[Plant Signaling & Behavior 4:4, 324-327; April 2009]; ©2009 Landes Bioscience

Article Addendum

Plant growth promotion by 18:0-*lyso*-phosphatidylethanolamine involves senescence delay

A. Keith Cowan

Institute for Environmental Biotechnology; Rhodes University; Grahamstown, South Africa

Key words: invertase, *lyso*-phosphatidylethanolamine, phosphatidic acid, phospholipids, senescence

***Lyso*-phosphatidylethanolamine (LPE) is a minor membrane glycerolipid and egg-derived 18:0-LPE is used commercially as a plant bio-regulator to improve plant product quality. Physiological responses initiated by LPE treatment included delayed senescence in leaves and fruits, improved shelf-life of products post harvest, and mitigation of ethylene-induced process. However, the biochemical and molecular mechanisms underlying LPE-induced responses in plants and harvested plant parts remain unclear. In this paper, commentary is presented on the effects of LPE at the biochemical level in an effort to develop a mode of action. Implications, although tentative, are that LPE exerts its effect via lipid-protein interaction to attenuate ethylene (ETH)-mediated responses and impact pathogenesis-related proteins which together delay senescence progression.**

In all plant production systems from nursery to greenhouse/field, product harvest and packing, transport and storage, to resale and final consumption an important criterion is the ability to manage the senescence processes. Senescence occurs intrinsically as a normal part of the course of plant and plant product development but can be induced by extrinsic factors such as climate change, stress (nutrient, water, light, temperature, etc.,), pests and pathogens, mechanical events (harvest, transport, storage, etc.,), and at any point in the production chain. In fact productivity of any biological system, however measured and evaluated, is constrained by the time to onset of senescence. Once initiated, reserve mobilization and nutrient cycling which are integral components of the plant senescence process, are for the most part irreversible. It is the irreversibility of senescence that compromises crop production and product quantity/quality. Since senescence is an inevitable event most studies have concentrated on the development of management mechanisms to mitigate its deleterious effects at every step in the production chain. These include use and selection of appropriate cultivars with the desired traits, management of light penetration and utilization, control of fertilizer and irrigation schedules, use of fungicides and pesticides, and application of synthetic and natural plant bio-regulators. Post harvest, the use of step-down temperature acclimation, controlled atmosphere storage, inhibition of ethylene (ETH) production and/or sensitivity and prevention of pathogen proliferation have all yielded positive results. More recently, the introduction of molecular biology has seen the emergence of technologies based on autoregulated cytokinin production and/or the stay-green phenotype both of which cause senescence delay and appear to improve product yield and quality.[1-3]

Both intrinsic and extrinsic stimuli are coupled to response mechanisms wholly or at least in part through changes in phospholipid turnover and metabolism. In particular, changes in phosphoinositides, phosphatidic acid (PA), diacylglycerol pyrophosphate, *lyso*-phospholipids, and phospholipases A_2, C and D are amongst the key lipid signaling components affected.[4] *Lyso*-phospholipids are present in biological membranes in trace amounts and levels of these change rapidly and dramatically on exposure of plants to a range of biotic and abiotic stimuli. *Lyso*-phosphatidylethanolamine (LPE), a minor glycerolipid present in extra-chloroplastic membranes, is formed from the parent phospholipid, phosphatidylethanolamine (PE) by the action of phospholipase A_2. Although several molecular species of LPE have been identified as endogenous plant compounds (e.g., 16:0, 16:1, 18:1, 18:2 and 18:3)[5] only the 18:0 species has been used to study the effect of exogenous LPE on plant developmental processes.

PA, and Not PLD, is the Signalling Intermediate Affected by LPE

Treatment of plants and plant parts with 18:0-LPE delays fruit softening when used postharvest, mitigates the defoliation action of ethephon, and delays leaf and fruit senescence in tomato, cranberry, potato and grape.[6-9] Based on these and other similar observations a specific role for LPE as a mediator of aging and senescence processes in plants and plant parts was proposed.

Correspondence to: A. Keith Cowan; Institute for Environmental Biotechnology; Rhodes University; PO Box 94; Grahamstown 6140 South Africa; Tel.: +27.46.6222.656; Fax: +27.46.6222.656; Email: a.cowan@ru.ac.za

Submitted: 02/15/09; Accepted: 02/16/09

Previously published online as a *Plant Signaling & Behavior* E-publication: http://www.landesbioscience.com/journals/psb/article/8188

Addendum to: Hong JH, Chung G, Cowan AK. Delayed leaf senescence by exogenous *lyso*-phosphatidylethanolamine: towards a mechanism of action. Plant Physiol Biochem 2009; In press; DOI:10.1016/j.plaphy.2008.12.014.

In fact, it was argued that the decrease in electrolyte leakage in LPE-treated leaves and fruits postharvest arose as a consequence of the protective effects of LPE on membrane integrity by inhibition of lipid breakdown. Furthermore, in view of reports that PLD activity increased with senescence progression and that PLD activity together with leaf senescence were stimulated by abscisic acid (ABA) but attenuated by kinetin, it was mooted that a potential mode of action of LPE might be inhibition of PLD. Detailed biochemical investigations suggested LPE-specific inhibition of PLD (presumably PLDα) activity, an enzyme involved in the selective degradation of membrane phospholipids.[10] Inhibition of PLD activity in these crude cell-free extracts was concentration dependent and increased with length and desaturation of the LPE acyl chain. Although *lyso*-phosphatidylinositol also inhibited PLD activity in vitro, other *lyso*-phospholipids such as *lyso*-phophatidylserine, *lyso*-phosphatidylglycerol, and *lyso*-phosphatidic acid did not. It was also demonstrated that 18:0- and 18:1-LPE were potent inhibitors of ETH production and delayed senescence in cranberry fruit whereas the 14:0- and 16:0-chemical species displayed little or no effect. The authors concluded that *lyso*-phospholipids such as LPE are catabolites of hormone-activated PLA_2 and that they likely serve a second messenger function in plants to modulate activity of PLD. Circumstantial evidence to support this hypothesis emerged from studies using tomato expressing antisense PLDα in which the ETH climacteric was delayed and fruits showed increased firmness and red color.[11] Precisely the same responses were observed following application of LPE to tomato fruit.[12]

Unfortunately corroborative evidence for the above conclusion was not readily forthcoming. Also, several reports refuted the existence of PLD inhibitors and in particular that of LPE in this role. Lastly, pharmacological studies demonstrated that PLD activity or more accurately PA, plays a pivotal role in cytokinin signaling and thus in senescence delay.[13] Using a fusion of the cytokinin-responsive *ARR5* gene promoter and the *GUS* reporter gene it was shown that reporter gene activity in P_{ARR5}-*GUS* Arabidopsis seedlings was specific for cytokinin and was attenuated by *n*-alcohols. This particular study provided strong support for PA as the primary signaling molecule particularly as *n*-alcohols, which stimulate rather than inhibit PLD activity cause accumulation of phosphatidylalcohols and not PA, reduced GUS activity and *ARR5* transcript accumulation.

A Role for LPE in the Mitigation of Senescence Progression

To account for the senescence-delaying effects of exogenous LPE it seemed more likely therefore that LPE interacted either with the product of PLDα-catalyzed reactions, PA, or with downstream targets of PA to slow fruit ripening and promote senescence delay. To investigate this possibility more rigorously we chose to screen for potential LPE target proteins using a modified radish cotyledon bioassay. By manipulating the time to onset of senescence and by eliminating the confounding influence of wounding a range of senescence-associated enzymes (and their metabolic products) was analysed.[14] Results showed that exogenous LPE routinely induced activity of phenylalanine ammonia lyase (PAL) and acid invertase, (INV). More detailed studies revealed that the response was dose dependent. Also, the rise in PAL activity coincided with a decline in phenolic acid content and a rise in sinapine and lignin. Increased insoluble Ac INV by comparison occurred coincident with a reduction in sucrose concentration while levels of glucose and fructose were unaffected. Thus, and based on the LPE-induced change in sucrose/hexose ratio, it was proposed that applied LPE acts to co-ordinate carbohydrate partitioning locally to fulfil anabolic respiratory requirements usually associated with the propagation of systemic wound and stress responses.[15]

Source-sink relations and reserve mobilization are key processes in both developmental and stress-induced ontogenic transitions and in the senescence process.[16] Generally speaking, allocation of resources is governed by changing metabolic gradients established by supply and demand that arise as a consequence of the dynamic between anabolic and catabolic respiratory processes. Of the carbohydrate-metabolizing enzymes, Ac INV contributes significantly to increased sugar availability for the establishment of metabolic sinks[17-19] it has been identified as an integral component of the molecular mechanism of cytokinin-mediated senescence delay[20] and activity is induced on exposure of tissue to LPE.[15]

PA is apparently involved in the induction of cell death but almost nothing is known about its role in senescence. If we assume that senescence is similar to programmed cell death and that the two are fully synchronous as suggested by van Doorn and Woltering [21] it could be argued that PA is central to both. The significance here is that *lyso*-phospholipids possess detergent-like activity and application of LPE to plant tissues could elicit a wound-like response to increase the tissue concentration of PA both locally and systemically and enhance senescence progression. Using a modified leaf-disc bioassay we were able to demonstrate that exogenous PA initiates a type of programmed cell death-associated leaf senescence and that endogenous PA is indeed an intermediate in hormone-mediated (i.e., ABA and ETH) senescence progression.[22] LPE was unable to reverse senescence induced by either ABA or ACC (the immediate precursor to ETH). However, exogenous LPE completely reversed PA-induction of phosphatidylglycerol hydrolase and chlorophyllase, negated the PA-induced decline in activity of total Ac INV by increasing activity of both the soluble and insoluble forms of this enzyme. Together, these observations indicate that LPE-induced senescence-delay arises by a pathway or process distinct from phytohormone-induced senescence progression initiated by either ABA and/or ETH and mediated in part by PA.

Towards a Mechanism of Action for LPE

As outlined above a characteristic feature of LPE treatment of plant tissues is induction of Ac INV activity. Phenylalanine ammonia lyase is another enzyme typically induced by LPE treatment. Both Ac INV and PAL are important metabolic enzymes and pathogenesis-related proteins which would seem to indicate that the senescence delaying effect of LPE arises as a consequence of the initiation of plant defense responses which are manifest by increases in activity of these enzymes, anabolic metabolism and lignin formation.[15,22] Another characteristic feature of LPE-treated tissues is the reduction in ETH production and repression of

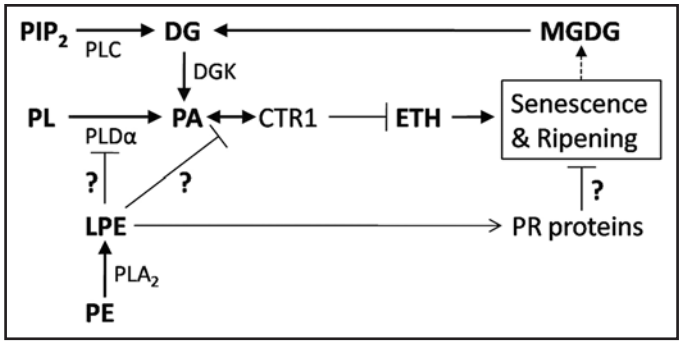

Figure 1. Scheme depicting hypothetical mechanism of action initiated by treatment of plant tissues with LPE. Application of LPE or formation of LPE from PE by PLA_2 increases endogenous LPE concentration. Increased LPE may act to attenuate PLDα activity and reduce formation of PA from structural phospholipids (PL) for PA-CTR1 interaction and/or directly inhibit PA-CTR1 binding to suppress downstream ETH-mediated responses. This coupled with the LPE-induced increase in activity of PR proteins such as Ac INV and PAL acts to delay senescence progression and the further deleterious effects of elevated PA. PA also arises from diacylglycerol kinase (DGK)-catalyzed phosphorylation of diacylglycerol (DG) derived either from monogalactosyl DG (MGDG) or the sequential action of phospholipase C (PLC) and DGK after hydrolysis of phosphastidylinositol-4,5-bisphosphate (PIP_2).

ETH-mediated responses. Numerous PA binding proteins have been identified from plants and many appear to function in abiotic and biotic stress responses.[23] Since the endogenous LPE concentration of plants changes coincident with the onset of abiotic and biotic stress, it is distinctly possible that exogenous LPE mitigates these stress effects and does so by attenuating the endogenous PA signal by an as yet unknown mechanism and, by inducing defense enzymes such as extracellular Ac INV to delay progression of stress-induced senescence and/or programmed cell death. One potential target for LPE is the signaling mechanism in ETH-mediated responses. This is particularly so given the recent demonstration that PA binds CTR1 (constitutive triple response 1) to inhibit its kinase activity and block interaction of CTR1 with ETR1, an ETH receptor, to initiate downstream ETH responses.[24] Although very little is known about the biochemical regulation of CTR1 activity which may not be the only signaling pathway, the scheme in Figure 1 sketches one possible interrelationship between LPE and CTR1 activity in ETH-induced ripening and/or senescence progression.

In brief, the size of the endogenous PA pool changes in response to extrinsic and intrinsic factors by PLD-catalyzed conversion of structural phospholipids (PL) and/or through phosphorylation of diacylglycerol (DG) by DG kinase (DGK) to either initiate senescence-like programmed cell death or senescence progression; the LPE pool changes as a consequence of PLA_2-catalyzed de-acylation of PE or, as a consequence of LPE treatment; the PA signal is attenuated by PA kinase, PA phosphatase and/or PLA_2-catalysed conversion of PA to *lyso*-PA; LPE-induced inhibition of PLDα and/or inhibition of PA-CTR1 binding coupled with induction of pathogenesis-related proteins such as Ac INV also attenuates the PA signal and suppresses downstream ETH responses and senescence progression. Many organic compounds interact with the ETH receptors. Some are agonists that mimic ETH whereas others are antagonists and prevent ETH action by blocking receptor signalling. Binding of PA to CTR1 which blocks its interaction with ERT1 leading to ETH responses and acyl-CoA ester binding by acyl-CoA-binding proteins which facilitates interaction with the ETH-responsive element binding protein, AtEBP[25] may offer clues to the molecular mechanism of action of LPE. This is supported by recent observations that the functional role of an enzyme can be switched via interaction with a specific *lyso*-phospholipid.[26] In addition, LPE displays chaperone-like properties and like molecular chaperones promotes the functional folding of citrate synthase and α-glucosidase in *E. coli* and prevents the aggregation of citrate synthase following exposure to heat stress,[27] which suggests that LPE can and does affect the structure and function of proteins.

Conclusion and Perspective

Accumulating evidence suggests an ever expanding role for the phospholipids and *lyso*-phospholipids in plant cell signaling processes and in a variety of response mechanisms. Thus, it is perhaps not surprising that commercial agriculture has investigated and developed individual phospholipids and mixtures of phospholipids and *lyso*-phospholipids as potential plant bio-regulators. As is the situation for all synthetic and natural plant growth regulators, a mode of action is required to facilitate implementation and use of these as crop management tools.

It is now well established that LPE treatment delays progression of leaf and fruit senescence, enhances fruit quality (e.g., fruit colour and firmness), and decreases susceptibility to abiotic and biotic stresses. Central to these responses is induction of pathogenesis-related proteins and in particular Ac INV and attenuation of ETH action. The molecular mechanism responsible seems to be lipid-protein interaction but this requires validation and proteomic characterization of the full spectrum of LPE target proteins. Finally, without some knowledge and understanding of a mode of action for exogenous LPE it is difficult to address aspects of specificity of response. Nonetheless, an important aspect in our opinion which requires investigation is LPE-induced PA formation. Specificity of LPE we believe can then be addressed with confidence in respect of PA-induced senescence-like programmed cell death, ethylene synthesis and action, and increased activity of pathogenesis-related proteins such as Ac INV.

Acknowledgements

The above commentary arises from work carried out at Nutra-Park Inc., Middleton, WI. Claire Leung and Chandra Santori are thanked for their technical expertise and assistance.

References

1. Nooden LD, Penney JP. Correlative controls of senescence and plant death in *Arabidopsis thaliana* (Brassicaceae). J Exp Bot 2001; 52:2151-9.
2. Yoshida S. Molecular regulation of leaf senescence. Curr Opin Plant Biol 2003; 6:79-84.
3. Lim PO, Kim HJ, Nam HG. Leaf senescence. Annu Rev Plant Biol 2007; 58:115-36.
4. Cowan AK. Phospholipids as plant growth regulators. Plant Growth Regul 2006; 48:97-109.
5. Welti R, Li W, Li M, Sang Y, Biesiada H, Zhou H-E, et al. Profiling membrane lipids in plant stress responses: role of phospholipase Dα in freezing-induced lipid changes in Arabidopsis. J Biol Chem 2002; 277:31994-2002.

6. Farag KM, Palta JP. Improving postharvest keeping quality of vine-ripened tomato fruits with a natural lipid. HortSci 1991; 26:162.
7. Farag KM, Palta JP. Use of lysophosphatidylethanolamine, a natural lipid, to retard tomato leaf and fruit senescence. Physiol Plant 1993; 87:515-21.
8. Özgen M, Park S, Palta JP. Mitigation of ethylene-promoted leaf senescence by a natural lipid, lysophosphatidylethanolamine. HortSci 2005; 40:1166-7.
9. Hong J-H, Hwang SK, Chung G-H, Cowan AK. Inαuence of lysophosphatidylethanolamine application on fruit quality of Thompson Seedless grapes. J Appl Hortic 2007; 9:112-4.
10. Ryu SB, Karlsson BH, Özgen M, Palta JP. Inhibition of phospholipase D by lysophosphatidylethanolamine, a lipid-derived senescence retardant. Proc Natl Acad Sci USA 1997; 94:12717-21.
11. Pinhero RG, Almquist KC, Novotna Z, Paliyath G. Developmental regulation of phospholipase D in tomato fruit. Plant Physiol Biochem 2003; 41:223-40.
12. Farag KM, Palta JP. Use of natural lipids to accelerate ripening and enhance storage life of tomato fruit with and without ethephon. HortTechnol 1993; 3:62-5.
13. Romanov GA, Kieber JJ, Schmülling T. A rapid cytokinin response assay in Arabidopsis indicates a role for phospholipase D in cytokinin signalling. FEBS Lett 2002; 515:39-43.
14. Cowan AK, Leung C, Santori C. *Lyso*-phosphatidylethanolamine (LPE) as a plant bioregulator. Acta Hort 2006; 727:527-36.
15. Hong J-H, Chung G-H, Cowan AK. *Lyso*-phosphatidylethanolamine-enhanced phenylalanine ammonia-lyase and insoluble acid invertase in isolated radish cotyledons. Plant Growth Regul 2009; 57:69-78.
16. Cowan AK, Freeman M, Björkman P-O, Nicander B, Sitbon F, Tillberg E. Effect of senescence-induced alteration in cytokinin metabolism on source-sink relationships and ontogenic and stress-induced transitions in tobacco. Planta 2005; 221:801-14.
17. Roitsch T, Bittner M, Godt DE. Induction of apoplastic invertase of *Chenopodium rubrum* by D-glucose and a glucose analog and tissue-specific expression suggest a role in sink-source relations. Plant Physiol 1995; 108:285-94.
18. Roitsch T, Gonzalez M. Function and regulation of plant invertases: sweet sensations. Trends Plant Sci 2004; 9:606-13.
19. Wingler A, Roitsch T. Metabolic regulation of leaf senescence: interactions of sugar signalling with biotic and abiotic stress responses. Plant Biol 2008; 10:50-62.
20. Lara MEB, Garcia MG, Fatima T, Ehneß R, Lee TK, Proles R, et al. Insoluble invertase is an essential component of cytokinin-mediated delay of senescence. Plant Cell 2004; 16:1276-87.
21. van Doorn WG, Woltering EJ. Senescence and programmed cell death: substance or semantics? J Exp Bot 2004; 55:2147-53.
22. Hong JH, Chung G, Cowan AK. Delayed leaf senescence by exogenous *lyso*-phosphatidylethanolamine: towards a mechanism of action. Plant Physiol Biochem 2009; doi:10.1016/j.plaphy.2008.12.014.
23. Testerink C, Dekker HL, Lim Z-Y, Johns MK, Holmes AB, de Koster CG, et al. Isolation and identification of phosphatidic acid targets from plants. Plant J 2004; 39:527-36.
24. Testerink C, Larsen PB, van der Does D, van Himerbergen J, Munnik T. Phosphatidic acid binds to and inhibits the activity of Arabidopsis CTR1. J Exp Bot 2007; 58:3905-14.
25. Li HY, Xiao S, Chye ML. Ethylene- and pathogen-inducible Arabidopsis acyl-CoA-binding protein 4 interacts with an ethylene-responsive element binding protein. J Exp Bot 2008; 59:3997-4008.
26. Cho EY, Yun CH, Chae HZ, Chae HJ, Ahn T. Lysophosphatidylserine-induced functional switch of human cytochrome P450 1A2 and 2E1 from monooxygenase to phospholipase D. Biochem Biophys Res Comm 2008; 376:584-9.
27. Kern R, Joseleau-Petit D, Chattopadhyay MK, Richarme G. Chaperone-like properties of lysophospholipids. Biochem Biophys Res Comm 2001; 289:1268-74.