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DEGRADATIVE METABOLISM OF OXALIC ACID AND ASCORBIC ACID IN SPINACH (*SPINACIA OLERACEA* L.).

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Vitamin C, metabolic flux, ascorbic acid degradation, oxalate oxidase, germin like protein

Degradation of ascorbic acid via synthesis and degradation of oxalic acid was studied in spinach seedlings. L-ascorbic-acid (AscA) cleavage at the C_2-C_3 carbon bond is the recognised biosynthetic pathway of oxalic acid (OXA) for oxalate crystal synthesis, but its contribution to the control of the ascorbic acid content of plant tissue and to the control of the content of soluble oxalic acid in plant tissues is poorly known. OXA in plants can be degraded, by the enzymatic activity of oxalate oxidase (OXO, EC 1.2.3.4), in carbon dioxide and hydrogen peroxide nevertheless, the knowledge about the regulation of activity of this enzyme are largely lacking. AscA is present in all plant species and oxalate is widely distributed in the plant kingdom both as a soluble acid or insoluble salt, mainly calcium oxalate. Both, AscA and OXA, play important physiological roles in plants, are metabolically linked and possess opposite nutritional value for human health. The metabolic link between AscA and OXA and the biochemistry of OXA degradation in plant food are important in terms of plant physiology and nutritional quality of plant derived food. After feeding with 125 mM of AscA or OXA for 4, 10, 24 h, and after 4, 8, 24 h of further water feeding (washing), changes of ascorbic and oxalic acid concentration, of the activity of oxalate oxidase, of the gene expression of a putative H_2O_2 –producing oxalate oxidase germin like protein, were measured in spinach seedlings. Ascorbate or oxalate treatment produced a time-dependent accumulation of AscA and soluble OXA in hypocotyls and, at higher levels, in cotyledons of spinach seedlings. During washing, AscA and OXA contents decreased at a very fast rate. After feeding 99% $^{13}C_2$ AscA, the $^{13}C/^{12}C$ ratio in the CO_2 released by spinach tissues increased. An insoluble form of OXO was preliminarily found in different spinach tissues, including hypocotyls and cotyledons. The insoluble OXO activity increased several fold following metabolite feeding in both hypocotyls and cotyledons and remained high during washing. A soluble form of OXO appeared in the tissues, after AscA and OXA feeding. AscA and OXA feeding induced the gene expression of a putative OXO germin like protein. Our results suggest that a) AscA can be degraded in spinach tissues via OXA synthesis and that OXA can then be degraded to H_2O_2 and CO_2 via OXO activity, b) the mentioned degradative pathway can be induced by AscA and OXA feeding in a feed-forward mode, via the increase of OXO activity d) the increase of OXO activity is linked to the increase of gene expression of a putative germin like protein. We demonstrate that the AscA and soluble OXA pools are metabolically linked in spinach tissues, and that AscA can be degraded at such a fast rate that the amount normally present in spinach tissue can be cleaved in a few hours.