INDIRECT MECHANISMS OF ACTIVATION OF PLANT PROTEASES AS A MODEL FOR CANCER INVASION

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In cancer invasion, protease activation ranges among the most decisive features. But it is not the matter of overexpression of a specific proteinase -as in the case of the herpes virus induced trypsin- but rather; the hydrolytic activation cover the whole endoproteinase expression; catepsins, chollagenases of the Zinc containing MMP, plasmin, plus trypsin and the whole arsenal of (non proteinase) spreading factors as hyaluronidase and lysozyme. All together activated in the whole as an -impressive- phenomena of **derepression**, the loss of an inhibitor, or "brake removal". It is not the question of the specific prevention of Rouss sarcoma malignization by fetuin, inhibition of MMP collagenases by TIMMPs, or that of hyaluronidase by other mucoproteins, but, rather by the common mechanism of activation through a lowering in pH and redox potential as corresponds to the impairment of respiratory enzymes postulated by Warburg. Terminal oxidases furnish the most regulatory device of pH and rH homeostasis.
Seeds germination is a violent process based in protease activation, amplified to a total reserve hydrolysis (proteins starch and fat) and, finally, rebuilt in a well differentiated plant.

Cancer invasion runs through a parallel way: increased expression of proteolytic enzimes and loss of its specific inhibitors.

Most plants proteinases belongs to the Papainase group -SH dependent or "cysteine-proteinases" are inactive in the oxidiced form (disulphide). Cyanide prevents oxidation and, therefore activates the enzyme through an indirect mechanism of "inhibition of an inhibitor" or, in other words: inhibition of the (copper containing) terminal oxidase. Vernalization of seeds is based in the gaion in germinative power and seedling development by cold treatment. It the stimulating effect of the snow in wheat seeding and is based too in the indirect activation of plant proteinase through the selective inhibition of terminal oxidase by low temperature (as could be demonstrated by browning bananas kept in the refrigerator)

Methodology

As a source of experimental evidence about the selective inhibition of terminal oxidases as the mechanism of proteinase activation we undertake the following line of experiments: Chemical vernalization of seeds with KCN, treatment and activation of proteolysis in Agave extracts by some strong chelators: Kupferron, EDTA and Zn- EDTA solutions. All the experiments give very positive results with the peculiarity of Zn as an activator of the EDTA effects. A source of cytological confirmation was obtained from the roots tips (onion and Vicia faba) with EDTA, cyanide (Fig. 2) and the very specific copper chelator salicylaldoxime (SAO) (Fig. 1). As a common results for the said agents we obtained at 0.02 mM concentrations an, impressive, pattern of selective attack compatible with papainase-like activation: Bands spliting of genetic material give a peculiar pattern of symmetric twin chromatide cutting, with a marked effect in helix uncoiling and an -impressive- cell swelling (till 4 fold the original size) affecting also to interface cells (2,4).

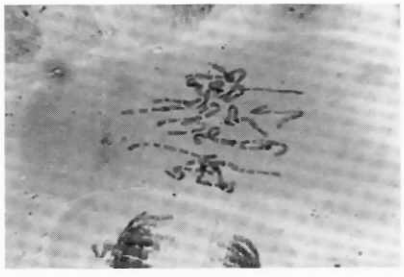

Fig. 1
0.0.2 mM salicylaldoxime:

Fig. 2
0.02 mM cyanide

Fig. 3
2.0 mM cyanide

All data suggesting an increase in osmotic pressure, as a very direct effect of an increased hydrolysis; uncoiling means a loss of the histone sheat integrity, while the chromatine fractures were more indicative of a phosphate bound cleavage (that could be attributed to the esterase action of papainase or, even, to a specific phosphatase or DNAase). Taking in the whole all these effects, give the pattern that would correspond to that of a the germinating seed: "Blocking of terminal oxidases, increased activity of hydrolytic enzymes and, thus increased osmotic pressure (mainly from carbohydrate splitting), till to accomplish the "chain reaction" as would correspond to an increase in free -SH groups. As an additional confirmation, this low (0.02 mM) concentration of salicylaldoxime (SAO) protects the roots toward the radiomimetic effects of radiation (2.000 r cesium source) and, that could seem more amazing, is that, while at 0.5 mM concentration, SAO increases the, cesium-induced-radiomimetic effects (4), in full concordance with the radiomimetic effects of high (2.0 mM) cyanide concentrations (anafasic bridges, fractures, satellites, agglutination) (Fig. 3).

Fig.4
Flowering of the "Centenary plant"*(Agave americana)* This monocarpic plant living from 10 to 30 years suffers a very short flowering process till a complete exhaustion (more than 1.000 kg sucrose are movilyzed from the basal leaves). Very few weeks are expended till plant dies.

Results

We would point out, as the most significative results, the increased papanaise expression in the agave ("century plant") flowering process with a concomitant decrease in peroxidase activity (3) and to recall, again, the attention about this -extremely violent process -in which the most outstanding feature is the complete consumption, till death, of the mother plant, a true **commotion** that in some way mimifies the brutal activation of proteinases in highly undifferentiated tumours. The equivalence of cyanide or EDTA with the germinating-vernalizating stimulus (1) allow to the concept of identity of effects between: photoperiod, cold stimulus an biochemical regulators and to translate it at the molecular level and we point to the well known facts that as: cytochrome oxidase undergoes activation from sun light, it is strongly inhibited by salicylaldoxime but not by other highly specific copper chelators as bathocuprein and, finally that is the first oxygen acceptor of the respiratory chain (also in animals).

Conclusions

From the foresaid it seems very reasonable to postulate that the low expression of cytochrome oxidase in the highly invasive (anaplasic) tumours could be considered as an important factor for its extreme status of differentiation from which the most apparent feature is the increases proteolitic activity. That is most important is that the natural proteinase inhibitors (doted of strong antiinvasive power) exert its specific activity through the mechanism of autoactivation of the metalloproteinases (5); in other words, a mechanism very closely related to that of the ZYMOGENS activation: in the case of trypsinogen, just TRYPSIN is the activator of both trypsinogen and chemotrypsinogen through an autocatalitic process or chain reaction. Plasminogen is activated by thyocianate, but not natural activator has been isolated. The customary way to plasminogen activation to plasmin is also a self activation mechanism or removal activating inhibitor.

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