Metabolic profiling of oxylipins upon sorbitol treatment in barley leaves

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Abstract

In barley leaves 13-lipoxygenases (LOXs) are induced by salicylate and jasmonate. Here, we analyse by metabolic profiling the accumulation of oxylipins upon sorbitol treatment. Although 13-LOX-derived products are formed and specifically directed into the reductase branch of the LOX pathway, accumulation is much later than in the cases of salicylate and jasmonate treatment. In addition, under these conditions only the accumulation of jasmonates as additional products of the LOX pathway has been found.

Jasmonates are known to function as a ‘master switch’ for various biotic and abiotic stresses [1]. In barley leaves osmotic stress by sorbitol treatment induces accumulation of α-linolenic acid (α-18:3), jasmonates and octadecanoids such as 12-oxo-phytodienoic acid followed by expression of genes inducible by endogenous jasmonates [2]. α-18:3 represents the main polyenoic fatty acid constituent of chloroplastic membranes and occurs at a 10-fold-higher level than linoleic acid (18:2) [3]. Both compounds are substrates of lipoxygenases (LOXs), which catalyse the regio-selective and stereoselective insertion of molecular oxygen into position C-9 (9-LOX) or position C-13 [4]. In the case of α-18:3 and 13-LOX the resulting product is 13-HPOT [(13S,9Z,11E,15Z)-13-hydroperoxy-9,11,15-octadecatrienoic acid]. Besides the jasmonate pathway, the following enzymes use 13-HPOT in individual branches of the LOX pathway, thus contributing to the pattern of oxylipins (Figure 1, left-hand panel): (i) a peroxygenase and a reductase to form hydroxides, (ii) a divinyl ether synthase leads to divinyl ether fatty acids, (iii) a peroxidase activity of LOX catalysis forms keto conjugenedioic fatty acids and (iv) ω-keto fatty acids and aldehydes are formed by hydroperoxide lyase. Owing to the time-dependent increase of jasmonates and octadecanoids upon sorbitol treatment of barley leaves [2], we were interested to record metabolic profiles of all other oxylipins under these conditions. For comparison, the substrates of the LOX pathway, α-18:3 and 18:2, were detected, too. Both polyenoic fatty acids might be released from the chloroplastic membranes upon sorbitol treatment in the ratio 10:1 (Figure 1, right-hand panels A and B), thus reflecting their constitutive occurrence within this type of membrane [3]. The 9- and 13-hydroperoxy derivatives of α-18:3 and 18:2, respectively, accumulated weakly and only at later times of sorbitol treatment (Figure 1, right-hand panels C and D). The same is true for the corresponding hydroxy derivatives (Figure 1, right-hand panels E and F). In each case the original 10:1 ratios of α-18:3 and 18:2 were still reflected in the compounds derived therefrom.

Aldehydes such as (3Z)-hexenal and (2E)-4-hydroxy-2-hexenal exhibited no characteristic increase upon sorbitol treatment compared with
Figure 1
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Left-hand panel: metabolic routes for LOX-dependent catabolism of polyenoic fatty acids in plants. Right-hand panel: metabolic profiling of 18:2 (A), α-18:3 (B) and oxylipins derived therefrom (C-G) in barley leaf segments floated on water or 1 M sorbitol. The results represent the means±S.D. of two measurements performed with two independent flotation experiments. POX, peroxygenase; DES, divinyl ether synthase; HPL, hydroperoxide lyase; AOS, allene oxide synthase; HHE, (2E)-4-hydroxy-2-hexenal; LA, linoleic acid; LeA, linolenic acid; KOD, keto-octadecadienic acid; KOT, keto-octadecatrienic acid.

The water control (Figure 1, right-hand panel G). Obviously, the effect of sorbitol treatment on the oxylipin formation is moderate and unspecific, except in the case of jasmonates and octadecanoids. Here, recent data [2] showed an earlier increase in the case of jasmonates and octadecanoids starting, at 12 h of sorbitol treatment, in contrast to the late accumulation of 13-HPOT starting at 48 h (Figure 1, right-hand panel D). Therefore, the jasmonate branch of the LOX pathway seems to be the only one that is preferentially activated by sorbitol treatment. This corresponds to transcriptional activation of its biosynthetic enzyme by sorbitol treatment [2].

References

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