) but a similar stimulation of endogenous respiration was also observed, even in substrate depleted bacteroids (Fig. 4 *C*). Uncoupler also stimulated the faster endogenous respiration of bacteroids from plants in the light (not shown).

Intact bacteroids did not respond to added NADH (Fig. 4 *B*), indicating that mitochondrial contamination was negligible; plant mitochondria generally oxidize exogenous NADH rapidly, and those from nodules are no exception (Tab. 2). Sonicated bacteroids, on the other hand, did oxidize NADH (Fig. 4 *D*), presumably because sonication leads to inversion of at least some cytoplasmic membranes, allowing access to the internal



Figs. 3 *a–c*. Mitochondria and bacteroids purified by Percoll gradient centrifugation. *a* Purified mitochondria (*m*) embedded in L. R. White resin. *b* Purified mitochondria embedded in Spurr's resin. Note that mitochondria membranes have more contrast than those in Fig. 3 *a*, although the electron density of the matrix space is lower. *c* Purified bacteroids (*B*). *pbm* perbacteroid membrane

NADH dehydrogenase. In some preparations, this O_2 uptake with NADH as substrate showed some semblance of respiratory control, being stimulated by ADP (Fig. 4 *D*). This was not observed with all sonicated preparations and clearly conditions have yet to be optimized, but this system may facilitate the study of oxidative phosphorylation in bacteroids. Sonicated bacteroids were also capable of oxidizing succinate and, provided a suitable reductant was present (*e.g.*, ascorbate), could also oxidize added cytochrome c,

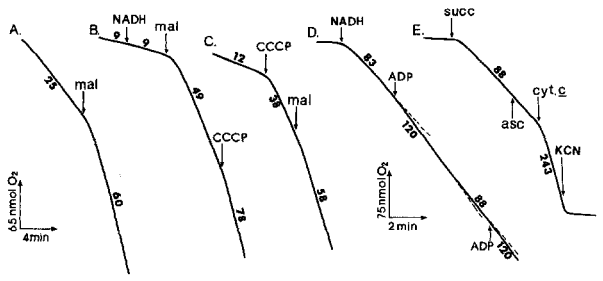


Fig. 4. Oxygen consumption by soybean bacteroids. O_2 consumption was measured polarographically as described in Materials and Methods. *A* Bacteroids isolated from control soybeans, 0.65 mg protein in 2 ml of standard reaction medium; *B* and *C* bacteroids from plants kept in the dark for two days prior to harvest, 0.75 mg protein in 2 ml; *D* and *E* bacteroids were sonicated (3 minutes, 100 W), centrifuged (10,000 g, 5 minutes) and the supernatant assayed, 0.4 mg protein in 3 ml of reaction medium. Note that the O_2 and time scales are different, in *D* and *E*, from those in *A*, *B*, and *C*. Additions as indicated were, 10 mM malate (*mal*), 1 mM NADH, 1 µm CCCP, 85 µM ADP, 10 mM succinate (*succ*), 25 µM cytochrome c (*cyt. c*), 5 mM ascorbate (*asc*) and 0.5 mM KCN. Numbers on traces refer to nmol $O_2 \cdot min^{-1} \cdot mg^{-1}$ protein

Table 2. *Alternative oxidase activity in root and nodule mitochondria.* The concentration of NADH was 1 mM and KCN 0.5 mM. Other details were as in Fig. 4. Experiments 1 and 2 were with different preparations of mitochondria. State 3 respiration refers to O_2 uptake in the presence of ADP. KCN-resistant O_2 uptake was inhibited by salicyl hydroxamic acid

Substrate	Oxygen consumption			
	Nodule mitochondria		Root mitochondria	
	State 3	+ KCN	State 3	+ KCN
	nmol $O_2 \cdot min^{-1} \cdot mg^{-1}$ protein			
Experiment 1				
Malate	126	14	138	68
Succinate	229	24	260	110
NADH	252	0	—	—
Experiment 2				
Malate	100	18	163	67
Succinate	226	33	381	190
NADH	303	0	265	96

indicating a very active cytochrome c oxidase. Intact bacteroids did not respond to added cytochrome c (not shown). It has been suggested that bacteroid cytochrome c oxidase is extrinsically located on the cytoplasmic membrane (APPLEBY 1984). This in turn implies that the outer (periplasmic) membrane of the bacteroid is impermeable to cytochrome c.

3.3. Mitochondrial Respiration

Nodule mitochondria readily oxidized all substrates tested, with good respiratory control. The oxidation of two NAD-linked substrates is illustrated in Fig. 5. α-ketoglutarate was rapidly oxidized, even in the presence of malonate which inhibits oxidation of the succinate produced (Fig. 5 *A*). Typical respiratory control ratios were 2.8–4.5 and ADP/O ratios 3.1–3.3.

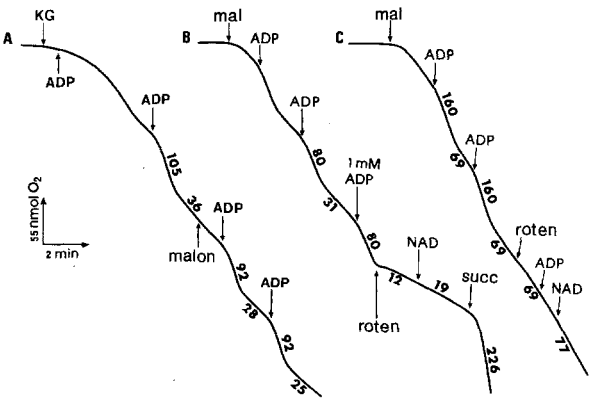


Fig. 5. NAD linked substrate oxidation by soybean nodule (*A* and *B*) and root (*C*) mitochondria. Oxygen consumption was measured as described in Materials and Methods, in 2 ml of standard reaction medium. In *A*, 0.2 mM thiamine pyrophosphate was included in the medium and 0.3 mM ADP was added as shown. In *B* and *C*, 10 mM glutamate was included in the medium and 0.2 mM ADP added as shown, except where indicated otherwise. Other additions, as indicated, were 10 mM α-ketoglutarate (*KG*), 10 mM malate (*mal*), 10 mM succinate (*succ*), 15 µM rotenone (*roten*) 2.5 mM malonate (*malon*) and 0.3 mM NAD^+. The amount of mitochondrial protein was 0.9 mg in *A* and *B*, and 0.4 mg in *C*. Numbers on traces are nmol $O_2 \cdot min^{-1} \cdot mg^{-1}$ protein

Malate was also rapidly oxidized with good respiratory control (Fig. 5 *B*), typical respiratory control and ADP/O ratios being 2.5–4.0 and 2.2–2.5 respectively. Neither malate nor α-ketoglutarate oxidation responded to addition of NAD^+, suggesting that nodule mitochondria possess a full complement of this variable co-factor; this is a property typical of actively respiring and growing plant tissues (DOUCE *et al.* 1985). Other substrates tested included exogenous NADH and succinate (Tab. 2). NADH in particular was rapidly oxidized.

Mitochondria from nodules were compared to those from roots, with particular regard to their sensitivity to respiratory inhibitors (Fig. 5 and Tab. 2). Malate oxidation by nodule mitochondria was much more sensitive to inhibition by rotenone than that of root mitochondria, and this difference could not be attri-

buted to different levels of matrix NAD^+ (Figs. 5 *B* and *C*, exogenous NAD^+ did not stimulate). Rotenone-resistant O_2 uptake was 24% of the control rate in nodule mitochondria and 48% in roots. Higher inhibitor concentrations produced the same effects. This difference was observed consistently and indicates that the capacity of the non-phosphorylating bypass of complex I of the respiratory chain (PALMER and WARD 1985) is less in nodules than in roots.

The same is also true of the capacity of the KCN-insensitive alternative oxidase, which branches from the main chain at the level of ubiquinone. Rates of KCN-resistant O_2 uptake were dramatically slower, with all substrates tested, in nodule mitochondria than in their root counterparts (Tab. 2). This result was obtained with all preparations and a range of KCN concentrations (two typical experiments are shown in Tab. 2) and represents the most striking difference between nodule and root mitochondria observed to date. Mitochondria from soybean cotyledons and leaves are also markedly KCN-resistant (DAY, unpublished results). Thus the nodule differs from all other tissue in this respect, at least in soybeans.

4. Discussion

4.1. Organelle Purity

The isolation procedure outlined here provided intact and functional nodule mitochondria and bacteroids, free from contamination by each other. Although there was some contamination of the mitochondrial fraction by peroxisomes (Tab. 1) this did not interfere with the assay of mitochondrial function; any cross-contamination between bacteroids and mitochondria, on the other hand, would make independent assay of their respective oxidases very difficult. The procedure outlined in Fig. 1 should therefore be of use for further studies of nodule bioenergetics. The results also provide an estimate of cross-contamination between mitochondria and bacteroids, using NADH oxidase and β-hydroxybutyrate dehydrogenase respectively as markers.

4.2. Bacteroid Respiration

The stimulation of bacteroid endogenous O_2 uptake by both uncoupler and exogenous substrate is an interesting observation which may give a clue to the nature of the endogenous substrate, which to date is unknown (APPLEBY 1984). The stimulation by organic acids shows that the rate of endogenous substrate oxidation was not limited by the available activity of the respiratory chain, since oxidation of malate and succinate yield NADH and FADH respectively, which in turn are oxidized by the respiratory chain. The main effect of uncoupler was not, therefore, on the electron transport chain but rather on some other reaction not involved in malate and succinate oxidation. This is most likely to be an adenylate-sensitive reaction, with uncoupler acting via a type of Pasteur-effect, as seen in some plant tissues (AZCON-BIETO *et al.* 1983). Reactions which are sensitive to adenylate regulation often involve ATP formation. This suggests that the oxidation of endogenous substrate in soybean bacteroids involved such a step, perhaps implicating sugars as the endogenous substrate with the pyruvate kinase reaction being inhibited by ATP and hence stimulated (indirectly) by uncoupler.

It is also noteworthy that the rate of endogenous O_2 uptake is slower than that with added organic acids, and that even the slow endogenous rate of bacteroids from starved plants is stimulated by uncoupler (Fig. 4). This implies that bacteroid respiration *in situ* is regulated by adenylate control, so that some endogenous reserves are maintained. This regulation does not, however, directly involve the respiratory chain, allowing the bacteroid to respond rapidly to substrate import from the host cells.

Finally, if indeed endogenous substrate breakdown does involve an energy-conserving step other than those of the respiratory chain, then that might explain why endogenous substrate has been found to be more efficient than added organic acids in supporting nitrogen fixation by isolated bacteroids (BERGERSEN and TURNER 1980). However, it should also be noted that our bacteroid preparations may have been heterogenous with respect to differentiation, function and, consequently, metabolic status, with one population responding to uncoupler and another to added substrates.

4.3. Mitochondrial Respiration

Ammonia fixation in the soybean nodule cytoplasm requires, in addition to ATP, NADH and α-ketoglutarate. Yet both of these compounds are rapidly oxidized by isolated mitochondria. The α-ketoglutarate could be provided by transamination reactions in the cytosol but net synthesis must take place in the tricarboxylic acid cycle; in either case, oxidation of α-ketoglutarate must be restricted *in vivo*. The same is also true of NADH oxidation. Clearly, further studies are needed to identify possible control mechanisms.

The plant mitochondrial respiratory chain is branched,

with bypasses of all the three energy transducing (proton-translocating) sites associated with the conventional chain. These bypasses act as non-phosphorylating alternative routes of electron flow from NADH to O_2 (MOORE and RICH 1985, PALMER and WARD 1985), and allow plant respiration to proceed in the face of high cellular energy charge levels (LAMBERS 1985). The non-phosphorylating branches can be distinguished by their insensitivity to the inhibitors rotenone and KCN. The high sensitivity of nodule mitochondria to these inhibitors shows that the non-phosphorylating pathways are virtually absent from the nodule (and may be completely absent from the infected tissue since our preparations used the whole nodule, with some organelles no doubt originating from the uninfected cells). Root mitochondria, on the other hand, were highly cyanide and rotenone insensitive (Fig. 5, Tab. 2).

The low capacity of non-energy conserving respiratory pathways of nodule mitochondria is yet another metabolic change accompanying differentiation of root cells during nodule formation, and fits well with the postulated high energy demand of nodule cells during ammonia fixation. These results are also consistent with the analysis of root and nodule respiration performed by DE VISSER and LAMBERS (1983), who showed that nodule respiration was more efficient. Since the O_2 tension within infected cells of nodules is much less than that in root cells (BERGERSEN 1982), it is possible that synthesis of the alternative oxidase is subject to regulation by O_2 concentration.

Acknowledgements

This research was partly supported by Agrigenetics Research Associates Ltd. We thank Ms. SUE YOUNG for her expert technical assistance and Mr. WALTER GOYDYCH (Bruce T.A.F.E., Canberra) for use of electron microscope facilities and the printing of micrographs.

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