Acyl Chain Length of Phosphatidylserine Is Correlated with Plant Lifespan

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Abstract

Plant lifespan is affected by factors with genetic and environmental bases. The laws governing these two factors and how they affect plant lifespan are unclear. Here we show that the acyl chain length (ACL) of phosphatidylserine (PS) is correlated with plant lifespan. Among the detected eight head-group classes of membrane lipids with lipidomics based on triple quadrupole tandem mass spectrometry, the ACL of PS showed high diversity, in contrast to the ACLs of the other seven classes, which were highly conserved over all stages of development in all plant species and organs and under all conditions that we studied. Further investigation found that acyl chains of PS lengthened during development, senescence, and under environmental stresses and that increasing length was accelerated by promoted-senescence. The acyl chains of PS were limited to a certain carbon number and ceased to increase in length when plants were close to death. These findings suggest that the ACL of PS can count plant lifespan and could be a molecular scale ruler for measuring plant development and senescence.

Introduction

Plant lifespan is a complex manifestation of genetic and environmental factors that encompass both development and senescence. It greatly influences crop productivity, biodiversity, and ecological equilibrium. Lifespan may be extended by factors with genetic and environmental bases. The laws governing these two factors and how they affect plant lifespan are unclear. Here we show that the acyl chain length (ACL) of phosphatidylserine (PS) is correlated with plant lifespan. Among the detected eight head-group classes of membrane lipids with lipidomics based on triple quadrupole tandem mass spectrometry, the ACL of PS showed high diversity, in contrast to the ACLs of the other seven classes, which were highly conserved over all stages of development in all plant species and organs and under all conditions that we studied. Further investigation found that acyl chains of PS lengthened during development, senescence, and under environmental stresses and that increasing length was accelerated by promoted-senescence. The acyl chains of PS were limited to a certain carbon number and ceased to increase in length when plants were close to death. These findings suggest that the ACL of PS can count plant lifespan and could be a molecular scale ruler for measuring plant development and senescence.

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Materials and Methods

Plant materials, growth conditions and treatments

We grew Arabidopsis ecotype Columbia (Col) and Crucihimalaya in soil, hydroponically using Hoagland’s medium [15], and/or on plates in MS media, as indicated, at 22°C, under light of 120 μmol/m²/s, and a 12/12 h photoperiod. For phytohormone-induced senescence, we placed leaves that had been detached from 6-week-old plants onto filter paper that contained water, ABA (50 μM), or ethephon (50 μM, to release ethylene), and incubated them for the indicated number of days [16,17]. To induce senescence via gamma radiation, hydroponically grown plants aged 20 days were irradiated in a commercial 60Co facility (FX-648G) at the indicated dose, grown under normal conditions, and sampled at the indicated times. To induce stress from heat shock, we incubated plates with plants aged 10 days in water at 44°C for 2 h [17]. To induce stress from dehydration, we placed plate-grown plants onto filter paper and exposed them to air for the indicated time [18]. All reagents described above were obtained from Sigma.

Lipid analysis and data processing

We analysed lipid samples by ESI-MS/MS [13]. We processed the data in the same way that we have described in previous papers [14,19]. We quantified the lipids in each class in comparison to two internal standards. The lipid content was described as nmol/mg dry weight of plants. We analysed five replicates of each plant species at each sampling time. Paired values were subjected to a t-test to determine statistical significance. The average carbon number (C) of the acyl chains in a given lipid class was calculated using the formula: C = 100 × [mol% lipid]/100, where N is the total number of acyl carbons in each lipid molecule.

Results

ACLs of membrane glycerolipids in plants

We found that four species of Cruciferae contained 34:6 monogalactosyldiacylglycerol (MGDG) molecular species, which indicated that they were 16:3 plants (synthesizing lipids through both prokaryotic and eukaryotic pathways, Figure S5). In contrast, the four other species, which belonged to Asteraceae, Poaceae, Fabaceae, and Papaveraceae, did not harbour 34:6 MGDG molecular species, which indicated that they were 18:3 plants (synthesizing lipids through eukaryotic pathway, Figure S5). For the four 16:3 plants, mean ACLs for all classes of glycerolipid were clustered in a very narrow range of 34.59–34.89 carbon (C); the maximum relative variation of ACL among all eight species was 0.95%. For the four 18:3 plants, the ACLs were clustered in the range 35.45–35.73 C, with a maximum relative variation of 0.78% (Figure 1a). The maximum relative variation of ACL among all eight species was only 3.3%; this variation resulted mainly from the different processes of growth, senescence, and death (Figure S7). The results showed that the ACLs for the seven lipid classes other than PS were highly conserved, and were clustered in a range of no more than 0.27 C. In contrast, the ACL for PS increased significantly by 2.6 C from 24 to 74 days, namely during the growth and senescence phases, and ceased to increase when the plants were dying (Figure 3a). The maximum rate of increase was 0.05±0.01 C/day up to about day 50 of the measured period of plant development (Figure 3b).

We then examined the ACL of PS in the main organs of plants to test whether an increase in the ACL of PS during development is a universal phenomenon in plants. We determined the ACLs for the eight classes of lipid in siliques at 15, 20, 25, and 30 days after flowering (Figure S8). The ACLs for DGDG, PC, PI, and PA remained unchanged over this period, and for MGDG, PG, and PE, decreased slightly by 0.1 C. In contrast, the ACL for PS increased significantly by 0.62 C (Figure 3c). The maximum rate of increase was 0.04±0.01 C/day during the period of silique development analysed (Figure 3d). We determined the ACL for PS in the roots of 15-day, 10-month, and 18-month pot-grown and wild perennial Crucihimalaya himalaica (Figure S2 and S9). The ACL for PS increased significantly by 1.78 C, whereas the ACLs for the other seven lipids remained within a narrow range (Figure 3e). These observations indicated that the acyl chains of lipids in the PS lengthened as development and that the maximum length was no more than 41 C.

Changes in ACL of PS during senescence

Senescence can be induced in leaves by detaching them from the plant and the senescence of detached leaves can be accelerated by the application of the phytohormones abscisic acid (ABA) and ethylene[16]. We examined the changes in ACL of lipids during senescence at 0, 3, and 5 days after leaves were detached and treated with ABA or ethylene. Hormone-treated leaves showed yellowing on their edges in comparison with control, meaning their senescence was promoted (Figure S10). The results showed that the ACLs for PS significantly increased by more than 2 C in these three treatments (Figure 4a), whereas in the other seven lipid classes, the ACLs were highly conserved (Figure S11). The maximum ACL for PS was ~41.5 C. The maximum rate of lengthening was 0.68±0.34 C/day, which was significantly higher than that under normal growth conditions (Figures 3b and 4b). Thus, it shows that the acyl chains of PS lengthened during detachment- and phytohormone-induced senescence, and that the rate of lengthening was related positively to the promotion of senescence.

Senescence can also result from DNA damage [20], which can be induced by gamma irradiation [21]. To induce DNA damage, we exposed Arabidopsis plants to gamma irradiation at 260 and 1010 Gy and examined the ACLs of all eight lipid classes for 20 days after irradiation. The yellowing of treated leaves showed that...
Figure 1. Acyl chain lengths of eight plants. (a) Acyl chain lengths of membrane lipids, (b) The acyl chain length of membrane glycerolipids of 16:3 plants, or (c) The acyl chain length of membrane glycerolipids of 18:3 plants. MRV, maximum relative variation. Values are means ± s.d. (n = 5). doi:10.1371/journal.pone.0103227.g001

Figure 2. The relative composition (mol%) of lipid molecular species during development of leaves after germination. Values are means ± s.d. (n = 5). doi:10.1371/journal.pone.0103227.g002
their senescence was induced. The growth of the plants was inhibited and they died slowly (Figure S12). The damage under irradiation at 1010 Gy was more severe than that at 260 Gy (Figure S12). The ACLs of all the lipids were highly conserved, apart from that of PS (Figure S13). After 260 Gy irradiation, the ACL of PS continued to increase throughout the 20-day experimental period and had increased by 1.09 C at the end of the period (Figure 4c and Figure S13). In contrast, after 1010 Gy irradiation, the ACL of PS ceased to increase after 9 days, although it had increased by 1.17 C at this point (Figure 4c and Figure S13). The maximum rate of lengthening for PS was 0.41±0.07 C/day (Figure 4d), which was significantly higher than that under normal conditions (Figure 3b and d). The rate of lengthening after irradiation at 1010 Gy was significantly higher than that after irradiation at 260 Gy, especially during the first 9 days. The earlier cessation of acyl chain lengthening for PS under irradiation at 1010 Gy than under irradiation at 260 Gy could be due to earlier cell death under the former condition (Figure S12). These observations show that the acyl chains of PS lengthened as senescence was induced by DNA damage and that the rate of lengthening was related positively to the severity of damage.

Changes in ACL of PS under environmental stresses

Plant growth and senescence are affected by environmental factors, such as temperature [22] and the availability of water [18].

Hence, we investigated whether the acyl chains of PS responded to heat shock (Figure S14) and dehydration. After the plants had been subjected to heat shock for 2 hours and had been dehydrated for 100 minutes, the acyl chains of PS have lengthened significantly by 0.67 and 0.20 C, respectively, whereas the length of the acyl chains of the other lipids varied much less or remained unchanged (Figure 5). These observations indicated that acyl chains of PS lengthen in response to environmental stresses and can sense corresponding physiological events that have a duration of 1–2 h.

Discussion

We have shown that ACLs of membrane glycerolipids MGDG, DGDG, PG, PE, PI and PA are conserved among various plant species, during the plant lifespan, and under different environmental stresses and that the ACL of PS increased during the plant lifespan and positively responded to environmental stresses. The increment of ACLs of PS can sense developmental and/or environmental events lasting years, months, days, and hours. Particularly in Arabidopsis, total ACL in PS increases from ~37 to ~41 C during growth and senescence; lengthening stops when plants are close to death, i.e. when ACL of PS reaches 41 C. This is true irrespective of how 41 C is reached, from development or from environmental stress.

Organisal lifespan is determined by the organ lifespan of key functions in animals. In contrast to animals, plants could...
continuously grow new organs during their lifespan. Even so, however, their lifespans are determined by their organ’s lifespan. Yet, the problem is that, the organismal lifespan of different types of plants is determined by the lifespan of different organs. For examples, the lifespan of annual plants is along with their leaf’s lifespan; the lifespan of perennial plants is tightly associated with their taproot’s lifespan. Given that organ lifespan is correlated with ACL of PS, we therefore suggest that lifespan of a plant species can be measured by the ACL of PS of its particular organ.

PS is one of low abundant membrane lipids (Figure 2 and S3) [23,24]. PS is located in the inner leaflet of plasma and contributes to maintain membrane asymmetry. Its outward movement disrupts the asymmetry and triggers cell death [25]. Fatty acids of membrane lipids are usually conservative, while fatty acids of storage lipids exhibit diversity [10]. It is not clear why the ACLs of other lipids were conserved and only those of PS increased; we speculate that PS is the only membrane glycerolipid that harbours VLCFAs of greater than 40 C [11,26]. VLCFAs are thought to be needed for forming highly curved membrane structures [10], thus transgenic plants containing VLCFAs can mimic structural role of sphingolipids, which usually have VLCFAs [27]. Accumulation of VLCFA is deleterious to cells because their special physical and

Figure 4. Acyl chains of PS lengthen with detachment-, ABA- and ethylene-induced senescence, and gamma irradiation in Arabidopsis. (a) The acyl chain length of PS treated with water, ABA, and ethylene in detached leaves, (b) The lengthening rate of PS treated with water, ABA, and ethylene in detached leaves, (c) The acyl chain length of PS in leaves at 0, 1, 3, 5, 7, 9, and 20 d after irradiation, (d) The lengthening rate of PS in leaves after irradiation. Values are means ± s.d. (n = 5). An asterisk indicates that the value is different from that of control or that of 260 Gy at same time (p<0.05).

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Figure 5. Acyl chains of PS lengthen under stresses in Arabidopsis. (Left panel) The acyl chain length of membrane glycerolipids under heat shock at 44°C for 2 h. (Right panel) The acyl chain length of membrane glycerolipids under dehydration for 100 min. Values are means ± s.d. (n = 5). An asterisk indicates that the value is different from that of the control (p<0.05).

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chemical properties can perturb the integrity of membrane structure [10]. It has been proposed that plants have a process to screen-out VLCFA from membranes [28]. In yeast, membrane lipid ergosterol involves in vacuole-vacuole fusion which is required for lifespan extension, however, sphingolipids have no same effects [29]. In animals, PS also plays a unique role in rescaling the plasma membrane after it has been damaged [30]. Given that the mechanism by which the plasma membrane is rescaled in plants is similar to that by which it is rescaled in animals, the lengthening of PS acyl chains could signify an attempt to rescale membranes that have been damaged by senescence and environmental stress. VLCFAs could increase in PS due to their time-dependent accumulation during the lifespan of the plant and due to the response of cells to stress-induced damage. We hypothesize that the tolerance of membrane bilayers to the lengthening of PS acyl chains is limited and that once a critical length is reached, the membrane structure is damaged irreparably and that cause cell death.

In summary, we identified a biomolecule, membrane glycerolipid PS, whose primary structure lengthened during development and natural and artificially-induced senescence of plants. The lengths of the PS acyl chains are limited to \( \sim 41 \) C and achieving this length appears to be a predictor of the end point of a plant’s life. Also the changing length of PS acyl chains between the initial and end stage of development can indicate the lifespan of plants. Thus evidence suggests that ACL of PS could be a molecular scale ruler measuring plant lifespan.

Supporting Information

Figure S1  Head-group classes of membrane glycerolipids and the structure of phosphatidylycerine (PS). a, The head group classes of membrane glycerolipids determined, and b, The glycerol backbone and acyl chains in the structure of PS. (TIF)

Figure S2  The plant species investigated for lipids in the study. The information of habitats and features of Saussurea medusaare taken from website (http://db.kib.ac.cn/eflora/view/search/chs_contents.aspx?name =Saussurea%20medusa%20Maxim; the information on other plant species is from Flora of China. (TIF)

Figure S3  The contents (nmol/mg) of lipid molecular species during the development of leaves after germination in Arabidopsis. Values are means ± s.d. (n = 5). (TIF)

Figure S4  The ACLs of membrane lipids during the development of leaves in Arabidopsis after germination. Values are means ± s.d. (n = 5). An asterisk indicates that the value is different from that of control (p<0.05). (TIF)

Figure S5  The composition (mol%) of MGDG molecular species (36:6 and 34:6) in eight plant species. A, thaliana, S-L, lineariafolia, D, oreades, and T, halophila harbour both 36:6 and 34:6MGDG. S. medusa, V, angularis, M, racemosa, and O, sativa harbour only 36:6MGDG. (TIF)

Figure S6  The range of ACLs of membrane lipids in eight plants. Values are means ± s.d. (n = 5). (TIF)

Figure S7  Leaf development of Arabidopsis at 24, 52, 74, and 99 days after germination. (TIF)

Figure S8  The development of Arabidopsis siliques at 15, 20, 25, and 30 days after flowering. (TIF)

Figure S9  The roots of 15-day, 10-month, and 18-month pot-grown and wild perennial (W) Crucihimalaya himalaica. (TIF)

Figure S10  The senescence of detached leaves treated with water, 50 µM ABA, and 50 µM ethephon for 5 days. (TIF)

Figure S11  The acyl chain length of membrane lipids during leaf detachment, ABA, and ethylene-induced senescence in Arabidopsis. Values are means ± S.D. (n = 5). An asterisk indicates that the value is different from that of control (p<0.05). (TIF)

Figure S12  The senescence of Arabidopsis after 260 and 1010 Gy of gamma-irradiation. a, Control; b, 260 Gy; and c, 1010Gy. (TIF)

Figure S13  The ACLs of membrane lipids during the gamma irradiation-induced senescence in Arabidopsis. Time the days after irradiation. Values are means ± s.d. (n = 5). An asterisk indicates that the value is different from that of control (p<0.05). (TIF)

Figure S14  The accumulation of heat-shock protein 70 (HSP70) during head-acclimation and head shock. Isolated total proteins were conducted by Western. (TIF)

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Author Contributions

Conceived and designed the experiments: WQL. Performed the experiments: YL, GWZ, XMY, BZY, DDW, YLZ, XJT. Analyzed the data: YL, GWZ, YXJ. Wrote the paper: WQL, XDZ.

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