CATALASE (CAT)

Catalase (hydrogen-peroxide: hydrogen-peroxide oxidoreductase, EC 1.11.1.6)

1) Introduction

Oxygen is a very reactive molecule that easily converts into reactive oxygen species (ROS), toxic compounds for the cell, inducing oxidative stress.

Within the cell, electrons are continuously moving from place to place through transporters, namely riboflavins and niacins. During the transport of electrons, if one of them approaches an oxygen molecule, it can accept it and turn into ROS, such as superoxide radicals, singlet dioxygen, hydroxyl radical and hydrogen peroxide (H_2O_2). Hydrogen peroxide is not, by itself, very reactive, however it can react with metal ions which ultimately convert H_2O_2 into hydroxyl radicals, the most critical known ROS, which can cause DNA mutations.

In plants, the formation of ROS, namely hydrogen peroxide, is more pronounced in biotic and/or abiotic stress situations such as pests, water stress, salinity, temperature and radiation, among others. Plants react to these stresses by activating defense mechanisms, notably by increasing the activity of enzymes such as catalase and ascorbate peroxidase, both responsible for the degradation of H_2O_2.

The catalase enzyme has a vital function in cells, as it catalyzes the decomposition of hydrogen peroxide in water and oxygen, always requiring the binding of two molecules of H_2O_2 to the active site to initiate the reaction:

$$2\ H_2O_2 + \text{CATALASE} \rightarrow 2\ H_2O + O_2$$

In most organisms, catalase has a heme group (iron ion) as cofactor. The catalases that have a heme group degrade the hydrogen peroxide molecule into two phases, oxidizing and alternately reducing the hemic iron in the active site.

The enzyme is a tetramer with 60 to 75 kDa, composed of four identical subunits, each having its active site accessible through hydrophobic channels. The four chain structures form an extremely stable structure resistant to a large amplitude of pH's, thermal denaturation and proteolysis. It is one of the most efficient enzymes found in cells: each catalase molecule can decompose millions of molecules of H_2O_2 per second.

In addition to the degradation of hydrogen peroxide, catalase also catalyzes ethanol degradation reactions, oxidation of methanol to formaldehyde, and is involved in the metabolism of tryptophan, glyoxylate and dicarboxylate.

2) **Enzyme extraction**

Extraction medium: phosphate K/Na buffer 50 mM, pH 7.6; EDTA 1 mM; 3% (w/v) PVP insoluble

Procedure (all at 4ºC)

- Homogenize ≈ 0,15 g FW leaf material (take note of the exact weight) with 3 mL of extraction medium, in a mortar.
- Centrifuge at 16 000 g X 2 minutes
- Collect the supernatant for an *eppendorf* micro tube and keep in ice

3) **Enzyme assay** (spectrophotometric determination)

Temperature =RT

λ = 240 nm

ε $H_2O_{2\ 240\ nm}$= 42,3 M^{-1} cm^{-1}

Assay medium: phosphate K/Na buffer 50 mM, pH 7.6; 2-20 mM H_2O_2; 50 μL crude extract (1 mL)

- Determine catalase activity, after thinking a little about the best way will be.
- Change de substrate concentration, between the range indicated above.
- Comment the results.
- Calculate the catalase activity of *Vitis vinifera* leaf found at different substrate concentrations, expressed in U g^{-1} FW
- Compare the results obtained