Relationship between Activities of Enzymes for the Removal of $O_2^{\bullet -}$ and H_2O_2 and Nitrogenase in Root Nodules of *Phaseolus vulgaris* L.

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Oxygen Derivates, *Phaseolus vulgaris* L., *Rhizobium leguminosarum*, *Rhizobium tropici*, Symbiotic Effectivity

Nodules of *Rhizobium leguminosarum* bv. *phaseoli* in symbiosis with *Phaseolus vulgaris* were compared with regard to their nitrogenase activity and activities of enzymes involved in the removal of $O_2^{\bullet -}$ and H_2O_2 as well as total ascorbate content. Activities of catalase (EC 1.11.1.6), ascorbate peroxidase (EC 1.11.1.11), and total ascorbate content were consistently higher in nodules inhabited by bacterial strains with higher nitrogenase activity. Values for superoxide dismutase (EC 1.15.11), and guaiacol peroxidase activity did not differ for the bacterial strains compared. On the other hand, when different plant cultivars were inoculated with the same bacterial strain, high nitrogenase activity did not correlate with a higher activity of the oxygen scavenging enzyms or a higher content of total ascorbate. In this case, values for guaiacol peroxidase activity were greatly enhanced in nodules with lower nitrogenase activity. This may be part of a hypersensitive reaction of the plant cultivar against the bacterial symbiotic partner.

Inhibition of catalase activity in the nodules by addition of triazole to the nutrient solution did not alter nitrogenase activity within the first nine hours after addition. It can be concluded that the activity of catalase, ascorbate peroxidase, and superoxide dismutase is not generally coupled to nitrogenase activity in root nodules of *P. vulgaris*.

Introduction

The maintenance of optimal concentrations of oxygen within the infected cells represents a key problem for the actively N_2-fixing nodules. The process of N_2-fixation itself is very sensitive to oxygen, with synthesis of nitrogenase being repressed and its activity rapidly reduced or halted in response to high pO_2 (Appleby, 1984). On the other hand there is the requirement of maintaining high respiration rates within the nodules to satisfy the high energy demand of the reactions catalyzed by nitrogenase. Especially activated forms of oxygen can damage the legume root nodules severly (Gallon, 1992). These activated forms include the superoxide anion radical ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), and the hydroxyl radical ($OH^{\bullet}$). Possible sites of $O_2^{\bullet -}$ generation in nodules are leghemoglobin autoxidation (Puppo *et al.*, 1982), or mitochondrial respiration chains (Rich and Bonner, 1978). The defence system against $O_2^{\bullet -}$ includes superoxide dismutase (SOD), an enzyme that is ubiquitous in aerobic eu- and prokaryotes (Fridovich, 1986). SOD-activity has been detected in bacteroids and free living bacteria of *Rhizobium leguminosarum* bv. *phaseoli* (Dimitrijevic *et al.*, 1984), as well as in nodule host cells (Puppo *et al.*, 1982). Its reaction is one of the major sources for H_2O_2 in nodules. However, in nodules of *P. vulgaris* hydrogen peroxide can also be generated by the urate oxidase reaction, which is the key step for the formation of ureides. Together with $O_2^{\bullet -}$, H_2O_2 is involved in the oxidation of leghemoglobin as has been documented for soybean nodules (Puppo *et al.*, 1982). Two different enzymatic reactions have been described for the removal of toxic H_2O_2 in nodule tissues. The first involves catalase activity which can be found in peroxisomes (Lorenzo *et al.*, 1990) and bacteroids (Becana *et al.*, 1986) of legume nodules, but effectiveness of the enzyme may be reduced by its high K_m-value (Dalton *et al.*, 1986). An alternative system consists of the joined action of an ascorbate specific peroxidase together with two alternative pathways

Abbreviations: AsA, ascorbate; ARA, acetylene reduction assay; DAsA, dehydroascorbate; GSSG, oxidized glutathione; MDAsA, monodehydroascorbate; SOD, superoxide dismutase.

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for the regeneration of the co-substrate: monodehydroascorbate reductase (Hossain *et al.*, 1984) or dehydroascorbate reductase together with glutathione reductase. In soybean root nodules, the enzymatic activities of the ascorbate-glutathione cycle have been detected in association with a soluble fraction from plant cells and in isolated mitochondria (Dalton *et al.*, 1986, 1991, 1993a), but only glutathione reductase activity could be recorded in bacteroids (Dalton *et al.*, 1993a).

If not removed by the above mentioned enzymes superoxide radical and hydrogen peroxide can react in a "Haber-Weiss" reaction to generate the hydroxyl radical which is the most potent oxidant known (Scandalios, 1993). Especially important is its role in the destruction of biological membranes by peroxidation of polyunsaturated lipids (Rabinowitch and Fridovich, 1983). The only way to deal with such chain propagating substances that remains in biological tissues is the reaction with antioxidants, such as ascorbate (Rabinowitch and Fridovich, 1983). There is evidence that SOD and catalase may be involved in maintaining membrane integrity in legume root nodules (Puppo *et al.*, 1991).

If the action of SOD, catalase, and ascorbate peroxidase is a prerequisite for an optimal nitrogenase reaction in legume root nodules, a correlation between the activities of these enzymes can be predicted. Data obtained from soybean root nodules indicate such a correlation between ascorbate peroxidase activity and nitrogenase activity (Dalton *et al.*, 1986). There are also reports of a total lack of ascorbate peroxidase activity in nodules without nitrogenase activity derived from ineffective *Bradyrhizobium* strains (Staehelin *et al.*, 1992) or at least of a substantial decrease in the activity of enzymes of the ascorbate-glutathione pathway in soybean root nodules inhabited by ineffective bacterial strains (Dalton *et al.*, 1993b).

Data obtained with two different genotypes of alfalfa indicate a correlation between plant ineffectiveness and activities of the enzymes of the ascorbate glutathione pathway (Dalton *et al.*, 1993b).

The present paper evaluates the role of enzymes for the removal of toxic oxygen derivates in nodules formed on *P. vulgaris*. Two experiments were performed with symbioses differing in their rhizobial strains (experiment 1) or in their plant cultivars (experiment 2). Nodules were compared with regard to their nitrogenase activity and the activities of SOD, catalase, ascorbate peroxidase, and guaiacol peroxidase. Additionally, the activities of enzymes for the regeneration of the co-substrate in the ascorbate peroxidase reaction, as well as the total ascorbate content of the nodules were determined.

In a third experiment, a decrease of catalase activity was induced *in vivo* by the addition of a herbicide (3-amino-1,2,4-triazole). Subsequently, the effect on ascorbate peroxidase activity, glucose-6-phosphate dehydrogenase activity, and nitrogenase activity was followed.

Materials and Methods

Bacterial culture

Rhizobium leguminosarum bv. *phaseoli* WPBS 3644, and WPBS 3605 originate from the Welsh Plant Breeding Station, Aberystwyth, Wales, U.K. *Rhizobium leguminosarum* bv. *phaseoli* Ph 24 was purchased from Tokachi Nokio Ren, Hokkaido, Japan. *Rhizobium tropici* CIAT 899 was supplied from CIAT, Cali, Colombia. Strains of rhizobia were grown in yeast extract mannitol (YEM) medium (Jordan, 1984) at 28 °C to late stationary phase. Prior to inoculation, cultures were diluted with sterile YEM medium to obtain a concentration of 10^9 cells ml^{-1}.

Plant culture

Seeds of *Phaseolus vulgaris* cv. Brilliant or cv. Rico were surface sterilized and subsequently inoculated by immersion in the corresponding bacteria suspension for 50 min while gently shaking at 28 °C. Sowing took place under sterile conditions in dishes containing a 1:1 mixture (v/v) of coarse sand and vermiculite. Seedlings were raised in a growth cabinet under a constant temperature regime (24 °C) and a 16 h illumination of 300 μE $m^{-2}s^{-1}$. Five days after germination the seedlings were transferred to water culture pots fitted with a gas tight lid pierced by five sealable holes of 1 cm diameter. Four of those were used for the placement of plants, and the fifth served as a service hole for the aeration tube. Plants were held in position and root systems sealed by modelling clay (Bostik-Prestek, Renner, Stuttgart, Ger-

many). The solution level in the pots was lowered and adjusted to leave 1 dm^3 airspace in the pots. Essentially all nodules developed in the area of the roots not directly exposed to the nutrient solution, resulting in almost perfect crown nodulation. The composition of the constantly aerated nutrient solution was as follows (M): 2×10^{-3} $CaSO_4$, 0.75×10^{-3} $MgSO_4 \cdot 7\ H_2O$, 0.2×10^{-3} KH_2PO_4, 0.9×10^{-3} K_2SO_4, 0.1×10^{-4} KCl, 4×10^{-5} FeEDTA, 1×10^{-5} H_3BO_4, 1×10^{-6} $MnSO_4 \cdot H_2O$, 1×10^{-6} $ZnSO_4 \cdot 7\ H_2O$, 2×10^{-7} $CuSO_4 \cdot 5\ H_2O$, 1×10^{-8} $(NH_4)_6MoO_{24}$, 2×10^{-9} $CoCl_2 \cdot 6\ H_2O$, (pH 6.5). The nutrient solution was replaced twice a week.

In the first two experiments, several harvests were carried out at different growth stages of plants in weekly time intervals, starting at three weeks after sowing. Parallel to acetylene reduction assays nodule samples of 0.6 g were separated, instantly frozen in liquid nitrogen, and stored at −15 °C for later analyses.

Catalase inhibition was achieved by adding 3-amino-1,2,4-triazole (aminotriazole) to the nutrient solution in a final concentration of 5 mM. In this experiment, determinations of nitrogenase activity took place 3, 6, and 9 h after the addition of the herbicide to the nutrient solution. Plants were cultivated in hydroponic culture for 4 weeks prior to the commencement of the treatment.

Acetylene reduction assays

Undisturbed, intact plants were assayed by closed system ARA in the pots used for hydroponic plant cultivation. Three pots, each containing four plants of the plant/*Rhizobium* combination investigated, were sealed and 6% of the air surrounding the roots was replaced by a corresponding amount of instrument grade acetylene (Carroll *et al.,* 1987). Preliminary experiments had shown maximal ethylene production in the interval between 5 and 10 min after injection of the acetylene. Gas samples (1 cm^3) were, therefore, withdrawn at these times and analyzed for their ethylene content utilizing a gas chromatograph (HP 5890, Series II), equipped with a flame ionization detector (FID) and a Porapak-N column. Oven temperature was set constant at 130 °C. After the ARA, nodules were separated from the roots and their fresh weight was determined.

Photometric enzyme determinations

Nodule samples (0.6 g) were homogenized in 6 ml 25 mM EPPS buffer (N-2-hydroxyethylpiperazine propane sulphonic acid, pH 7.8) containing 0.2 mM EDTA and 2% PVP. The homogenate was filtered through a nylon mesh and then centrifuged at 15000 x*g* for 15 min. The supernatant was used for enzyme analyses. All operations before analysis were carried out at 3 to 5 °C. With the exception of SOD, enzyme activities were measured in a final volume of 1 ml using various aliquots of the supernatant.

The photochemical method described by Giannopolitis and Ries (1977) was employed to evaluate the activity of SOD. One unit of SOD activity was defined as the amount of enzyme that caused 50% inhibition of the rate of *p*-nitro blue tetrazolium chloride reduction at 560 nm.

Activity of catalase was assayed in a reaction mixture containing 25 mM phosphate buffer (pH 7.0), 10 mM H_2O_2, and enzyme. H_2O_2 decomposition was followed at 240 nm (ε = 0.039 mM^{-1} cm^{-1}).

According to Nakano and Asada (1981), analyses of ascorbate peroxidase activity were conducted in a reaction mixture of 25 mM phosphate buffer (pH 7.0), 0.1 mM EDTA, 1.0 mM H_2O_2 , 0.25 mM ascorbate, and the enzyme aliquot. There was no change of absorption in the absence of H_2O_2. The rate of ascorbate oxidation was measured at 290 nm (ε = 2.8 mM^{-1} cm^{-1}).

A reaction mixture of 25 mM phosphate buffer (pH 7.0), 0.05% guaiacol, 10 mM H_2O_2, and enzyme served to investigate guaiacol peroxidase activity (Staehelin *et al.,* 1992). The increase in absorption due to the formation of tetraguaiacol (ε = 26.6 mM^{-1} cm^{-1}) was followed at 470 nm.

The assay for monodehydroascorbate reductase utilized the procedure of Hossain *et al.* (1984). The reaction mixture contained 25 mM EPPS buffer (pH 7.8), 0.2 mM EDTA, 0.1 mM NADH, 0.1 mM ascorbate, 5 units of ascorbate oxidase, and enzyme. The rate of NADH oxidation was determined at 340 nm (ε = 6.2 mM^{-1} cm^{-1}).

Determinations of dehydroascorbate reductase were carried out according to Nakano and Asada (1981) by following the rate of ascorbate formation at 265 nm (ε = 14 mM^{-1} cm^{-1}). The reaction mixture consisted of 25 mM phosphate buffer (pH

7.0), 2.5 mM reduced glutathione, 0.4 mM dehydroascorbate, and enzyme. The results were corrected for the nonenzymatic reduction of dehydroascorbate.

According to Foyer and Halliwell (1976), glutathione reductase activity was recorded by measuring the decrease in absorption at 340 nm due to NADPH oxidation ($\varepsilon = 6.2\ mM^{-1}\ cm^{-1}$). The reaction mixture contained 25 mM EPPS buffer (pH 7.8), 0.2 mM EDTA, 0.5 mM GSSG, 0.12 mM NADPH, and enzyme. Data were corrected for the rate of NADPH oxidation in the absence of GSSG.

Activity of glucose-6-phosphate-dehydrogenase (EC 1.1.1.49) was determined according to Kuby and Noltmann (1966). The reaction mixture contained 100 mM triethanolamine buffer (pH 7.6), 6.6 mM $MgCl_2$, 1.15 mM glucose-6-phosphate, 0.37 mM NADP, and 100 µl enzyme aliquot. The rate of NADPH formation was followed at 340 nm ($\varepsilon = 6.2\ mM^{-1}\ cm^{-1}$).

Total ascorbate content

Total ascorbate content in the nodules was determined following the method of Law *et al.* (1983). Nodule tissue (0.6 g) was homogenized in 6 ml of 5% meta-phosphoric acid and centrifuged at 22000 x*g*. For the reduction of dehydroascorbate the reaction mixture contained 0.4 ml aliquot of the supernatant, 0.6 ml of 150 mM phosphate buffer (pH 7.8) containing 5 mM EDTA, and 0.1 ml of 10 mM DTT. Samples were incubated for 10 min at R.T. before the addition of 0.1 ml of 0.5% N-ethylmaleimide for the removal of excess DTT. The color reaction was induced by adding 0.4 ml of 10% TCA, 0.4 ml of 44% *ortho*-phosphoric acid, 0.4 ml of 4% á-dipyridyl in 70% ethylalcohol, and 0.2 ml of 3% $FeCl_3$. The mixtures were incubated for 40 min at 40 °C before spectrophotometric measurement being carried out at 525 nm. Concentrations were calculated according to a previously established standard curve.

Results

Nitrogenase activities on a per plant basis followed a different pattern for each symbiotic system (Fig. 1A and D). Values obtained with nodules on bean cultivar Brilliant generally showed declining values during the course of plant development. Data obtained for this cultivar in symbiosis with the bacterial strains Ph 24 and WPBS 3605 declined from the beginning of the experiments through week five, whereas symbiosis with WPBS 3644 reached maximal nitrogenase activity in the fourth week. In contrast, nodules deriving from bean cultivar OAC Rico in symbiosis with WPBS 3605 revealed increasing values for total nitrogenase activity with growing time (Fig. 1 D).

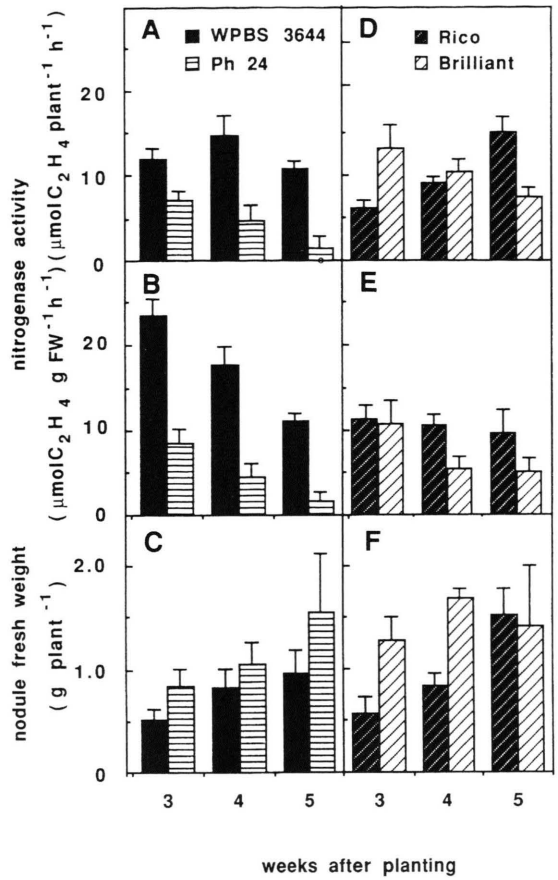

Fig. 1. Nitrogenase activity per plant (A, D), per g nodule fresh weight (B, E), and fresh weight of nodules (C, F) on one genotype of *P. vulgaris* (cv. Brilliant) inoculated with two different strains of *Rhizobium leguminosarum* bv. *phaseoli* (WPBS 3644, and Ph 24, Fig. A–C) or two different plant genotypes (cv. Brilliant or cv. OAC Rico) inoculated with an identical strain of *Rhizobium leguminosarum* bv. *phaseoli* (WPBS 3605, Fig. D–F). The standard deviation is indicated by vertical bars ($n = 3$).

Comparisons involving the activities of enzymes responsible for the removal of oxygen derivates were based on nitrogenase activity per g nodule fresh weight. In all nodules investigated, nitrogen-

ase activity per g fresh weight declined with progressing age. In the first experiment, nitrogenase activity in nodules derived from Ph 24 was always lower than in those derived from WPBS 3644 (Fig. 1 B). In the second experiment, both symbioses exhibited similar nitrogenase activities three weeks after sowing but, thereafter, the symbiosis with cv. Brilliant as the macrosymbiont revealed a steeper decline in activity than the symbiosis with cv. OAC Rico (Fig. 1 E).

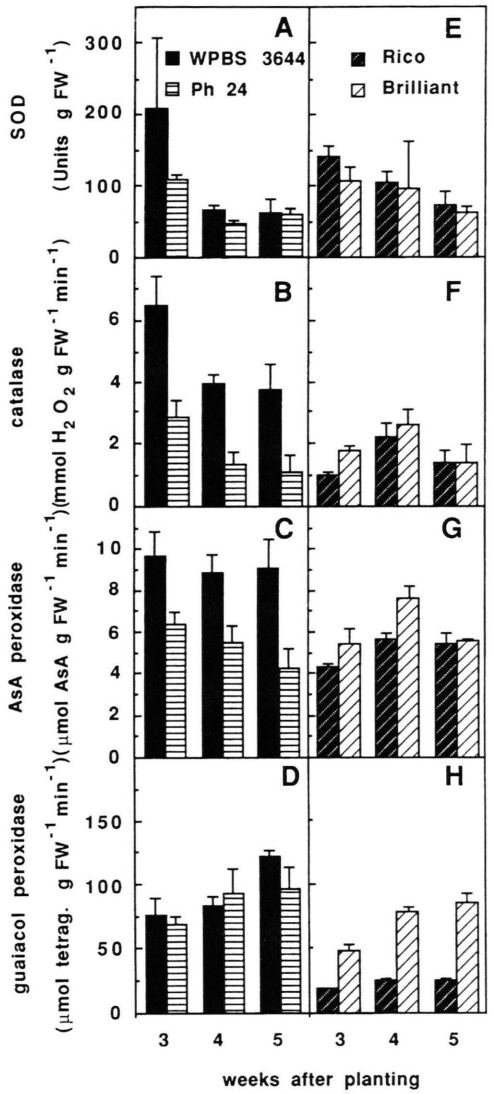

Fig. 2. Activity of SOD (A, E), catalase (B, F), ascorbate peroxidase (C, G), and guaiacol peroxidase (D, H) in nodules on one genotype of *P. vulgaris* (cv. Brilliant) inoculated with two different strains of *Rhizobium leguminosarum* bv. *phaseoli* (WPBS 3644, and Ph 24, Fig. A–D) or two different plant genotypes (cv. Brilliant or cv. OAC Rico) inoculated with an identical strain of *Rhizobium leguminosarum* bv. *phaseoli* (WPBS 3605, Fig. E–H). The standard deviation is indicated by vertical bars ($n=3$).

Nodule mass increased with progressing plant age in both experiments (Fig. 1 C, and F). In the first experiment, only small differences were observed between the strains, indicating equal growth characteristics for nodules with different nitrogenase activities. In the second experiment, nodule weight of cv. Brilliant was elevated during the first four weeks. However, five weeks after planting, both symbiotic systems reached equal amounts of nodule biomass (Fig. 1 F).

In both experiments, SOD activity declined with progressing nodule age (Fig. 2 A, and E), while differences between the compared symbiotic systems were negligible. Values found for catalase activity and ascorbate peroxidase activity revealed clear differences between the two experiments. Comparison of bacterial strains always revealed relationship between nitrogenase, catalase, and ascorbate peroxidase activity, with the strain exhibiting higher nitrogenase activity (i.e. WPBS 3644) also showing higher activity of the two latter enzymes. No such relationship was found when different nitrogenase activities were the result of different plant partners in the symbioses. Catalase and ascorbate peroxidase activities were nearly identical between nodules derived from bean cultivar OAC Rico or Brilliant (Fig. 2 F, and G).

Total peroxidase activity measured as guaiacol peroxidase activity increased with time in both experiments reflecting a contrary pattern to all of the above mentioned enzymes (Fig. 2 D, and H). Values for guaiacol peroxidase activity were exceeding those found for ascorbate specific peroxidase. While in the first experiment nodules inhabited by bacterial strains with different nitrogenase activity revealed nearly identical values for guaiacol peroxidase (Fig. 2 D), a different situation arose when plant genotypes were compared. Here, nodules with lower nitrogenase activity showed very high values for total peroxidase activity (Fig. 2 H).

Activity of dehydroascorbate reductase was almost not detectable in both experiments (Fig. 3 A, and E) and relatively low values were found for glutathione reductase activity compared to those

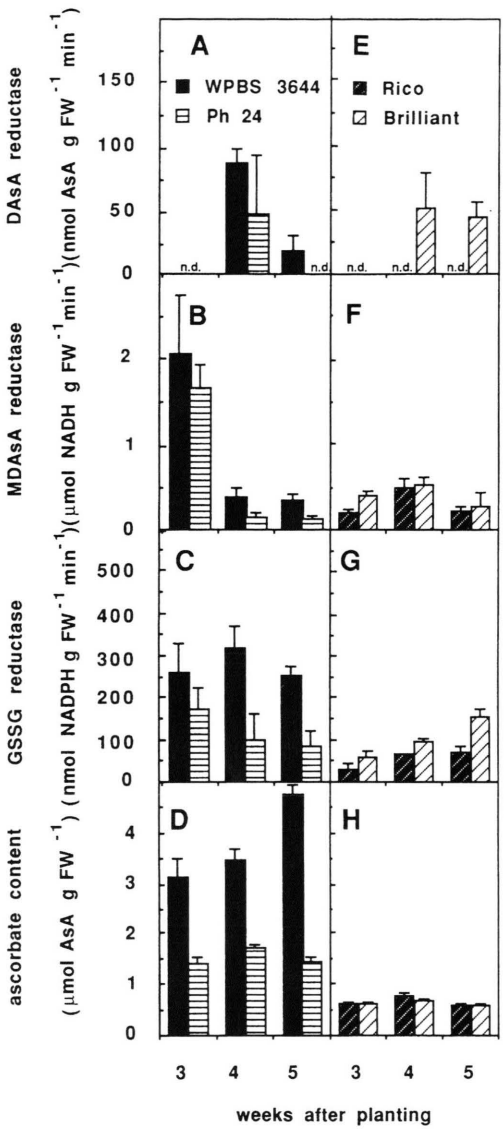

Fig. 3. Activity of dehydroascorbate reductase (A, E), monodehydroascorbate reductase (B, F), glutathione reductase (C, G), and total ascorbate content (D, H) in nodules on one genotype of *P. vulgaris* (cv. Brilliant) inoculated with two different strains of *Rhizobium leguminosarum* bv. *phaseoli* (WPBS 3644, and Ph 24, Fig. A–D) or two different plant genotypes (cv. Brilliant or cv. OAC Rico) inoculated with an identical strain of *Rhizobium leguminosarum* bv. *phaseoli* (WPBS 3605, Fig. E–H). The standard deviation is indicated by vertical bars ($n = 3$, n.d. = not detectable).

found for ascorbate peroxidase (Fig. 3C, and G). In contrast, high values were recorded for activity of monodehydroascorbate reductase (Fig. 3B, and F). The first experiment revealed partly higher monodehydroascorbate reductase and glutathione reductase activities for the strain with higher nitrogenase activity (Fig. 3B, and C), while no differences were found by comparing different plant genotypes (Fig. 3F, and G).

In the first experiment, values for the total ascorbate content were greatly enhanced in nodules with higher nitrogenase activity compared to those with lower nitrogenase activity (Fig. 3D). Data obtained for this parameter in the second experiment were generally lower compared to the first which may reflect a characteristic of nodules derived from the bacterial strain WPBS 3605. Here, similar values of total ascorbate content were found in both symbioses (Fig. 3H).

The addition of aminotriazole to the nutrient solution led to a rapid decrease in the catalase activity in the nodules (Fig. 4A). Equal values for ascorbate peroxidase and glucose-6-phosphate dehydrogenase activities were measured in treated nodules and controls (Fig. 4C, and D). Constant values were determined for nitrogenase activity during the complete time course of this experiment (Fig. 4B).

Discussion

The data presented here indicate a relationship between the effectivity of the enzymatic removal of toxic oxygen derivates and the activity of nitrogenase in nodules of *P. vulgaris*, when different rhizobial strains are compared. The strain with lower nitrogenase activity also revealed lower activities in catalase, ascorbate peroxidase, and partly in SOD. For guaiacol peroxidase activity no differences were found. Nodules derived from the strain with lower nitrogenase activity also revealed lower values for total ascorbate content. We found identical results in two further experiments comparing different rhizobial strains on *P. vulgaris* (data not shown). It can be concluded that lower values for total ascorbate content and lower activities of the enzymes which play a key role in the removal of H_2O_2 are a characteristic of nodules on *P. vulgaris* inhabited by rhizobial strains with lower nitrogenase activity.

The situation is different when reduced nitrogenase activity in the nodules can be attributed to a less effective plant genotype. Data obtained from

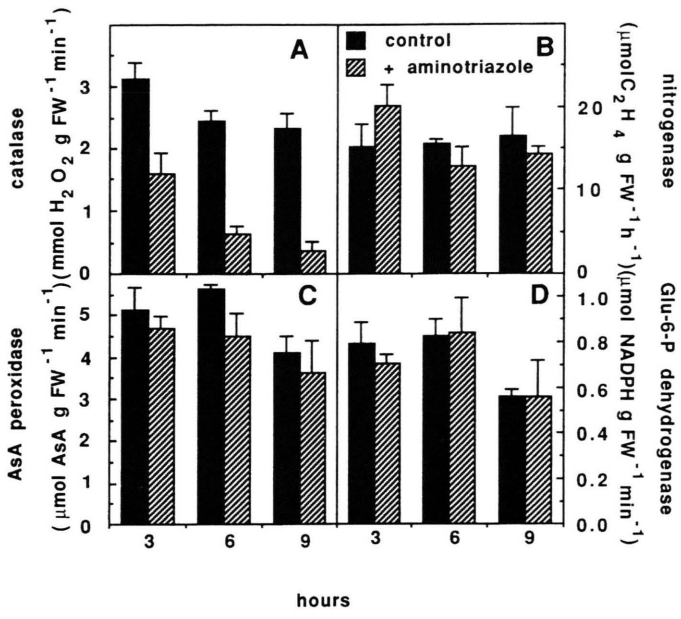

Fig. 4. Activity of catalase (A), nitrogenase (B), ascorbate peroxidase (C), and glucose-6-phosphate dehydrogenase (D) in nodules on *P. vulgaris* cv. Rico inoculated with *Rhizobium tropici* CIAT 899 at different time points after addition of 3-amino-1,2,4-triazole (5 mM) to the nutrient solution. The standard deviation is indicated by vertical bars ($n=3$).

the comparison of different genotypes of alfalfa revealed a correlation between nitrogenase activity and activity of ascorbate peroxidase, monodehydroascorbate reductase, and glutathione reductase (Dalton *et al.*, 1993b). In our experiment, higher values for nitrogenase activity found with one of the two bean genotypes were not correlated with higher activities of the above mentioned enzymes. This suggests that the amount of activity of these enzymes reflects the influence of the bacterial genotype in the symbiosis, but is not a general prerequisite for higher symbiotic effectiveness with *P. vulgaris*.

On the other hand, there was an influence of the plant genotype on the expression of guaiacol peroxidase activity in the root nodule (Fig. 2H). The identical guaiacol peroxidase activities found in a comparison of different bacterial strains (Fig. 2D) are in marked contrast to the great differences for this activity found in the comparison of two plant genotypes. Higher values of guaiacol peroxidase activity found in the symbiosis with the less effective plant genotype could represent part of an incompatibility between both symbiotic partners. High values of guaiacol peroxidase and chitinase activity have been found in ineffective soybean root nodules and were interpreted as characteristic for a plant hypersensitive reaction against incompatible bacterial strains (Staehelin *et al.*, 1992). Such a hypersensitive reaction may be a reason for the lower symbiotic effectiveness of certain bean genotypes. Furthermore, our data indicate that low nitrogenase activity in the symbiosis of *P. vulgaris* with *Rhizobium* is caused by either plant or bacterial ineffectiveness.

According to Dalton *et al.* (1993a) dehydroascorbate reductase is the most important enzyme for the cytosolic ascorbate regeneration in soybean root nodules. The main role of monodehydroascorbate reductase is probably the reduction of monodehydroascorbate generated in the cell wall by reactions with phenolic compounds. In contrast, our results indicate that in root nodules of *P. vulgaris* monodehydroascorbate reductase may be the key enzyme in the regeneration of the co-substrate in the ascorbate peroxidase reaction.

The inhibition of catalase by aminotriazole is well known (Margoliash and Novogrodsky, 1958; Margoliash *et al.*, 1960) and has been used in several studies for the examination of the distribution and role of catalase in plant tissues (Amory *et al.*, 1992; Havir, 1992). Besides a specific effect on catalase activity there have been numerous reports about other physiological effects of the herbicide (Fedtke, 1982). Conclusions with regard to catalase activity are limited to short time effects during the first hours after addition of aminotriazole.

The constant values found for the nitrogenase activity after inhibition of catalase activity demonstrate that H_2O_2-removal by catalase is not essential for nitrogenase reaction in the root nodules of *P. vulgaris*. In our experiment, there was no concommitant increase in ascorbate peroxidase activity in response to lower catalase activities as it was described for the endosperm of *Ricinus communis* seeds cultivated for four days with aminotriazole (Klapheck *et al.*, 1990). Previous reports of a protection of nitrogenase components from O_2 inactivation by the addition of SOD and catalase (Mortensen *et al.*, 1974) demonstrate the sensitivity of nitrogenase subunits to reactive oxygen derivates but obviously do not reflect the situation *in vivo*. During the first 9 h after addition of triazole, the inhibition of catalase was not limiting for nitrogenase activity in our study excluding a direct relationship between the removal of H_2O_2 by catalase and the nitrogenase activity in the bean root nodule.

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