MOLECULAR AND PHYSIOLOGICAL ASPECTS OF PLANT PEROXIDASES

ISOPEROXIDASES, MARKERS OF SURROUNDING AND PHYSIOLOGICAL CHANGES, IN SITU IN LEAVES AND IN VITRO IN CALLI OF PEDILANTHUS TITHYMALOIDES L. VARIEGATUS, EUPHORBIACEAE: CELL COMPARTMENTATION AND POLYFUNCTIONALITY, CONTROL OF ACTIVITY BY PHENOLS AND SPECIFIC ROLES

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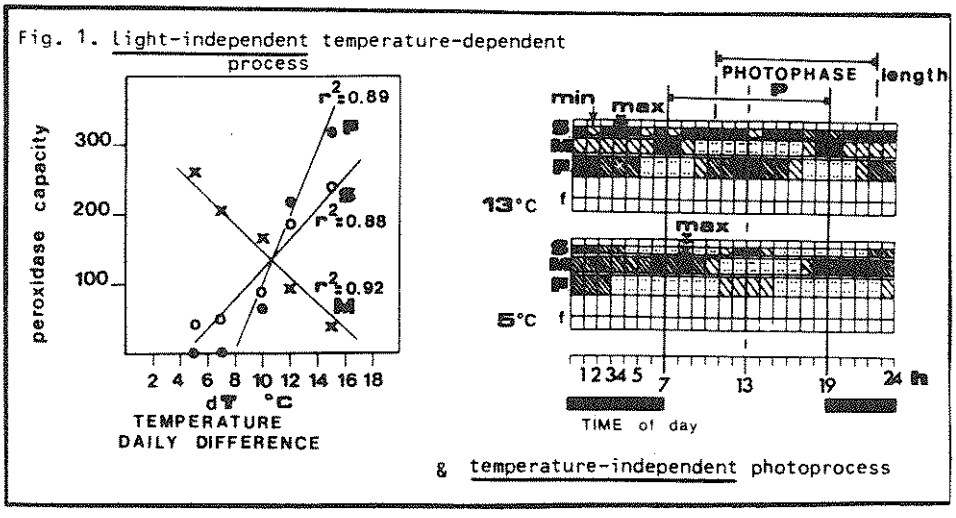
Introduction

When you purify enzymes and when you work in a "test-tube" with "pure" chemicals you are making chemistry, when you work with crude extracts or partially purified extracts you are attempting to make biochemistry, and if you want to make physiology you must always have in mind that ,whatever the purity of your extracts, you are always looking only at the phenotype and you will never know the genotype (except if you look at DNA). And because all peroxidases are actually glycohemoproteins their biosynthesis is submitted to the variability of the protidic and the glycosidic machineries. It could be said that every cell is enclosed into and balanced between "le hasard et la nécessité". "La nécessité" is the need for the cell to make enzymes to fulfil functions to make the machinery work. "Le hasard" is the variability of the post-transcriptional and post-translational events in protein biosynthesis, it is the variability of the really active enzymes which act in building the glycosidic parts and in their linking to the proteic parts of the molecule.



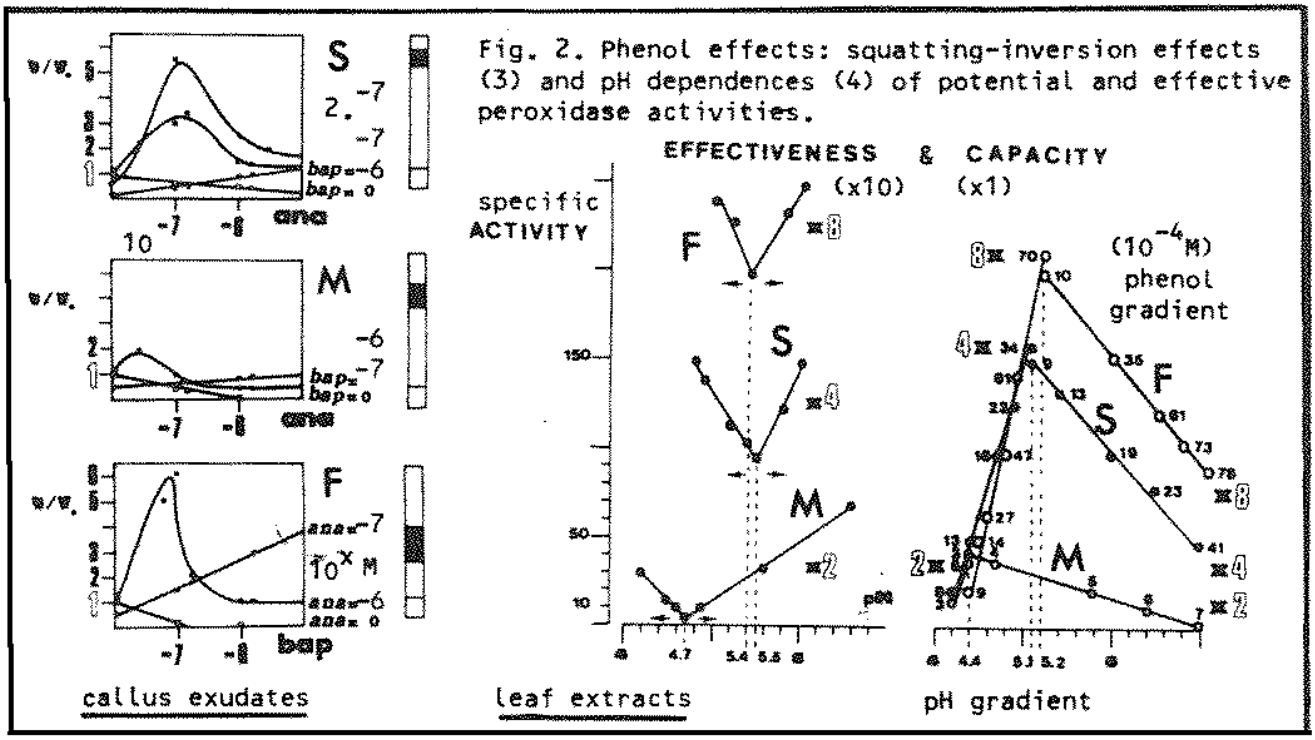


Fig. 2. Phenol effects: squatting-inversion effects (3) and pH dependences (4) of potential and effective peroxidase activities.

Three groups of isoperoxidases were extracted from leaf tissues (1) and calli of Pedilanthus tithymaloides L. variegatus, a Crassulacean Acid Metabolism plant, and characterized by their specific activities at different pH and temperatures, their isozyme patterns and physicochemical properties (2). The peroxidase capacity and phenolic content changes could be explained both through the 2 same processes: a temperature-independent photoprocess, responsible for the position of the day and night-related peroxidase bursts, and a light-independent temperature-dependent process, responsible for the burst amplitudes (Fig. 1).

All isoperoxidases, previously shown to be glycohemoproteins (2), using in vivo vital stainings of cells in optimal pH and temperature conditions (2) were associated to cell compartments and exhibited in vitro another more specific but also more labile activity (Table 1). Kinetic studies with callus culture exudates indicate squatting-inversion effects (4) between phenols and auxin ana or cytokinin bap, which give activation or inhibition of peroxidase activities depending on their respective concentrations and the enzyme types; with leaf extracts, a pH feedback control of peroxidase activity, with steady-state potential (capacity) and effective (effectiveness) pH dependences (5), indicates a membrane immobilization of peroxidases depending on phenolic content and isozyme types (Fig. 2).

The light-dark control of peroxidase related activities is effective through both dependent processes: a phenol- and a pH-feedback controls of nonperoxidative oxidation properties. The oxidation and peroxidation activities could take place at different sites of multi-isozyme complexes (3) with competitive effects of phenols explained either by their binding at these sites or by their pH-induced polar changes in membrane bindings.

Conclusion

I think we often argue for just nothing because actually I think that there is at least 2 types of peroxidases. The one I could name the "true" peroxidases and the other I will name the "dual" peroxidases.

What are true peroxidases? They are enzymes that only act in peroxidasic reactions with H_2O_2 and other kind of substrates (amine, phenol...). What are dual peroxidases? These are enzymes that act in other enzyme reactions (for example:catalases, auxinoxidases, polyphenoloxidases) but because they are glycohemoproteins they can act also as peroxidases!

Actually I do not know why, where, how and when true or dual peroxidases act in cells, but I can make some remarks. Auxin-oxidases, polyphenoloxidases... surely need a more specific restricted cellular surrounding and have less steric freedom than true peroxidases, they have thus more labile activities and if the stericity or the surrounding is deterred these enzymes lose their most specific activity and it still remains a less specific one but more resistant one, the peroxidasic one. I do think that "peroxidase" is often the "last face" of a glycohemoprotein enzyme, just because it is a resistant face. I dont know in situ which activity is really expressed or more functionally important. But I think the peroxidasic state could be really used

Table 1. Cell compartmentation and polyfunctionality of the isoperoxidases.

ELECTROPHORETIC ISOZYME TYPES	OPTIMAL TEMPERATURES	CELL COMPARTMENTATION	ASSOCIATE ACTIVITIES
slow migrating type: S	increasing temperatures	spongy layer, circumamyloplast	catalase rather than peroxidase in leaves, peroxidase rather than than catalase in calli
medium migrating type: M	lowest temperatures	palissade layer, circumchloroplast	polyphenoloxidase in leaves
fast migrating type: F	highest temperatures	guard cell tonoplasm	auxinoxidase in leaves and calli polyphenoloxidase in calli

as marker of the disorder stericity of these dual enzymes (like the pH is used to measure the electropotential state of the cell) and it is surely much more easy to measure a peroxidasic activity rather than a Ca^{2+} efflux or the fat content of membranes.

For example with my plant material it can still exist "true" peroxidases but they are always located within the cell walls in situ and I did not extract these activities because they could not be extracted, or because they had lost activity, and into extracts without cell wall but with membrane fractions it can only be found "dual" peroxidases.

I have made a review in the literature about conformational changes, dual side-effects of enzymes, ligand stericity, membrane binding and release of peroxidase-like enzymes that is in agreement with those findings and some falsification tests could be built to challenge...but this is another story.

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