Short communication

Phytomonas spp: superoxide dismutase in plant trypanosomes

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In 1909, Donovan [1] proposed the creation of a new genus, Phytomonas, to differentiate plant from animal trypanosomatids. Phytomonas spp. live in the latex, sap, sieve tubes, and fruit of many plant species [2]. At first, considerable controversy surrounded the pathogenicity of phytozoans in plants, as initially these parasitic protozoans were not thought to be particularly harmful. However, evidence from a number of plants of great economic significance, such as coffee, coconut, and palm, has shown that Phytomonas infections can have devastating economic consequences [3].

The defence mechanisms of trypanosomatids against the toxic products of O2 reduction – e.g. superoxide anion, hydrogen peroxide or hydroxyl radicals – are not completely understood. Trypanosomatids are protected from the damaging effects of reactive oxygen intermediates by scavengers such as trypanothione and specific enzymes such as superoxide dismutase (SOD, EC 1.15.1.1) and trypanothione peroxidase. Catalase, involved in the elimination of hydrogen peroxide, is absent from most trypanosomatids, although it has been detected in Crithidia luciliae [4] and in Phytomonas spp. [5].

Cyanide-insensitive SOD activity has been reported in Trypanosoma cruzi [6], as well as in other trypanosomatids such as Crithidia fasciculata [7] and Trypanosoma brucei [4]. The SOD activities of Leishmania tropica and T. cruzi are also cyanide-insensitive but peroxide-sensitive [7]. SOD of C. fasciculata is located in the cytosol and exists in three forms, which may represent three distinct isozymes. Comparisons of the amino-acid sequence of this SOD with those of SODs from other sources suggest that the crithidial enzyme is closely related to bacterial FeSOD of the alga Euglena gracilis. SOD activity has also been detected in L. donovani and Phytomonas spp., although its nature has not been determined [8,9].

In the present work, we confirm and quantify the SOD activity in three trypanosomes isolated from different plants: from phloem of the Coco nucifera (Hartrot disease) [11], from latex vessels of Euphorbia characias [12], from the fruits of Lycopersicon esculen-
Fig. 1. (A) Isoenzymatic profile with IEF of superoxide dismutase in total homogenates. (1) Cu/Zn-SOD. (2) E. coli Fe-SOD (Sigma). (3) phloem isolate. (4) fruit isolate. (5) insect isolate and (6) latex isolate. The homogenates were obtained as described in Table 1. The gels were stained [18] with 10 ml of the stock solution (27 ml of 50 mM potassium phosphate buffer, pH 7.8, 1.5 ml of l-metionine (300 mg, 10 ml⁻¹), 1 ml of NBT (1.41 mg, 10 ml⁻¹) and 0.75 ml of Triton X-100 1% (v/v), added with 0.3 ml riboflavin (0.44 mg, 10 ml⁻¹)) and illuminated with UV light until enzyme activity appeared as a colourless band on a blue background. (B) Isoenzymatic IEF (3–9) profile of superoxide dismutase in tomato fruit isolate subcellular fraction obtained as follow: after cell disruption by grinding with silicon carbide on a mortar, unbroken cells and nuclei were sedimented at 1500 × g for 10 min. From the resulting supernatant (post-nuclear fraction), the large organelle fraction was obtained after centrifugation (5000 × g, 10 min). The supernatant was centrifuged (14 500 × g, 10 min) to precipitate the small organelle fraction. Finally, the resulting supernatant was centrifuged at 1 40 000 × g for 1 h. The pellet was kept as the microsomal fraction, and the supernatant as the cytosolic fraction. Lane 1: homogenate; Lane 2: postnuclear fraction; Lane 3: large organelles; Lane 4: small organelles; Lane 5: microsomal fraction; Lane 6: cytosolic fraction. The gels were stained as described above. (C) Isoenzyme profile after native electrophoresis in a polyacrylamide 8–25% gradient gel. The isoenzymatic distributions of the phloem isolates (1), fruit (2), insect (3) and latex (4) are shown. (D–E–F) IEF (3–9) isoenzymatic profile of superoxide dismutase. (D) control; (E) revealed in the presence of 20 mM CN⁻; (F) revealed after incubation for 45 min with 5 mM H₂O₂, 0.1 mM disodium EDTA. Lane 1; E. coli Fe-SOD, lane 2; bovine erythrocyte superoxide dismutase Cu/Zn-SOD, lane 3; E. coli Mn-SOD (all control enzymes from Sigma), lane 4; homogenate from phloem isolate, lane 5; homogenate from tomato isolate, lane 6; homogenate from insect isolate, and lane 7; homogenate from latex isolate.

tum [13] and another isolated from the insect Fabric-tilis gonagra (Coreidae, 19/231G41, TCC-110) [14], all previously described as belonging to the genus Phy-tomonas [10,15,16].

The enzymatic activity was determined by two methods. One was based on NADP(H) oxidation in the presence of superoxide ions, generated by a chemical reaction [17]. This method is able to detect 2 ng of purified SOD, far below the limit of most traditional methods. The second method measures the reduction of NBT by superoxide ions [18].

The levels of SOD activity in the four isolates were not expected to differ greatly. The four isolates strongly resemble each other, not only morphologically but also biochemically [19]. However, the homogenates from the flagellates isolated from the insect presented SOD levels that were far higher than those from the other isolates (Table 1). This higher activity could be understood as an adaptation to the colonized microhabitat, or as a greater necessity to detoxify free radicals generated through as yet unknown metabolic pathways. In support of the first hypothesis, it is important to note that the phloem vessels of plants are considered to be microaerobic, or even anaerobic, environments, as opposed to body fluids (lymph, saliva, etc.) of insects. However, the Phy-tomonas used in the present work have been obtained by in vitro culture under aerobic conditions, for which the stress induced by the presence of oxygen should be similar to all strains. On the other hand, it is known that the metabolism of the trypanosomatids in the insect stage depends mainly on the catabolism of amino acids. This catabolism is only possible with the intervention of mitochondrial enzymes. Despite not having yet been described, it is possible that, as occurs in aerobic organisms with fully functional mitochondria, these metabolic pathways generate free radicals from oxygen.
Fig. 2. Distribution profile of superoxide dismutase activity in the fractions obtained by differential centrifugation (Fig. 1(B) and Ref. [5]). SOD activity was determined in the different fractions according to Paoletti and Mocali [17]. The relative specific activity (RSA, ratio between the percentage of total activity and percentage of total protein for each fraction, Y-axis) is plotted against the cumulative percentage of protein (X-axis, from left to right: nuclear, N; large organelle fraction, L; small organelle fraction, S; microsomal, M; and cytoplasmic fraction, C). As organelle markers, PK (A), PFK (B) and ME (C) activities were plotted in the same way, from *Phytomonas* isolated from phloem. Distribution profile of SOD activity from *Phytomonas* isolated from phloem (D), fruit (E), insect (F), and latex (G), respectively.
The presence of SOD activity in Phytomonas spp. was confirmed by isoelectric focusing in polyacrylamide gels (pI 3–9) of the four homogenates (Fig. 1(A)). No significant differences between them were observed. The enzyme appeared in two isoenzymatic forms, one with a pI of 4, while the other had a pI of 7.65. As in other trypanosomatids [20,21], the SOD activity detected by isoelectric focusing in polyacrylamide gels (pl 3–9) did not inhibit the activity, but that was abolished by incubation with hydrogen peroxide (Fig. 1(E)).

By native electrophoresis in 8–25% polyacrylamide gradients (Fig. 1(C)), superoxide dismutase activity was distributed into two zones, in agreement with the isoelectric focussing experiments. Native molecular weight reveal slight differences between the different isolates tested.

Finally, the distribution of the SOD activity, expressed as relative specific activity (RSA), is shown in Fig. 2. The SOD activity was found to be located mainly in the cytosol fraction of the four isolates, although a small amount of activity was also present in the small-organelle fraction (glycosomes). These results of differential centrifugation were confirmed by isoelectric focusing (Fig. 1(B)). The gel shows the distribution of the SOD activity in the tomato-fruit isolates, and similar results were obtained using the other three isolates (data not shown). This result is not unexpected, given that most metabolic processes for gaining energy are known to be located in the glycosomes and the cytosol within the genus Phytomonas [5,19].

In conclusion, Phytomonas spp. Isolated from different hosts, showed two different isoenzymes for superoxide dismutase activity, both with an iron-dependent nature. The activity was mainly located at the cytoplasmic level, but some activity could be associated to the glycosome. As with others protist pathogens, certain enzyme peculiarities that are not found in the host, might be exploitable for the design of specific inhibitors [22]. Since the SODs of protozoan parasites (including some members of the family Trypanosomatidae) are iron dependent and different from host SODs, protozoan SODs have been proposed as potential chemotherapeutic targets [23].

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References

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