Hypothesis

Superoxide dismutase: an essential role in the protection of the nitrogen fixation process?

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The oxygen consumption process by cells is fraught with danger: in playing its classical role of electron acceptor to form a molecule of water, oxygen can be partially reduced and generate several toxic intermediates [1]. The first one is the superoxide anion which is the precursor of more potent oxidants, including interconversion products derived from this radical [2]. A primary defence is provided by superoxide dismutase (SOD) an enzyme scavenging superoxide anion with remarkable efficiency [3]. On the other hand, one of the most intriguing characteristics of nitrogen-fixing organisms is the rapid and irreversible inactivation by oxygen of the key enzyme of this process: nitrogenase. At the cellular level, different strategies to ensure an adequate oxygen level compatible with an optimal nitrogenase activity have been described [4]. Unfortunately little information is available concerning the molecular mechanism involved in the protection of nitrogenase against oxygen [5] and therefore we propose here to consider the role of SOD. However, to take into account a frequent variability in the measurement of SOD activities due to the nature and the concentration of the detector molecule, the comparisons will be made only between assays realized under the same experimental conditions.

Free-living bacteria like *Azotobacter, Azomonas* and *Beijerinckia* able to fix nitrogen exhibit high SOD levels in the range of 32–86 units/mg of protein, determined by the xanthine oxidase-cytochrome *c* assay [6]; by comparison the SOD content of *Escherichia coli*, used as control and tested under the same experimental conditions, reaches only 14 units/mg of protein [6]. In the same way, SOD activity determined in the facultative anaerobe diazotroph *Klebsiella pneumoniae* using the xanthine oxidase-cytochrome *c* test exhibits 27 units/mg of protein [7]. Under the same conditions, the level of SOD appears significantly lower in five strains of *Streptococcus*, a common facultative anaerobe (mean value of 12.6 units/mg of protein) [7]. Thus it appears that the SOD content of free-living nitrogen-fixing bacteria is significantly higher than that encountered in non-fixing ones. On the other hand, the repression of nitrogenase by ammonium is associated with a decline in SOD activity (measured by the xanthine oxidase-cytochrome *c* test) of *Azospirillum brasilense* growing under similar oxygen tensions [8].

The situation of some blue-green algae capable of fixing nitrogen appears even more complex since they contain an oxygen-evolving system associated with photosynthesis. The location of nitrogenase in non-photosynthetic specialized cells, the heterocysts, is considered as a first strategy to limit the oxygen damage [9]. However, these cells, in which an oxygen diffusion barrier is found [4], are fitted with a SOD level which reaches 27% of that encountered in the vegetative ones having a functional photosynthetic system [10]; in the two cases, the same xanthine oxidase-

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NBT assay has been used [10]. On the other hand, in *Gloeothece* which fixes nitrogen aerobically even though it does not possess heterocysts, nitrogenase and SOD activities (xanthine oxidase-cytochrome *c* test) are maximal at the same stage of growth [11].

In the case of legume symbiotic associations, the total level of SOD in free-living *Rhizobium* is slightly higher than that of the corresponding bacteroids [12], although the oxygen environment is strongly different (70 and 0.05 mmHg pO_2, respectively). On the other hand, the exceptionally high ability of *Sesbania* root and stem nodules to reduce atmospheric nitrogen [13] is on a par with the presence in the corresponding bacteroids of significant SOD levels: 36 and 39 units/mg of protein, respectively [14]. The SOD activity of bacteroids extracted from French bean nodules, measured under the same conditions, is 12 units/mg of protein [12]. All the tests concerning legume symbiosis have been performed with the same xanthine oxidase-NBT assay and their comparison is thus valid. As observed for *Gloeothece*, the maximal activities of nitrogenase and SOD are encountered at the same stage of growth [14]. Recently, the coordinate induction of SOD activity (measured by the xanthine oxidase-cytochrome *c* assay) with derepression of nitrogen fixation has been pointed out in the symbiotic system *Alnus-Frankia* [15]. In the case of these slow growing actinomycetes issued from Casuarinaceae, SOD (xanthine oxidase-NBT test) appears as a marker for the identification of strains [6].

All the above data strongly suggest that SOD is involved in the protection of cellular components that are crucial to the overall process of dinitrogen fixation. As SOD appears generally correlated with superoxide anion production, the high levels of this enzyme can result either from higher superoxide anion production by the metabolism of nitrogen-fixing microorganisms or from their active respiration [12]. At which step(s) of the nitrogen fixation process SOD action could be involved remains unknown. Although superoxide anion has been implicated in the inhibition of nitrogen fixation in some cases [11,17], a direct interaction with nitrogenase is not proved [5]. If it exists, the inactivation could be due to hydroxyl radical produced by a Fenton reaction of the reduced metal of nitrogenase with hydrogen peroxide, as suggested for another oxygen-sensitive enzyme: succinate dehydrogenase [18].

On the other hand, damage to other steps of the nitrogen fixation process may be prevented by SOD. Thus its involvement in the protection of the proton-donating systems participating in nitrogen fixation and hydrogen metabolism in heterocysts [10] and of leghemoglobin against oxidation in soybean nodules [19] has been suggested. Moreover the symbiotic form of *R. japonicum* contains a group of heme proteins with properties similar to cytochrome P-450 proteins [20]. Since the role of this molecule as an oxygen activator is well established, its presence can enhance the superoxide anion production, thus contributing an explanation for the significant SOD content of the bacteroids [12]. In symbiotic systems, the influence of SOD could occur during the first steps of infection by reference to its association with virulence in different strains of pathogenic bacteria such as *Neisseria meningitidis* [21] or *Staphylococcus aureus* [22] and with resistance to microbicidal activities of the host cell in the case of *Nocardia asteroides* [23].

Anyhow, the correlation between high SOD levels and nitrogen-fixing activity is a field which appears of great interest and may explain how diazotrophs protect their nitrogen-fixing system from damage caused by oxygen.

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