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Effect of oxygen pressure on synthesis and export of nitrogenous solutes by nodules of cowpea

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Abstract. Nodules of cowpea plants (*Vigna unguiculata* (L.) Walp. cv. Vita 3: *Bradyrhizobium* CB756) cultured for periods of 23 d with their root systems maintained in atmospheres containing a range of partial pressures of O_2 (pO_2; 1–80%, v/v, in N_2) formed and exported ureides (allantoin and allantoic acid) as the major products of fixation at all pO_2 tested. In sub-ambient pO_2 (1 and 2.5%) nodules contained specific activities of uricase (urate: O_2 oxidoreductase; EC 1.7.3.3) and allantoinase (allantoin hydrolyase; EC 3.5.2.5) as much as sevenfold higher than in those from air. On a cell basis, uninfected cells in nodules from 1% O_2 contained around five times the level of uricase. Except for NAD: glutamate synthase (EC 1.4.1.14), which was reduced in sub-ambient O_2, the activities of other enzymes of ureide synthesis were relatively unaffected by pO_2. Short-term effects of pO_2 on assimilation of fixed nitrogen were measured in nodules of air-grown plants exposed to sub-ambient pO_2 (1, 2.5 or 5%, v/v in N_2) and $^{15}N_2$. Despite a fall in total $^{15}N_2$ fixation, ureide synthesis and export was maintained at a high level except in 1% O_2 where formation was halved. The data indicate that in addition to the structural and diffusional adaptations of cowpea nodules which allow the balance between O_2 supply and demand to be maintained over a wide range of pO_2, nodules also show evidence of biochemical adaptations which maintain and enhance normal pathways for the assimilation of fixed nitrogen.

Key words: *Bradyrhizobium* – Oxygen pressure and root nodule function – Root nodules – Symbiosis (legume – *Rhizobium*) – Ureide synthesis – Uricase – *Vigna*

Introduction

Recent research has demonstrated the dependence of legume-nodule functioning on mechanisms which effectively regulate endogenous O_2 supply so as to match closely demand for O_2 within the N_2-fixing tissue (Witty et al. 1986; Dakora and Atkins 1989). As a consequence the free O_2 concentration close to the bacteroids is maintained at around 10 nM (Appleby 1984) and inactivation of nitrogenase by O_2 prevented. The major O_2-consuming reaction in nodules is the terminal oxidase of bacteroid respiration which functions to provide ATP for N_2 fixation. The particular oxidase involved is uniquely adapted, through having a K_m (O_2) close to 5 nM (Appleby 1984), to function effectively in the microaerobic conditions within the infected tissue of the nodule. However, a number of other important reactions of nodule metabolism also require a ready supply of O_2. These include the terminal oxidase of mitochondrial electron transport (both in infected and uninfected cells) and, in those symbioses forming ureides as products of N_2 fixation, urate oxidase.

Urate oxidase, isolated from cowpea nodules, has a very low affinity for O_2 (K_m (O_2) = 29 μM; Rainbird and Atkins 1981) when assayed in vitro. Most of the enzyme in nodules is effectively separated from the bacteroids, being localised in microbodies within uninfected cells (Nguyen et al. 1985; Van den Bosch and Newcomb 1986). Despite this more favourable location, in terms of competing with bacteroid respiration for O_2, it seems unlikely that gaseous diffusion to uninfected cells would lead to free O_2 levels greatly different from those in adjacent infected cells. Similarly, although mitochondria are located principally at the periphery of infected cells and usually close to extracellular spaces (Bergersen and Goodchild 1973), they too are unlikely to have an oxygen supply which approaches their K_m (O_2) (100 nM; Rawsthorne and LaRue 1986; Day et al. 1986). Thus, it is possible that reactions outside the bacteroid, which are dependant on ATP, as well as the oxidation of urate to form allantoin, are likely to be limited by a lack of free O_2.

Although there is some evidence that short-term decreases in external gas-phase pO_2 around the roots of nodulated soybean result in proportionally less labeled ureides being formed as products of $^{15}N_2$ fixation (Ohyama and Kumazawa 1980), the data are not conclusive as to the role of O_2, if any, in these changes. In

Abbreviations and symbol: GOGAT = glutamate synthase (glutamine oxo-glutarate amidotransferase) GS = glutamine synthetase; pO_2 = partial pressure of oxygen in the gas phase

fact there is considerable evidence for the operation of a variable diffusion barrier in nodules which, under conditions of altered external pO_2, would function to preclude substantial change in internal O_2 concentration (Dakora and Atkins 1989).

Studies of the adaptation of nodules to long-term development in rooting atmospheres containing a range of pO_2 from 1 to 80% have indicated that, as a consequence of changes in the permeability of nodules to gases, relatively constant rates of N_2 fixation were maintained from 5 to 60% O_2 (Dakora and Atkins 1990a, b). These nodules showed a range of morphological and anatomical changes which might account for their altered gas exchange (Dakora and Atkins 1990c). In the present study we have used these materials to determine whether or not adaptation to changes in pO_2 also involved the biochemistry of nodules, particularly with respect to metabolic pathways associated with assimilation of fixed N and synthesis of nitrogenous solutes translocated to the host.

Material and methods

Plant material. Cowpea (*Vigna unguiculata* (L.) Walp. cv. Vita 3) plants, inoculated with *Bradyrhizobium* strain CB756 (University of Western Australia Culture Collection), were grown in liquid culture with their nodulated root systems maintained in a range of pO_2 (1 to 80%, v/v, O_2 in N_2) as described in Dakora and Atkins (1990a).

Collection and analysis of xylem exudate. Xylem sap was collected as exudate bleeding from root stumps of decapitated plants. The first drop of exudate forming at the cut surface was discarded to avoid contamination from damaged cells. Subsequent drops were collected onto ice at 5–10-min intervals for 30 min and then stored at −20° C. Collections from each plant in a pot or, in some cases in a treatment, were pooled for analysis of solute composition. Amino acids and amides were separated and measured using an ion-exchange high-pressure liquid chromatography (HPLC) technique which incorporated Li elution buffers and post-column ninhydrin detection (Pate et al. 1985). Allantoin and allantoic acid were measured together as the phenylhydrazone of glyoxylate (Trijbels and Vogels 1966).

Exposure to $^{15}N_2$. Plants, grown for 28 d in liquid culture with their nodulated roots in air, were transfered to nutrient solution from which air has been removed by a vacuum, and maintained in a flowing atmosphere containing 1, 2.5, or 5% (v/v) O_2 (in N_2) in the the gas phase surrounding the roots for 1 h to allow equilibration. Plants with their roots maintained in air served as controls. Following the removal of 50 ml gas from the closed and sealed rooting atmosphere of each pot, a similar volume of $^{15}N_2$ (98.6 A%/X ^{15}N) was introduced into the gas space surrounding the nodulated roots to give a final ^{15}N content ranging from 16.8 to 26.0 A%X. After 4 h, plants were harvested and nodules separated and extracted as described above. The experiment was repeated with plants maintained for 120 h in sub-ambient pO_2 before the introduction of $^{15}N_2$. Ureides, amides and amino acids were separated by cation-exchange chromatography and recovered for assay of ^{15}N content by mass spectrometry (Atkins et al. 1988). The $^{15}N/^{14}N$ isotopic ratio of samples was compared with an atmospheric reference (0.3663% ^{15}N) to estimate A%/X.

Enzyme assays. Enzymes of nitrogen and ureide metabolism were assayed in extracts of nodules from plants cultured for 28 d with their roots in a range of pO_2 (1–80%, v/v, in N_2) throughout. In each case nodules were harvested under Ar and extracted using two volumes of chilled (4° C) buffer equilibrated with Ar. However, once the tissue had been homogenised there was no further attempt to exclude O_2. The extraction buffer contained 0.1 M Tricine (N-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine)-KOH (pH 7.5), 10 mM $MgCl_2$, 0.4 M sucrose, 15% (v/v) glycerol, 0.5% (w/v) bovine serum albumin and 50 mM 2-mercaptoethanol. Following filtration through 100-μm nylon mesh to remove particulate material, the homogenate was centrifuged (1000·*g*, 20 min, 4° C) and the supernatant desalted by passage through a Sephadex G-25 (Pharmacia Australasia, Sydney, Australia) column equilibrated with extraction buffer. This desalted, soluble extract served as the source of enzyme in all assays.

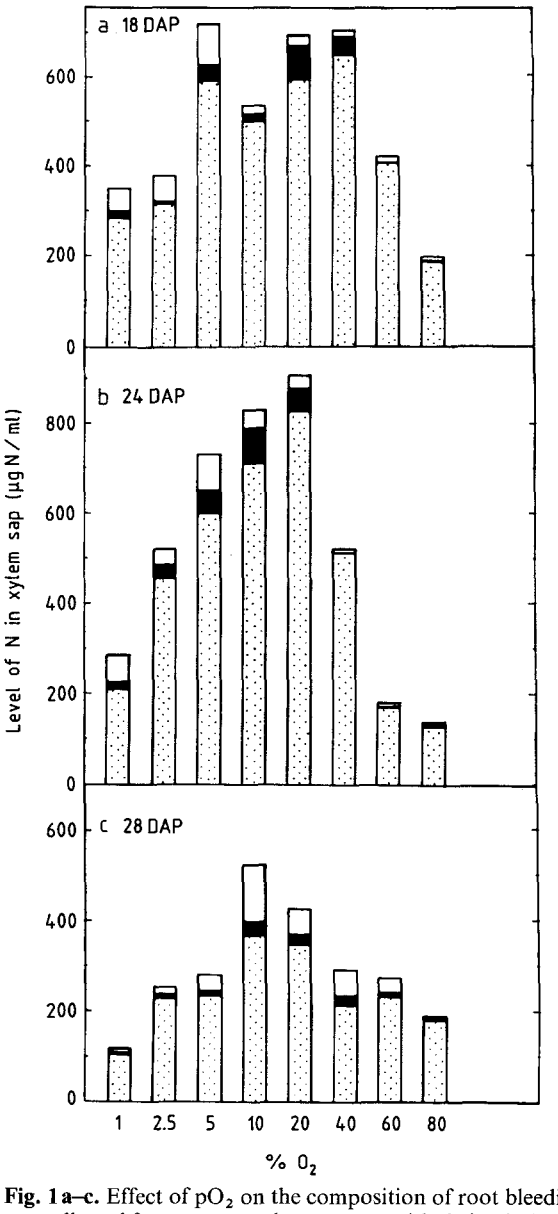

Fig. 1a–c. Effect of pO_2 on the composition of root bleeding xylem sap collected from cowpea plants grown with their whole nodulated root systems maintained in different pO_2 from 5 to 18 (**a**), 24 (**b**) or 28 (**c**) d after planting (*DAP*). *Ureide* = allantoin + allantoic acid ▨; *Amide* = Gln + Asn ■; *Amino acid* = sum of Asp, Glu, Thr, Ser, Gly, Ala, Pro, Ile, Leu, γ-aminobutyrate, Tyr, Phe, Met, Cys, Lys, His and Arg □. Sap was collected separately from three pots each containing four plants and equal volumes pooled for analysis of N solutes

Glutamate synthase (GOGAT; EC 1.4.1.14), glutamine synthetase (GS; EC 6.3.1.2), xanthine dehydrogenase (EC 1.2.1.37), urate oxidase (EC 1.7.3.3) and allantoinase (EC 3.5.2.5) were assayed as in Atkins et al. (1980), inosine 5'-monophosphate dehydrogenase (EC 1.2.1.14) as in Atkins et al. (1985), and nucleosidase (EC 3.2.2.1) as in Atkins et al. (1989). All enzymes were assayed at 30° C. The soluble protein in enzyme extracts was measured, after precipitation with 12% (w/v) trichloroacetic acid, using the method of Lowry et al. (1951) with bovine serum albumin as standard.

Results

Effect of long-term growth in different pO_2 levels on products of N_2 fixation. The nitrogenous solute composition of xylem sap from cowpea plants grown with their nodulated root systems in a range of pO_2 is shown in Fig. 1. Although the level of N in xylem varied with pO_2, showing a peak around 20%, within each treatment the content of ureides was always high (85–98%) relative to that of amides or amino acids. The amount of N in xylem also showed variation between the sampling times but there was little change in the relative composition of nitrogenous solutes. At each time ureides comprised more than 95% of xylem N at the highest pO_2 used (60 or 80%) compared with a slightly lesser proportion (around 85%) at ambient or sub-ambient pO_2. As a consequence the ratio of ureide-N to the sum of glutamine- and glutamate-N in xylem, though variable, remained similar to that of air-grown plants in those cultured at sub-ambient pO_2 but became progressively wider at supra-ambient pO_2 (Fig. 2), reaching values close to 200 mg ureide-N per 1 mg glutamate-N + glutamine-N at 80% O_2.

Effect of short-term exposure to different pO_2 on products of N_2 fixation. Transferring 25-d-old cowpea plants from having their nodulated roots in air to a range of higher or lower pO_2 for 2 or 5 d resulted in a significant decrease in the levels of N in xylem relative to those in xylem of plants maintained in air (Fig. 3). The concentration of all nitrogenous solutes was reduced. However, this was most marked for amino acids and amides, with the consequence that, at both sub- and supra-ambient

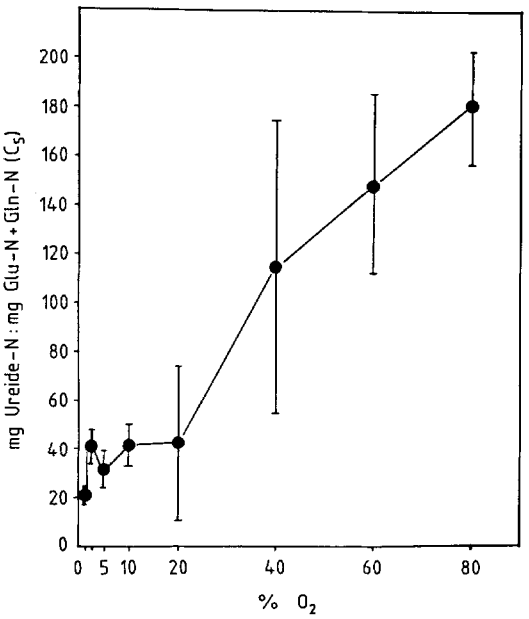

Fig. 2. Effect of pO_2 on the ratio of ureide-N to glutamate-N + glutamine-N (C_5) in xylem bleeding sap collected from cowpea plants grown with their whole nodulated root systems maintained in different pO_2 for 18, 24 or 28 d after planting. At each harvest, sap was collected separately from three pots each containing four plants and equal volumes pooled for analysis of N solutes. Values are means ± SE ($n = 3$ harvests). (*Ureide* = allantoin + allantoic acid)

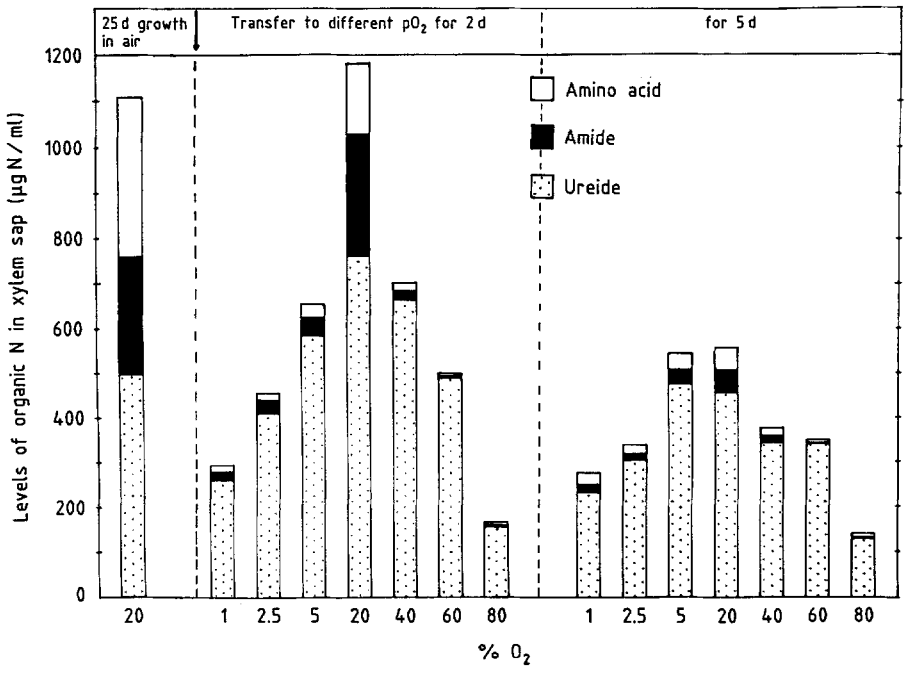

Fig. 3. Effect of pO_2 on the composition of xylem bleeding sap collected from cowpea. Plants were grown in air from 5 to 25 d after planting and transferred to different pO_2 for 2 or 5 d. Sap was collected separately from four plants in each of three pots and equal volumes bulked for analysis of N-solutes. (*Ureide* = allantoin + allantoic acid; *Amide* = Gln + Asn; *Amino acid* = the sum of the rest of the amino acids in the xylem stream, see Fig. 1)

Table 1. Effect of pO_2 on ^{15}N labeling of nitrogenous solutes from $^{15}N_2$ in cowpea nodules exposed to sub-ambient pO_2 for 5 or 120 h. Plants used had been grown in liquid culture with ambient air around the nodulated roots for 28 d. They were transferred to rooting atmospheres of 1, 2.5, 5% O_2 or air for 1 h to equilibrate, then $^{15}N_2$ was added for a further 4 h. The same labeling protocol was applied to plants maintained for 120 h at the treatment pO_2 before addition of $^{15}N_2$

Length of O_2 treatment (h)	Organic N compd.	Air → air			Air → 5% O_2			Air → 2.5% O_2		
		μgN/g nod FW	Labeling ng ^{15}N	% distribution ^{15}N	μgN/g nod FW	Labeling ng ^{15}N	% distribution ^{15}N	μgN/g nod FW	Labeling ng ^{15}N	% distribution ^{15}N
5	Ureides	22.10[a]	265.2	39.0	37.30	126.8	38.9	41.30	90.9	34.0
	Asn	31.31	9.4	1.4	25.79	5.2	1.6	24.23	4.8	1.8
	Gln	1.62	46.2	6.8	0.55	8.6	2.6	1.42	21.6	8.1
	Asp	4.15	45.7	6.7	5.40	29.2	9.0	5.74	19.5	7.3
	Glu	6.60	188.1	27.7	12.30	108.2	33.2	15.80	102.7	38.5
	Gly	0.62	5.0	0.7	0.97	3.2	1.0	0.94	3.2	1.2
	Ala	3.90	61.2	9.0	3.53	17.3	5.3	4.66	9.8	3.7
	Thr	2.77	53.2	7.8	2.88	9.5	2.9	4.44	1.3	0.5
	Ser	3.18	4.8	0.7	3.40	18.0	5.5	4.13	13.2	4.9
	Total	76.3	678.8		92.1	326.0		102.7	267.0	
120	Ureides	75.97	645.8	50.4	42.95	360.8	48.9	–	–	–[b]
	Asn	1.64	14.1	1.1	2.85	7.4	1.0	–	–	–
	Gln	6.32	170.6	13.3	1.58	26.9	3.6	–	–	–
	Asp	3.96	66.5	5.2	4.36	45.3	6.1	–	–	–
	Glu	12.18	291.1	22.7	15.00	204.0	27.7	–	–	–
	Gly	0.17	9.8	0.8	0.61	9.6	1.3	–	–	–
	Ala	0.91	39.0	3.0	4.35	56.1	7.6	–	–	–
	Thr	3.17	12.4	1.0	3.17	6.3	0.9	–	–	–
	Ser	2.25	32.6	2.5	2.63	20.8	2.8	–	–	–
	Total	106.6	1281.9		77.5	737.2				

[a] Single pots each containing four plants were used and nodules from each plant combined for solute analysis and $^{15}N/^{14}N$ determination
[b] Not determined

O_2, ureides accounted for an increased proportion of xylem-mobile N. The effects resulting from transfer to different pO_2 were still evident in xylem composition after 5 d (Fig. 3) although the content of all N solutes exported from nodules in xylem had declined at this time.

The effects of short-term changes in pO_2 on the nature of products of N_2 fixation in nodules were assessed by measurements of the distribution of ^{15}N following addition of $^{15}N_2$ to the rooting atmosphere. The nodulated roots were exposed to the labeled gas for 4 h following their transfer from air to 1, 2.5 or 5% O_2 for 1 or 120 h. Thus the root systems were exposed for 5 or 120 h to their respective sub-ambient pO_2 and to $^{15}N_2$ for the final 4 h in each case.

The predominant labeled products of fixation by nodules maintained in ambient air were the ureides and glutamate with lesser amounts of ^{15}N in the two amides (glutamine and asparagine), aspartate, alanine and threonine (Table 1). Transfer to 5 or 2.5% O_2 had little effect on the distribution of label although the total amount of ^{15}N fixed into all solutes declined. After 5 h in 1% O_2 the total ^{15}N recovered was reduced almost 90% compared with those in air and the proportion as ureide had fallen to 14% of the total fixed (Table 1). At this pO_2, ^{15}N accumulated mainly in glutamate. After 120 h exposure to 2.5 or 5% O_2 the labeling pattern remained similar to that at 5 h. As at 5 h, the amount of ^{15}N recovered among all nitrogenous solutes was reduced progressively with the fall in pO_2. However, after 120 h in 1% O_2 the amount of ^{15}N fixed was increased compared to that fixed by nodules exposed to $^{15}N_2$ during the first 5 h. After 120 h in 1% O_2 labeling of ureides also increased as a proportion of fixed N though glutamate remained the principal product (Table 1).

Effect of O_2 level on enzymes of N metabolism. The specific activity of some of the enzymes of N metabolism measured in cowpea nodules at 28 d varied markedly with the pO_2 around the roots during growth while others were little affected (Fig. 4a, b). The GS activities, though higher at the sub- and supra-ambient extremes of pO_2 used, did not fall below those in air while GOGAT levels were reduced to less than 25% those in air at sub-ambient pO_2 and increased slightly at 60 and 80% O_2. The activity of inosine 5′-monophosphate dehydrogenase showed no marked effect of pO_2 while purine nucleosidase and xanthine dehydrogenase levels declined slightly at supra-ambient pO_2 and increased slightly at sub-ambient O_2. By comparison both uricase and allantoinase showed sharply increased levels in nodules cultured with sub-ambient pO_2, especially in 1 and 2.5% (Fig. 4b).

Discussion

Transport of N in xylem or the level of ^{15}N recovered in nodules following $^{15}N_2$ fixation showed a sharp response to pO_2, both as a consequence of growth in dif-

Table 1. (continued)

Air → 1% O_2		
μgN/g nod FW	Labeling ng ^{15}N	% distribution of ^{15}N
25.78	10.3	14.1
19.04	3.8	5.2
0.20	3.1	4.3
3.36	7.7	10.6
20.39	32.6	44.7
0.43	2.0	2.7
5.13	7.2	9.9
3.43	6.2	8.5
4.32	0.0	0.0
82.1	72.9	
18.51	90.7	25.3
2.00	8.0	2.2
0.22	15.7	4.4
–	–	–
29.23	169.5	47.2
0.32	5.4	1.5
9.74	51.6	14.4
1.28	3.3	0.9
3.72	14.9	4.1
65.0	359.1	

ferent pO_2 and following short-term changes from air to sub-ambient O_2. Generally this response was what might have been expected from the effects of pO_2 on nitrogenase activity, N_2 fixation and plant growth noted previously for this symbiosis when the plants were cultured under similar experimental conditions of varied pO_2 (Dakora and Atkins 1990a, b).

Despite a decline in xylem-N content at the extremes of pO_2, the composition of nitrogenous solutes in xylem was little affected by the gas-phase pO_2. Ureides were, in all cases, predominant. Thus, even though ureide synthesis in nodules depends directly on O_2, through the activity of uricase (Rainbird and Atkins 1981), at levels as low as 1% O_2 around the roots, their rate of synthesis was apparently sufficient to account for the assimilation of most of the fixed N and export to the rest of the plant.

Adaptation of cowpea nodules to alterations in the external gas-phase pO_2, such that internal O_2 supply was apparently optimised, resulted in a relatively constant rate of N_2 fixation from 5 to 60% O_2 (Dakora and Atkins 1990a, b). This was no doubt reflected in the relatively constant composition of N solutes in xylem over this range. At pO_2 below 5% the reduced level of nitrogenase activity (Dakora and Atkins 1990a, b) might be expected to require less urate oxidation so that a reduced level of ureide synthesis was still sufficient to fill the demand for ammonia assimilation. However, at low pO_2, uricase activity in nodule extracts was increased more than sevenfold compared with those grown in air, indicating that increased catalytic capacity of the enzyme was required to maintain a high level of ureide export.

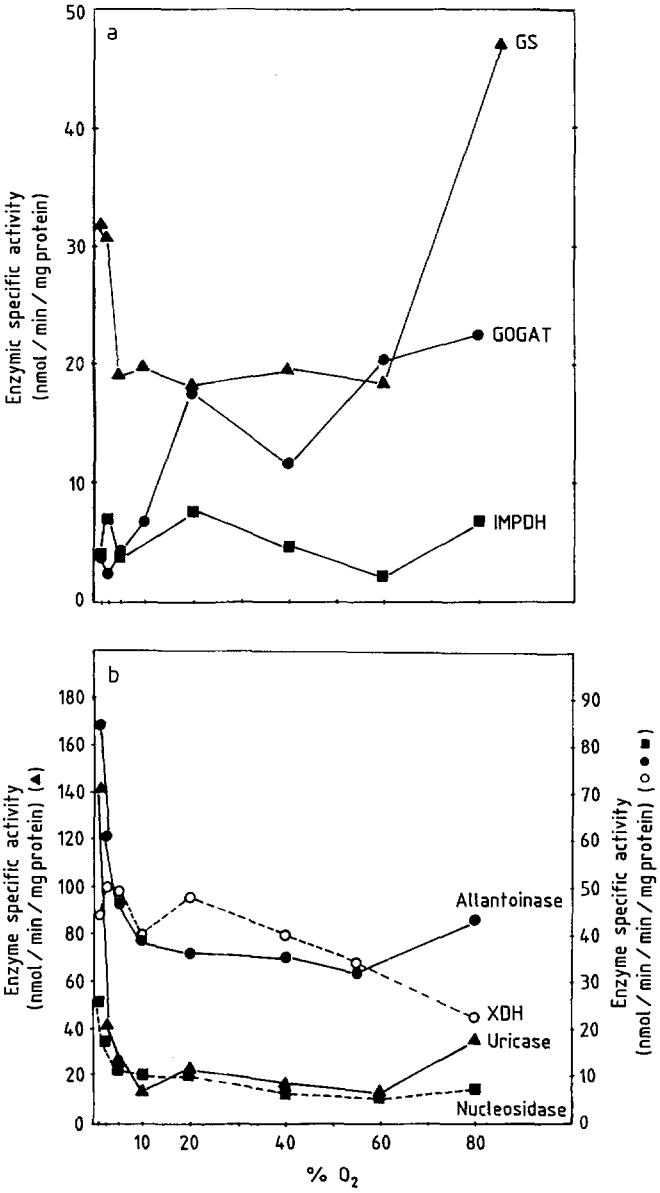

Fig. 4a, b. Effect of pO_2 on the activity of enzymes of N assimilation and purine oxidation in cowpea nodules grown in different O_2 levels from 5 to 28 d after planting. Extracts were prepared under an atmosphere low in pO_2 at 28 d. *GS*=glutamine synthetase; *GOGAT*=glutamate synthase; *IMPDH*=inosine 5'-monophosphate dehydrogenase; *XDH*=xanthine dehydrogenase. Values in each case are averages of two assays of two extractions of tissue. The average percent SE was ±12% for the enzymes shown in **a** and ±10% for those shown in **b**

Govers et al. (1986) have provided evidence to show that low O_2 is not essential for induction of nodule-specific proteins like uricase. Thus the elevated activity seen at low pO_2 could have been the consequence of an amplified level of expression as noted earlier by Larsen and Jochimsen (1986, 1987) in soybean root and callus cultures. However, the enzyme has been shown to be localised in enlarged microbodies in the uninfected

Table 2. Activities of uricase and allantoinase in nodules of cowpea from plants cultured from 5 to 28 d after planting with their nodulated root systems in a range of pO_2. Enzyme activities are expressed on a per-uninfected-cell basis[a]

pO_2 (%)	Activity (nmol·min^{-1}·uninfected cell^{-1})×10^{-4}	
	Uricase	Allantoinase
1	27.4±2.9	16.3±1.8
2.5	7.2±0.9	10.7±1.3
5	4.7±0.6	8.8±1.1
20	5.6±1.0	9.3±1.5
40	5.3±0.7	8.5±1.0
60	3.4±0.7	8.7±1.8
80	2.5±0.6	3.1±0.7

[a] Enzyme activities from data of Fig. 4 were used together with data for nodule numbers and the frequency of uninfected cells/nodule from plants cultured under identical conditions in a previous study (Dakora and Atkins 1990c). Values are means±sum of SE for enzyme assays and uninfected cell counts

cells of the central tissue of both soybean (Nguyen et al. 1985; Van den Bosch and Newcomb 1986) and cowpea (Webb and Newcomb 1987) nodules and an increase in the frequency of these cells, or of microbodies within them, could have been responsible for the sharp rise in activity. Allantoinase, which is also localised in uninfected cells (Hanks et al. 1981, 1983), showed a similar increase in activity in low pO_2 while enzymes located primarily in infected cells (xanthine dehydrogenase, nucleosidase, inosine 5′-monophosphate dehydrogenase, GOGAT and GS; Shelp et al. 1983) showed little or no tendency to increase in low O_2. A significant change in the relative frequencies of infected and uninfected cells in the central tissue zone of cowpea nodules at low pO_2 has been recorded (Dakora and Atkins 1990c). At 1, 2.5 or 5% O_2, the ratio of uninfected to infected cells was, in this sequence, 2.8, 1.8 and 1.7, compared with a constant value around 1.0 in pO_2 from 20 to 80%. Despite the greater number of uninfected cells in nodules in low pO_2, activities of both allantoinase and uricase, expressed on a cell basis, also increased (Table 2), indicating that apart from the effect of O_2 on cell differentiation their expression was specifically enhanced.

While uricase has been localised within microbodies in nodules, allantoinase has been recovered with a considerably less dense particulate fraction in sucrose density gradients (Hanks et al. 1981). In view of the fact that both these enzymes have been recovered in peroxisomes from other plant tissues (Theimer and Beevers 1971), together with their apparently co-ordinate expression in relation to pO_2, a re-examination of the question of their subcellular location in ureide-forming nodules seems justified.

The response of GS to pO_2 is notable in that at no O_2 level did activity fall below that in nodules maintained in air. Although in this study we did not attempt to differentiate the relative proportions of the various isoforms of GS which are known to occur in nodules (Cullimore et al. 1982), the high GS activities at the extremes of pO_2 indicate that the nodule's capacity for ammonia assimilation remained high. In this respect the very low levels of GOGAT found in nodules grown at sub-ambient pO_2 might indicate a significantly reduced capacity for transfer of amide-N from glutamine to glutamate. In view of the recent demonstration by Chen and Cullimore (1988) of a nodule-specific isoform of GOGAT (NADH-GOGAT II) it would be interesting to see whether or not it was this particular protein which was repressed at low pO_2. Apparently the low levels of GOGAT did not limit amide-group transfer as there was no proportionately greater accumulation of glutamine or a lesser pool of glutamate in nodules of plants grown at low pO_2 compared with those in air.

While nodules cultured in sub-ambient pO_2 showed clear evidence of adaptations which together served to maintain ureide synthesis at high rates, even in 1% O_2, these adaptations would be absent in air-grown plants exposed to low pO_2 for relatively short periods. However, even though transfer to 5 or 2.5% O_2 reduced the level of $^{15}N_2$ fixed, labeled ureides were formed in the same proportion as in plants maintained in air (Table 1). At 1% O_2, ureide synthesis was apparently limited by O_2 but in this case inhibition was only 50%. Continued exposure to sub-ambient pO_2 for 120 h resulted in increased levels of fixation as well as of ureide labeling, indicating that nodules had begun to adapt to low pO_2. These observations were consistent with those made in earlier studies showing that periods in excess of 24 h were required for the adaptation of gas-exchange characteristics of nodules to altered pO_2 (Dakora and Atkins 1990a, b).

A previous kinetic labeling study (Atkins et al. 1988) of $^{15}N_2$ fixation by cowpea found that the major products of fixation were ureides and C_5-compounds (glutamate and glutamine). Furthermore, asparagine in nodules appeared not to be a product of current fixation, and it was suggested (Atkins et al. 1988) that the nodule pool of this amide was derived largely from phloem delivery. The labeling patterns found in the present study confirm these findings and show also that, even under conditions of reduced O_2 supply, [^{15}N]asparagine was not formed from $^{15}N_2$ as an alternative product of ammonia assimilation.

Overall our results provide little support for the idea that ureide synthesis in cowpea nodules is limited by O_2 supply. The level of uricase activity was able to maintain urate oxidation, even at a pO_2 where fixation of N_2 was markely reduced, indicating either that the enzyme was greatly in excess of the metabolic requirement for its activity, or that the unfavourable kinetic properties determined in vitro (Rainbird and Atkins 1981) were not reflected in vivo. In this regard the fact that uricase is concentrated within a microbody, which also contains a potential O_2-generating mechanism (catalase; Hanks et al. 1981), should not be overlooked. Furthermore, the kinetic properties of many enzymes have been shown to be markedly altered through their confinement at high concentration in lipophyllic micellar structures (Martinek et al. 1987) which might be likened, to some extent, to microbodies. The enzyme has been successfully puri-

fied from nodules of a number of ureide-forming species (e.g. Rainbird and Atkins 1981) so that more detailed studies of its physical and kinetic properties are now feasible.

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