Role of some oxidative enzymes in root nodules of *Trigonella foenum-graecum*

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Summary. Levels of superoxide dismutase and peroxidase were found to be lower and that of catalase higher in the nodule cytosol and bacteroids as compared to roots. The significance of these enzymes has been discussed with respect to nitrogen fixation.

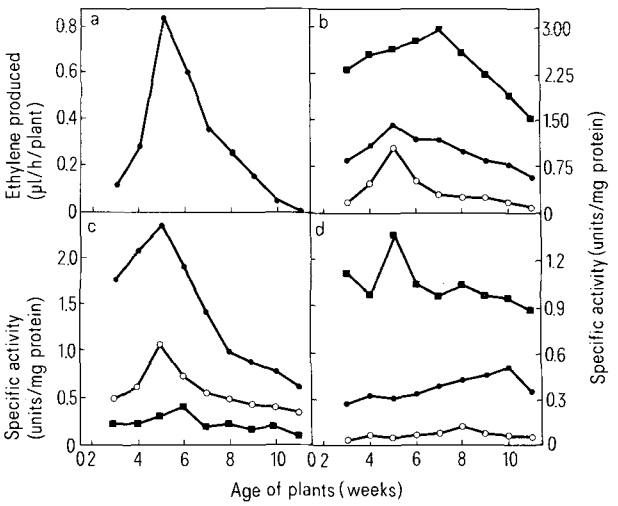
Nitrogenase is an oxygen-sensitive enzyme, thus nitrogen fixing systems which utilize oxygen to produce ATP must, at the same time, prevent oxygen from inactivating the nitrogenase[2]. The mechanisms which protect the nitrogenase from inactivation by oxygen are varied, and have been discussed exhaustively[3-6]. The most favored hypothesis to explain oxygen toxicity is that the products of oxygen metabolism, oxygen radical (O_2^-) and hydrogen peroxide (H_2O_2) are toxic agents[7]. In general, the organisms contain superoxide dismutase, which detoxifies O^-_2, and catalase and peroxidase which detoxify H_2O_2. The present communication deals with the probable role played by oxidative enzymes like superoxide dismutase, catalase and peroxidase in connection with the ontogeny of nodules and corresponding changes in the nitrogenase levels. The interrelationship between the oxidative enzymes, maintenance of reducing power and nitrogen fixation have been stressed.

The *Trigonella foenum-graecum* plants were grown and maintained as we have described earlier[8]. Extraction procedure for the nodule cytosol, bacteroidal and root cytosol enzymes was essentially the same as we have described earlier[9]. The nitrogenase (E.C. 1.7.99.2; nitrogen (acceptor) oxidoreductase) activity of the intact root nodule was assayed as described by Kurz and La Rue[10]. The superoxide dismutase (E.C. 1.15.1.1) was assayed by the method of Beauchamp and Fridovinch[11] and catalase (E.C. 1.11.1.6; hydrogen peroxide oxidoreductase) and peroxidase (E.C. 1.11.1.7; donor: hydrogen peroxide oxidoreductase) were assayed as described earlier[8]. The activities of all the enzymes are expressed in this paper in terms of specific activities (units/mg protein) and are the average values of 5 determinations. The protein was estimated by the method of Lowry et al.[12] using bovine serum albumin as standard. The amounts of NADPH and NADH oxidized were determined as described by Horecker and Kornberg[13], and Kornberg[14] respectively.

The basic requirements for the nitrogen reduction are the enzyme nitrogenase, reduced atmosphere, ATP and low oxygen tension[2]. In nodules, an elaborate system has been evolved for nitrogen fixation. Nodules use leghemoglobin in order to regulate oxygen concentration at the site of nitrogen fixation[15-18].

The nitrogenase activity of the intact root nodule was found to be maximal in the 5th week after the planting of *Trigonella foenum-graecum* (fig., a). The superoxide dismutase activity in the nodule cytosol and bacteroids was found to increase simultaneously with the increase in nitrogenase activity up to the 5th week of plant growth. On the other hand, the activity in roots increased up to the 7th week. However, in the case of bacteroids a significant change in superoxide dismutase activity was observed with the peak of nitrogen fixation, as compared to the nodule cytosol and roots (fig., b). The observation is of significance since the high levels of bacteroidal superoxide dismutase may scavenge the O^-_2, thus providing a necessary defence for the nitrogenase against oxygen damage. Earlier, we reported that nodules have much higher levels of catalase as compared to roots[8]. The activity of the nodule cytosol and bacteroidal catalase was found to increase simultaneously with the increase in the nitrogenase activity up to the 5th week of plant growth. However, the activity of catalase in the roots did not change significantly throughout the plant growth (fig., c). This finding demonstrates that catalase may be playing an important role in governing the levels of hydrogen peroxide in the nodule. High levels of catalase have also been reported in the effective nodules of soybean and white clover[19]. There also, it has been proposed that catalase might be a critical factor in governing the capacity of the 2 symbionts to function together in assimilating nitrogen, which may be by virtue of its role in destroying H_2O_2[19]. In this way it could nullify the toxic effects of the H_2O_2 which may be produced by the nodule cytosol or bacteroids. Thus, the inefficiency of nitrogen fixation may result from an accumulation of hydrogen peroxide, which may oxidize, and thus prevent the normal functioning or synthesis of, some substances essential for nitrogen assimilation.

The activity of root and bacteroidal peroxidase was found to increase simultaneously with the increase in nitrogenase activity up to the 5th week of plant growth. On the other hand, the nodule cytosol peroxidase activity tends to increase, though not to a significant extent, up to the 10th week of plant growth. However, the levels of peroxidase were very low in the nodule cytosol and bacteroids as compared to the roots (fig., d). The function of peroxidase is not only to remove H_2O_2 but it can also catalyze the oxidation of several reduced substances[20]. The *Trigonella foenum-graecum* nodule cytosol peroxidase catalyzed the oxidation of reduced nicotinamide adenine dinucleotide phosphate (NADPH) and reduced nicotinamide adenine



a Activity of nitrogenase (●) in developing root nodules of *Trigonella foenum-graecum*. *b* Activity of superoxide dismutase in developing nodule cytosol (●), bacteroid (○) and root (■) of *Trigonella foenum-graecum*. *c* Activity of catalase in developing nodule cytosol (●), bacteroid (○) and root (■) of *Trigonella foenum-graecum*. *d* Activity of peroxidase in developing nodule cytosol (●) bacteroid (○) and root (■) of *Trigonella foenum-graecum*.

dinucleotide (NADH) (data not presented). Several other workers have also reported the oxidation of NADPH and NADH by peroxidase[21-25]. In this context the low levels of peroxidase become significant in the nodules, as peroxidase if present in high amounts may oxidize NADPH and NADH, which are required for the removal of an excess of ammonia by the ammonia assimilatory enzymes like glutamate dehydrogenase, glutamine synthetase/glutamate synthase[9,26] and as the reductant for nitrogenase.

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Treatment of mice with monosodium glutamate does not inhibit a subsequent response to gold thioglucose

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Summary. Neonatal mice were injected with monosodium glutamate (MSG) followed 10 days later by treatment with gold thioglucose (GTG). Weight gain, food consumption and body lipid determinations were made. Results indicated that pretreatment with MSG does not block subsequent response to GTG and the resulting changes are similar to those induced by GTG alone.

Gold thioglucose (GTG) and monosodium glutamate (MSG) are frequently used to induce metabolic alterations in mice. Damage in the ventromedial area of the hypothalamus (VMN) after GTG administration results from pericapillary changes[1]. Physiological alterations induced by VMN damage include excessive weight gain, lipid accumulation, and changes in energy balance among others. For example, GTG-injected mice reached a body weight of 56.3 ± 0.9 g 4 months post-injection vs 30.7 ± 3.6 g for the controls. Mean body lipid content was 51.3% for the injected animals and 14.9% for the controls[2].

In 1969 Olney[3] reported that neonatal mice treated with MSG had increased body weight, substantially elevated amounts of depot lipid and a slight suppression in food intake compared to control animals. Examination of the hypothalamic region of the MSG-treated mice revealed extensive damage to the arcuate-median eminence area with no observable change in the VMN. Djazayery and Miller[2] reported little change in body weight or food comsumption of MSG-treated neonatal mice; however, at 4 months of age the treated animals had a body lipid content of 26.3% compared to 14.9% for the non-injected animals.

When GTG or MSG treatments are utilized one can expect to see changes in body lipid content; however, food intake and total body weight alterations depend on the compound administered and the protocol used. Since these 2 compounds seem to affect different hypothalamic regions and somewhat different metabolic parameters we sought to determine if treatment with MSG might eliminate a subsequent response to GTG. The results indicated that the MSG-treated mice are still susceptible to damage from GTG. Mice used in this study were a CF1 strain derived, from Sprague-Dawley stock. The animals were housed in plastic box cages in a temperature controlled room (22 ± 1 °C) with a 12:12 light cycle. Feed (Purina Chow) and water were available ad libitum. Upon delivery of the young, litter size was adjusted to 8-10 per female. Beginning at 5 days of age, one half of the pups in each litter were s.c. injected for 10 days with MSG in sterile saline