PLANT LYSOSOMES: ASPECTS AND PROSPECTS

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ABSTRACT

Lysosomes play an important role in the animal biology and pathology. Although initial reports on the existence of lysosomes in plant cells did not find general acceptance, structural and functional analogy of plant vacuoles with animal lysosomes established that vacuoles represented lysosomes in plant cells. Recent evidence indicates active involvement of lysosomic vacuoles of invading cells in the spread of infection within the plant tissue, and it presents the possibility of arresting such a spread through manipulation of host lysosomal membrane.

INTRODUCTION

LYSOSOMES were discovered in early 1950's by de Duve and his collaborators[1] in animal liver cells. Their work led to the characterization of lysosomes as sedimentable intracellular vesicles surrounded by a single-membrane containing hydrolases. There were repeated attempts to localize/characterize lysosomes in plant cells. In the beginning there were contradictory reports: while Berjak[2] claimed to have observed lysosomes in the root cap cells of *Zea mays* through electron microscope, Matile[3] suggested that the vacuoles represented lysosomes in plant cells. Since then the analogy of plant vacuoles with animal lysosomes has been established[4-11]. However, their role in plant pathology has not been discussed since the review of Wilson[12] appeared in 1973. The present article attempts to collate the work done in recent years on lysosomic nature of plant vacuoles and their possible role in disease spread/control.

LYSOSOMAL TENET IN PLANTS

It is generally agreed that the hydrolytic enzymes in plant cells are compartmentalized; but there were different opinions about the location of these compartments. Originally plant sphaerosomes were thought to contain lysosomal enzymes[13-18], but there was confusion over the enzyme content and also over the nature of the sphaerosomal membrane[19-21]. This confusion was due to the diversity of sphaerosomes in different plants and tissues. Ultrastructural study has shown sphaerosomes with[16] and without[22] membranes. These discrepancies were subsequently explained by Schwarzenbach[23, 24] who reported that the immature sphaerosomes of endosperm in *Ricinus communis* were bound by unit membrane whereas the matured sphaerosomes were not. Recent reports on the packaging of hydrolytic enzymes in small vacuoles raise the question as to whether early sphaerosomal particles were in fact small vacuoles.

There was another school of thought, contending that Golgi vesicles in the plants contained acid phosphatase and other hydrolytic enzymes[25, 26] and hence these vesicles might be considered as lysosomes. However, it was later demonstrated that the greatest concentration of hydrolytic enzymes was packaged in small vacuoles[27-30] and hence they should be called as lysosomes. But Gahan[31] opined that the term sphaerosome should be used for the plant lysosomes which are single membrane-bound bodies containing hydrolytic enzymes in plants. The proposal was premature and later a number of similarities were found between animal lysosomes and plant vacuoles and consequently the importance of vacuoles as plant lysosomes was emphasized. Also the origin of primary lysosome from endoplasmic reticulum was pointed out. It has recently been demonstrated that the genesis of vacuoles is a lysosomal multistep process in which Golgi-associated endoplasmic reticulum (GERL) produces provacuoles in which lysosomal enzymes appear to be concentrated and packaged; these GERL derived provacuoles cooperate in the programmed cellular autophagy, leading to young vacuoles, which swell and fuse together into a few large matured vacuoles[32]. This indicates that lysosomes should not be treated as an organelle but it is a system in which Golgi-bodies, endoplasmic reticulum, provacuoles and vacuoles are closely associated.

In order to confirm the contention that vacuoles are plant lysosomes, it would be appropriate to apply the criteria: (i) presence of two or more membrane-bound

hydrolases and (ii) latency of these enzymes; used for detection/characterization of animal lysosomes on plant vacuoles and see if these criteria are fulfilled by plant vacuoles; if so, this will then in turn establish their analogy with animal lysosomes.

(i) *Presence of membrane-bound hydrolases*

A number of hydrolytic enzymes in the vacuoles have been demonstrated[32-41]. Acid hydrolases have also been detected in plant tissue extract[5] and the evidence suggests that these hydrolases are localized within a separate cell compartment. Major portion of hydrolases could be sedimented and structures containing these hydrolases were fractionated. They appear to be small (0.1 to 1.5 μm diameter), heterogeneous, membrane-bound structures which could often be resolved into several sub-populations[35-40]. Lysosome-like structures from maize root-tip cells were separated into heavy and light fractions which differed in their relative proportions of hydrolases, transaminases and oxidoreductases[42]. Although the origin of many of the structures isolated in the above studies was not determined, it was suggested that some of the structures were provacuoles[42-47]. Many hydrolases have been detected in the vacuoles from a variety of cells like algae[48, 49], yeast[7] and numerous higher plant tissues (table 1). Six hydrolytic enzymes like acid protease, carboxypeptidase, phosphodiesterases, RNAse, phytase and β-glucosidase were reported in the isolated vacuole from castor bean endosperm[38]. Boller and Kende[41] have shown the intracellular activity of several acid hydrolases, *i.e.* α-manniosidase, nuclease, phosphatase and proteinase in the vacuoles of the higher plant tissues. More recently several other hydrolases have been demonstrated to be present in the vacuoles of the higher plants[50-56]. It can thus be concluded that the central vacuoles of the higher plant cells have an enzyme composition analogous to animal lysosomes.

(ii) *Latency of hydrolases*

Apart from the presence of hydrolases in vacuoles, the postulation of lysosomal nature of vacuoles requires the demonstration of latent activity of hydrolases *i.e.* the substrate should not be attacked by the hydrolases unless the membrane of isolated vacuoles are disrupted. Gahan[57] has already shown the concentration of acid phosphatase in lysosome-like particles in root meristem cells of *Vicia faba* and the enzymes were also solublized by treating the cells with triton X-100, a detergent known to destroy cytoplasmic membranes. Furthermore the latency has also been demonstrated in hydrolases of isolated vacuoles[58].

The demonstration of several tonoplast-bound hydrolases and latency of their enzymes establishes the structural analogy of plant vacuoles with animal lysosomes. The functional analogy can also be shown

Table 1 *Acid hydrolases in purified, intact vacuoles from mature higher plant tissues*

Tissue used	Hydrolases associated	Ref
Hippeastrum petals	RNase, DNase, acid phosphatase	33
Lycopersicon esculentum leaves	Acid phosphatase, carboxypeptidase	34
Bryophyllum daigremontianum leaves	Acid phosphatase, RNase	35
Beta vulgaris root storage organ	Acid phosphatase, acid invertase	36, 37
Ricinus communis endosperm	Acid phosphatase, acid protease, phosphodiesterase, RNase, phytase, β-glucosidase	38
Armoracia lapathifolia roots	Acid phosphatase	39
Sorghum bicolor leaves	Dhurrin β-glucosidase	40
Nicotiana tabacum pith-derived cells in culture	Nuclease, β-Fructosidase (invertase)	41
Tulipa sp. petals	α-galactosidase	41
Ananas comosus leaves	Proteinase, α-galactosidase	41
Nicotiana, *Ananas*, and *Tulipa*	α-Mannosidase, β-N-acetylglucosaminidase, acid phosphatase, phosphodiasterase	41
Triticum and *Zea mays* leaves	Proteases	50
Hordeum sp. leaves	Acid proteinase, α-Mannosidase, Acid phosphatase	51
Hippeastrum and *Triticum* leaves	Proteases	52
Triticum sp. leaves	Proteases	53
Bryophyllum daigremontianum	Acid phosphatase, α-Mannosidase, α-Galactosidase	54
Hordeum sp. leaves	Endoproteinases EP_1 and EP_2	56

if the functions of animal lysosomes can be demonstrated to be performed by plant vacuoles. It is known that acridine orange, an antimetabolite, when applied in excess tends to accumulate in animal lysosomes[59 60]. Through such accumulation acridine orange is effectively kept away from the metabolic sites. In other words adverse effects of acridine orange are detoxified by lysosomes. Parallel experiments on this line have been conducted on plant cells in which relatively simpler cells of *Rhoeo* staminal hair, when exposed to acridine orange solution, were found to accumulate the dye which was detected by the appearance of characteristic red flourescent particles under fluorescence microscope[61]. The deep-seated meristematic cells of root tip of *Vicia faba* too accumulate the dye in the vacuoles which have been observed under fluorescence[61] as well as electron microscope[61, 63]. It is now clear that plant vaculoes perform similar functions which are done by lysosomes in animal cells[64]. This establishes functional analogy of plant vacuoles with animal lysosomes[65]. It is, therefore, justified to corroborate that plant vacuoles represent lysosomes in plant cells[66, 67].

LYSOSOMES IN HOST-PARASITE INTERACTION

Lysosomic vacuoles in the host and parasite play important role during the course of host-parasite interaction through their hydrolytic enzymes. The parasite may labilize its enzymes to degrade the host into utilizable nutrients. On the contrary, hydrolytic enzymes of the host could also degrade the parasite. These interactions can be grouped as follows:-

(1) *Prepenetration interaction*: Immediately after contact between host and parasite, a battle between the enzymes of the host and parasite lysosomes might be waged with one perhaps winning by its ability to degrade or inactivate the enzymes of the other.

(2) *Penetration*: The parasite may penetrate the cell wall of the host through mechanical or enzymatic means. The mechanical penetration invariably causes injury with concomitant increase in the acid phosphatase presumably released from the lysosomes[68]. If the penetration of the parasite is to be facilitated through enzymatic means, the lysosomes would be of primary importance; as for example, the concentration of lysosome-like organelle in the penetration peg of lichen haustorium of *Parmelia sulcata* has been reported[69] and it was postulated that these bodies helped to provide hydrolytic enzymes for the cell wall penetration. Similarly the migration of lysosome-like bodies into appressorium of microparasite *Mycotypha microspora* on *Piptocephalis virginiana* has been reported by Armentrout and Wilson[70]. The increase in the concentration of the acid phosphatase in the haustorium and sheath after penetration lends support to the contention that the lysosomic vacuoles are instrumental in supplying the hydrolytic enzymes for the digestion of the host cell wall. Further, the partial or complete digestion of the host cell wall through enzymatic dissolution[71–75], also indicates the participation of lysosomes in the cellular penetration. This is corroborated by the demonstration of enzymatic disolution of the waxes, cuticle, cellulose, pectic substances and hemicellulose by many biotrophs[76].

(3) *Cellular injury*: Lysosomic plant vacuoles respond to cellular injury through release of the hydrolytic enzymes. The increase in the ribonuclease, phosphodiesterase, phosphatase and peptidase enzymes in response to the leaf excision, is attributed to the disruption of lysosomes[68]. The freeze injury is also known to cause an increase in the activity of hydrolytic enzymes presumably due to damage of lysosomes[77]. Likewise the increase in the acid phosphatase activity has been recorded during the ozone exposure of mesophyll cells of Ponderosa[78].

LYSOSOMES IN PLANT DISEASE RESISTANCE

Hypersensitivity is a phenomenon of extreme resistance accomplished through extreme susceptibility in which a host plant or a non-host plant is more than normally sensitive to an infecting agent and reacts with an early tissue necrosis. It has been reported to be prevalent against viruses[79], bacteria[80], and fungi[81]. The strong correlation of hypersensitive reaction with disease resistance was discovered by Ward[82] and was later accentuated by Stakman[83] who observed rapid killing of cells around the site of penetration of stem rust of wheat. Until recently hypersensitivity was considered to be the most common defence mechanism and the most widely distributed form of disease resistance[84]. The main characteristic that distinguishes this reaction is its rapidity in response to invasion which becomes apparent within a couple of hours of inoculation. The specific detailed mechanism underlying this phenomenon still remains elusive; however, the evidences present in the literature indicate the active participation of the lysosomic vacuoles.

The first symptom associated with the development of hypersensitive reaction against viruses is the change in the permeability of cell membranes[85], as a result electrolytes and water leak out. The production of ethylene is also enhanced[86, 87]. The formation of necrotic spots is accompanied by an increase in phenolic and flavanoid compounds and their metabolizing enzymes[88]. As a result of excess of phenols the necrotic lesions may also increase[89]. Most of these metabolic changes are detectable during the appearance of tissue necrosis; however, ethylene production, the increased electrolyte leakage and an increase in phenylalanine ammonia lyase activity precedes. It is hard to decide with certainty, as to which is the primary event, the end result of which is cell and tissue necrosis and rapid loss of water[84].

The mechanism of hypersensitive response in the case of fungal biotroph and a necrotroph is similar to viral local necrosis. The membranes play a key role[90]. The alteration in the structural and metabolic integrity of membranes causes decompartmentalization followed by uncontrolled mixing of enzymes and substrates otherwise normally compartmentalized in subcellular bodies.

The hypersensitive response, in case of bacterial pathogens, occurs when high concentrations of incompatible bacteria[80, 91] are infiltrated into tissue of non-host plant. Rapid cell collapse associated with cell necrosis is the characteristic of the hypersensitive reaction against bacteria. Alterations in the host cell permeability and electrolyte loss occur about 6 hr after inoculation[92]. The membranes of the cell organelles then become disorganised. Visible symptoms such as necrosis, tissue collapse and dessication develop within 6 to 8 hr after inoculation and the whole process is completed within 18 hr.

On the basis of available results discussed above, the general symptoms associated with hypersensitive reaction against viruses, bacteria and fungi are: the change in the cell membrane permeability, electrolyte losses, granulation and browning of the cytoplasm[93, 94] which ultimately result into necrosis of few cells around the site of contact between the host and pathogen. Various light and electron microscopic studies have revealed that the host cell necrosis precedes the death of invading fungi by several hours[95-97] and thus the inoculum along with the necrotic lesions is also killed. In the light of lysosomal role attributed to plant vacuoles the above observations may be explained by assuming that the change in cell membrane permeability is accompanied by a change in permeability of vacuolar membrane too. Amidst altered tonoplast permeability the host cell vacuoles release hydrolytic enzymes in the cytoplasm which start digesting their own cell substrates leading to the appearance of granulation and browning of the host cytoplasm. Ultimately these hydrolytic enzymes digest away a few host cells surrounding the point of contact and may also act on the pathogen through the labilization of their lysosomic membrane. On the basis of the above interpretation it is justified to assume that plant lysosomes play an important role in hypersensitive disease reaction. The killing of cells can be brought about so rapidly only by hydrolytic enzymes released from lysosomes. It is proposed that if the factor(s) responsible for inducing hypersensitive disease reaction (change in lysosome membrane permeability) can be identified, the hypersensitive reaction can be induced at will which would, in turn, help control various fungal, bacterial and viral diseases.

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NEWS

CHEST X-RAYS: OVERUSED?

. . . "Routinely taking chest X-rays of hospital patients, even when they show no symptoms of chest disease, has little medical value and adds as much as $1.5 billion to the cost of health care nationwide, a new study by physicians at the Long Beach Veterans Admin. Medical Ctr. has concluded. . . . Their report noted that about 52 million chest films are taken annually in the US, making the procedure the most frequently conducted of all X-ray examinations. About 30 million of them were for purposes that yielded little medical information that could not be gleaned from clinical histories or by other means. . . . "

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