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Concentration Does Matter: The Beneficial and Potentially Harmful Effects of Ascorbate in Humans and Plants

Szilvia Z. Tóth,[1] Tamás Lőrincz,[2] and András Szarka[2]

Abstract

Significance: Ascorbate (Asc) is an essential compound both in animals and plants, mostly due to its reducing properties, thereby playing a role in scavenging reactive oxygen species (ROS) and acting as a cofactor in various enzymatic reactions.
Recent Advances: Growing number of evidence shows that excessive Asc accumulation may have negative effects on cellular functions both in humans and plants; inter alia it may negatively affect signaling mechanisms, cellular redox status, and contribute to the production of ROS *via* the Fenton reaction.
Critical Issues: Both plants and humans tightly control cellular Asc levels, possibly *via* biosynthesis, transport, and degradation, to maintain them in an optimum concentration range, which, among other factors, is essential to minimize the potentially harmful effects of Asc. On the contrary, the Fenton reaction induced by a high-dose Asc treatment in humans enables a potential cancer-selective cell death pathway.
Future Directions: The elucidation of Asc induced cancer selective cell death mechanisms may give us a tool to apply Asc in cancer therapy. On the contrary, the regulatory mechanisms controlling cellular Asc levels are also to be considered, for example, when aiming at generating crops with elevated Asc levels. *Antioxid. Redox Signal*. 00, 000–000.

Keywords: ascorbate, ascorbate biosynthesis, cell death, pharmacologic ascorbate, photosynthesis, reactive oxygen species

Introduction

Ascorbate (Asc) is one of the most widely known vitamins, which has a range of essential functions both in animals and plants. Because of its hydrophilic nature, it is highly soluble in water (0.33 g/ml), less soluble in ethanol (0.02 g/ml), and insoluble in oils, fats, and fat solvents. Consequently, it needs various transport proteins to pass through biological membranes (104). This characteristic feature also provides the possibility to set different Asc concentrations in different cell types and cell organelles (11, 104).

The donation of one electron to a redox partner results in the formation of monodehydroascorbate (MDA) (Fig. 1), which is also called ascorbyl radical. On further oxidation of the radical, dehydroascorbate (DHA) is formed (Fig. 1). Several metals, such as copper and iron, catalyze the oxidation of Asc. DHA then undergoes irreversible hydrolysis to 2,3-diketo-L-gulonic acid, which may be further oxidized to oxalic acid and L-threonic acid (Fig. 1). The poor reactivity of ascorbyl radical makes Asc an excellent scavenger of reactive oxygen species (ROS), and thereby, it protects various cellular functions particularly under stress conditions. On the contrary, Asc is also a cofactor of several enzymes participating in a range of physiological processes and it also has other, recently discovered roles both in animals and plants.

Ascorbate is most often regarded as a protective agent and there has been considerable effort to increase its concentration

[1]Institute of Plant Biology, Biological Research Centre of the Hungarian Academy of Sciences, Szeged, Hungary.
[2]Laboratory of Biochemistry and Molecular Biology, Department of Applied Biotechnology and Food Science, Budapest University of Technology and Economics, Budapest, Hungary.

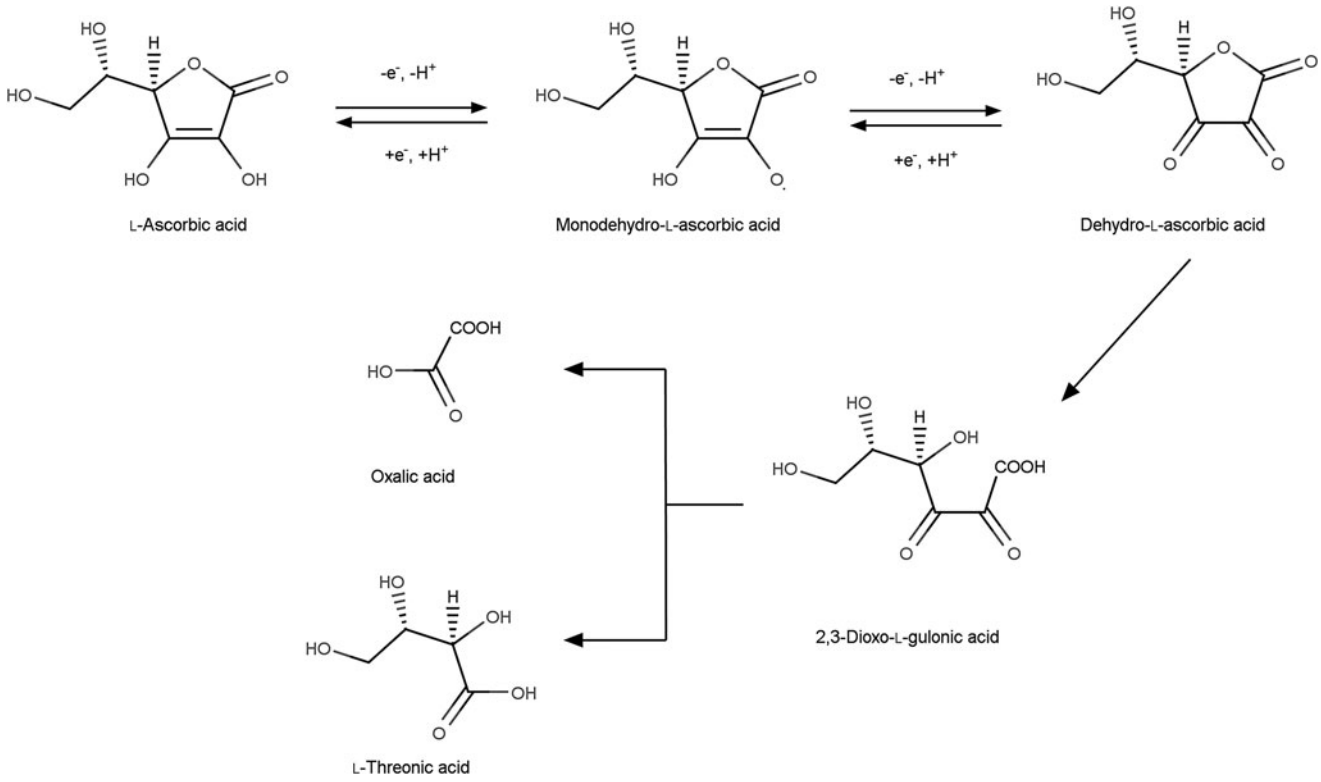


FIG. 1. The different redox forms and the degradation of Asc. MDA is formed from ascorbic acid by the donation of one electron. Further oxidation of the radical results in the formation of DHA. Ascorbate regeneration in biological systems from its oxidized forms should occur in a relatively short time, otherwise it would be lost because of the opening of the instable lactone ring of DHA. This irreversible hydrolysis of DHA yields dioxo-L-gulonic acid, which is further oxidized to oxalic acid and L-threonic acid. Asc, ascorbate; DHA, dehydroascorbate; MDA, monodehydroascorbate.

in fruits and vegetables, with the goal of both increasing stress tolerance of plants and to generate high-value agricultural products. However, these efforts have been accompanied with moderate success, possibly due to the strong feedback regulation of Asc on its own biosynthesis in plants. Furthermore, Asc at high concentration has been shown to behave as a pro-oxidant in isolated thylakoid membranes (163), just as well as in human cells (127). As reviewed here, the cellular Asc level in plants and humans seems to be regulated by various mechanisms, including its degradation and cellular transport, suggesting that maintaining its concentration in a certain range is of high physiological importance. This becomes evident when considering that Asc is a reducing agent and also has a regulatory role in various cellular functions. On the contrary, its pro-oxidant property has the potential to be applied in cancer therapy.

The Physiological Roles of Asc in Humans and Plants: More Than Just an Antioxidant

The roles of Asc in humans

Our knowledge on the biological functions of Asc is continuously expanding (Fig. 2A). All these known functions are based on its characteristic feature to be an excellent electron donor, that is, reductant. By this means, Asc efficiently scavenges ROS and reactive nitrogen species, RNS, which are by-products of oxidative metabolism and formed under various stress situations, leading to cellular damage (11). Asc also acts as a reducing cofactor for many enzymes, including copper-containing mono-oxygenases (22) and Fe(II)/2-oxoglutarate-dependent dioxygenases (97). These enzymatic reactions make Asc to be indispensable for the synthesis of carnitine (73) and catecholamines (9), and for the posttranslational modification of extracellular matrix proteins, including collagen (152). Asc may also be involved in another posttranslational modification, in the formation of disulfide bridges (152). It was recently discovered that 2-oxoglutarate-dependent dioxygenases are also epigenetic erasers by hydroxylating methyl-lysine residues in histones (Jumonji-C domain-containing histone demethylases) and 5-methyl-cytosine in ten-eleven translocases, TETs (86). Iron-containing 2-oxoglutarate-dependent enzymes also down-regulate hypoxia inducible factor 1 (85). As Asc is a specific cofactor for these enzymes, it definitely affects their activities. Asc is also a modulator of cellular iron metabolism. Beyond the known ability of dietary Asc to enhance nonheme iron absorption in the gut, accumulating evidence suggests that Asc can also regulate cellular iron uptake and downstream cellular metabolism (Fig. 2A) (89).

The roles of Asc in plants

In plants, the best-known function of Asc is to prevent the overaccumulation of ROS, which are formed during photosynthetic reactions occurring in the chloroplast, as a by-product of respiration in the mitochondria [reviewed by Ref.

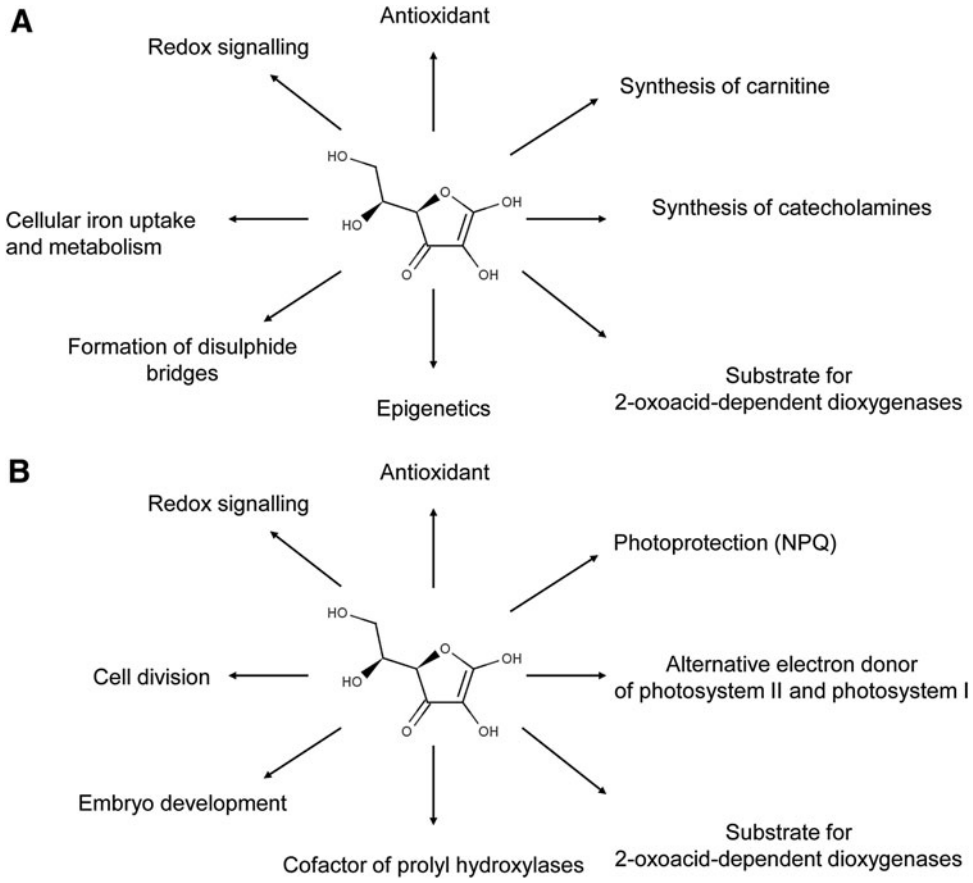


FIG. 2. Roles of Asc in humans (A) and plants (B). The figure depicts the presently known functions of vitamin C in plants and humans, however, it is worth noting that our knowledge on its biological functions is constantly broadening. All the known functions are associated with its excellent electron donor feature.

(72)] and ROS are also generated in the peroxisomes, due to their oxidative type of metabolism [reviewed by Ref. (41)]. As a nonenzymatic antioxidant, Asc is able to detoxify singlet oxygen ($^1O_2^-$) and hydroxyl radical ($OH^\bullet$). Asc can also scavenge lipid peroxyl radicals and thereby participate in the recycling of tocopheroxyl radicals to tocopherol in plants (Fig. 2B) [reviewed by Ref. (118)].

Asc plays an essential role in the highly regulated enzymatic scavenging of ROS in the so-called Mehler reaction or water/water cycle as well. In the Mehler reaction, superoxide ($O_2^{\bullet -}$) is produced at the acceptor side of photosystem I (PSI), *via* the reduction of O_2 by ferredoxin (Fd). It is then reduced to H_2O_2 by superoxide dismutase, SOD, and Asc peroxidase (APX) reduces H_2O_2 to water. MDA can be directly reduced back to Asc by PSI and/or in the Asc-glutathione cycle. Monodehydroascorbate reductase (MDAR) and dehydroascorbate reductase (DHAR) use NADPH as reducing power to regenerate Asc [for details of this reaction, see Ref. (5, 55); Ref. (38) for microalgae].

Besides its role in controlling the amount of ROS, Asc participates in a large number of enzymatic reactions in the plant cell. It serves as a cofactor of violaxanthin-deepoxidase (VDE), the enzyme responsible for the conversion of violaxanthin to zeaxanthin on illumination leading to thylakoid lumen acidification (67). Zeaxanthin accumulation results in the increase of excess excitation energy dissipation as heat (10, 68, 122), and zeaxanthin is also an efficient scavenger of ROS (39, 40, 42, 43). The role of Asc in the thermal dissipation of the excess excitation energy is well documented in seed plants, although it is unknown whether Asc is a cofactor of algal-type VDE as well (94).

In plants, Asc functions as a cofactor for prolyl hydroxylases (125, 174); 1-aminocyclopropane-1-carboxylate oxidase that catalyzes the last reaction of ethylene biosynthesis (14, 145). Asc is also a substrate for 2-oxoacid-dependent dioxygenases, which are involved in the synthesis of abscisic acid, gibberellins (99, 128), and Asc also influences ethylene (110) and salicylic acid biosynthesis (13) and anthocyanin accumulation on high-light exposure (126).

By being involved in abscisic acid signaling (99, 128), Asc also plays a role in the regulation of stomatal movement (52, 144). Asc also regulates embryo development (32) and cell elongation and progression through the cell cycle, *via* poorly understood mechanisms [reviewed by Ref. (59)]. It is conceivable that Asc plays a role in the cell cycle *via* its recently discovered role in epigenetic regulation (24), a possibility that has not been investigated in plants yet.

Thanks to its reducing properties, Asc is also an alternative electron donor to photosystem II (PSII) in higher plants and green algae under conditions where the oxygen-evolving complex (OEC) is impaired, for instance, upon heat stress (157, 158). The process of electron donation from Asc to Tyr_Z^+ is physiologically relevant as it slows down the inactivation of PSII reaction centers and allows a faster recovery from heat stress. Asc has also been shown to provide electrons to PSII and PSI in bundle sheath cells of NADP-malic enzyme-type species of C4 plants, which are deficient in oxygen evolution. The physiological role of this process,

most likely, is to poise PSI cyclic electron transport, responsible for the generation of ATP in bundle sheath cells (76).

The abovementioned functions demonstrate that Asc is a major player in cellular physiology (Fig. 2B), thus much more than just an antioxidant, as pointed out earlier (4). We also note that the antioxidant properties of Asc have been described in detail, but its roles in enzymatic reactions certainly warrant further investigations.

The Regulation of Cellular Asc Levels

The regulation of Asc concentration in human tissues and cells

Due to a large number of mutations in the gene of L-gulono-gamma-lactone oxidase, the ultimate enzyme of Asc biosynthesis (121), humans have lost the ability to synthesize Asc; therefore, they need to obtain Asc from their diets. The level of Asc in tissues and cells is determined by its absorption [intestinal and (sub)cellular transport] and reabsorption (in kidneys).

The major natural dietary sources of vitamin C are fruits and vegetables. These plant sources contain both the reduced form of Asc and the oxidized form of DHA, although the concentration of Asc largely exceeds that of DHA (53). Asc may get oxidized within the lumen of the gastrointestinal tract (87). It is also worth noting that DHA, similarly to Asc, can prevent scurvy (155), because it can be reduced to Asc by glutathione or in NADPH-dependent reactions (18). Both major forms of vitamin C, Asc and DHA, are absorbed along the entire length of the human intestine, as shown by the investigation of the transport activity of luminal (brush border) membrane vesicles (103). The transport of both forms showed saturation with an apparent K_M of $267 \pm 33\ \mu M$ for Asc and $805 \pm 108\ \mu M$ for DHA (103). The transport of Asc was proved to be Na^+ dependent, while the uptake of DHA was Na^+ independent. Asc crosses the apical membrane with two Na^+ ions, whereas DHA enters through facilitated diffusion. Asc uptake is inhibited by the increasing intracellular concentration of glucose (trans-inhibition). The external (cis side) glucose does not interfere with Asc uptake, and the observation that SCN^- inhibits Asc uptake while stimulating the glucose transport clearly rules out the mediation of Asc transport by the Na^+-dependent glucose transporter SGLT1. The uptake of DHA was not influenced by glucose (103). The relatively low affinity of DHA transport compared with Asc transport indicates that most vitamin C is absorbed in the form of Asc.

The colon carcinoma cell line CaCo-2 is widely used as an *in vitro* model for enterocyte-like cells. The kinetics, the inhibition profile, the Na^+ dependence of transport, and reverse transcriptase-PCR analysis indicate that the Na^+-Asc cotransporters SVCT1 and SVCT2, the DHA transporters GLUT1 and GLUT3, and a third DHA transporter with characteristics of GLUT2 are expressed in CaCo-2 cells. It is in agreement with the observations that DHA is taken up by different members of the facilitative glucose transporter family (Fig. 3) (solute carrier, [SLC2]). GLUT1, 2, 3, and 4

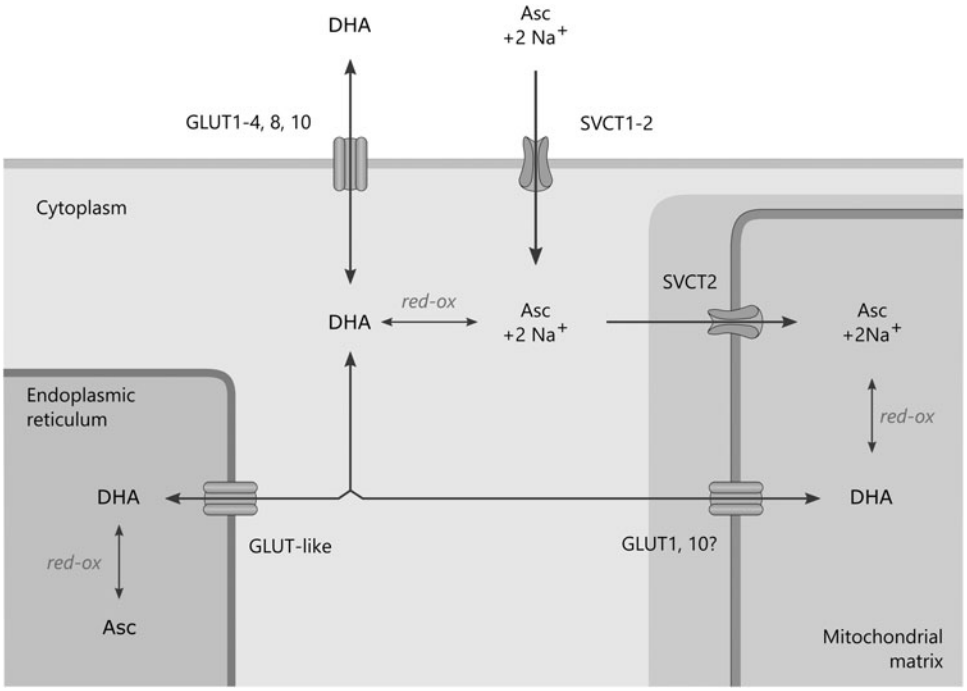


FIG. 3. Transport of Asc across the plasma and intracellular membranes. Ascorbate (Asc) is transported by SVCT1 and SVCT2 transporters. Both transporters cotransport Na^+ and Asc with a 2:1 stoichiometry along the electrochemical Na^+ gradient. The facilitated diffusion of DHA, mediated by GLUT1, 2, 3, and 4 transporters from class I and by GLUT8 and 10 transporters from class III glucose transporters. Mitochondrial Asc transport is the best characterized among the subcellular transports. Recently, the mitochondrial presence of SVCT2 was strengthened by experimental and *in silico* tools. GLUT1 has been described as a mitochondrial DHA transporter. No other transporter protein has been identified at the subcellular level, only a functional study found the preferential uptake of DHA in mammalian microsomal vesicles. SVCT, sodium-dependent Asc transporter.

from class I and GLUT8, 10 from class III glucose transporters are considered efficient DHA transporters (38, 92, 133, 134, 135, 137, 166). Since vitamin C can be detected in the human plasma practically only in its reduced form (43), the transport of DHA may be negligible under normal conditions. Directed localization of SVCT1 in the apical membrane of CaCo-2 cell monolayers was found (108). The apical cell surface expression of SVCT1 was also reinforced in renal and intestinal cells (147). Later, the accumulation of SVCT2 at the basolateral surface was described. This differential epithelial membrane localization suggests nonredundant functions of the two SVCTs (17). A basolateral targeting sequence in the N-terminus of SVCT2 is crucial for directing the protein to the basolateral membrane. Without this targeting sequence, SVCT2 was redirected to the apical side (165).

SVCT1 represents a high-capacity Asc transporter with lower affinity (K_M: 29–237 μM). It mostly occurs in epithelial tissues such as the intestine, lung, liver, kidney, and skin, where it is involved in the absorption and (renal) reabsorption of Asc to maintain the whole-body homeostasis (106, 161, 170, 171). Knockout of the *SVCT1* transporter gene resulted in 7–10-fold higher urinary loss and 50–70% lower blood level of vitamin C in mice compared to wild-type littermates (33).

SVCT2 can be characterized by lower capacity and higher affinity (K_M: 8–115 μM) than SVCT1. It is widely expressed in tissues such as the brain, lung, liver, skin, spleen, muscle, adrenal, eye, prostate, and testis to maintain and regulate the cellular redox state (21, 137, 161, 171). It is also necessary for prenatal transport of the ascorbic acid across the placenta (146).

Both SVCT1 and SVCT2 cotransport Na^+ and Asc with a 2:1 stoichiometry along the electrochemical Na^+ gradient and show a binding order of Na^+-Asc-Na^+ (Fig. 3) (63, 102).

In the case of oral administration, the plasma concentration of vitamin C is tightly controlled. Plasma vitamin C concentration reaches a plateau by increasing oral doses (64, 124). It can be explained by two factors. First, as we described above, the capacity of the Asc transporters is limited (as it is the case for all transport proteins). Second, the expression of *SVCT*s is fine tuned by their own ligand and by the redox state of the cell. The uptake of Asc and the expression of *SVCT1* were significantly decreased on elevated Asc levels (101). A similar self-regulatory role for Asc was demonstrated for SVCT2 in platelets, where *SVCT2* expression showed Asc concentration dependence at the translational level (135). It is not exactly clear whether vitamin C acts on its own carrier directly or indirectly by altering the redox state of the cell. This is a real dilemma since skeletal muscle cells modulated the expression of SVCT2 carrier according to their redox balance. The mRNA and protein levels of SVCT2 were upregulated in H_2O_2-treated myotubes, while antioxidant supplementation lowered the expression of *SVCT2* (136).

The investigation of the transcriptional regulation of human SVCT1 revealed that the basal transcription of SVCT1 depends on the binding of hepatic nuclear factor 1 (HNF-1) to the promoter of *SVCT1* (111). HNF-1 sites play an important role in ascorbic acid deprivation and supplementation on the activity and regulation of Asc transport systems (131). The promoter of *SVCT2* binds Yin Yang-1 (YY1) and interacts with specificity protein 1/3 (Sp1/Sp3) elements in the proximal promoter region. YY1 with Sp1 or Sp3 synergistically enhanced the promoter activity as well as the endogenous SVCT2 protein expression (130). Although Asc is absorbed along the entire length of the human intestine (103), it was reported that the carrier-mediated Asc uptake is significantly lower in the colon than in the jejunum (148). It was associated with a significantly lower level of expression of *SVCT1* and *SVCT2* at both protein and mRNA levels. The lower level of Asc uptake in colon can be at least partially attributed to differential levels of transcription of the SLC23A1 and SLC23A2 genes between these regions. Changes were found in both transcription factor abundance and histone modifications relevant to the control of *SVCT1* and *SVCT2* expression level in the colon and jejunum. As we saw, the basal activity of *SVCT1* and *SVCT2* promoters is regulated by HNF-1α and Sp1 (111, 130, 131). The levels of both transcription factors (HNF-1α and Sp1) were significantly lower in the colon compared to the jejunum (148).

Furthermore, two euchromatin markers for both genes were lower and a heterochromatin marker for SVCT1 was higher in the colon compared to the jejunum (148). At this point it is worth to note that vitamin C has been shown to regulate the epigenome, suggesting a possible role of vitamin C itself in the regional expression of genes.

As it can be expected, polymorphisms in the genes encoding SVCTs are strongly associated with plasma Asc levels and likely impact tissue cellular vitamin C status. A few SNPs in *SLC23A1* caused lower SVCT1 activity and consequently lower plasma or serum Asc concentration. Unfortunately, studies are lacking on the possible effects of genetic variation in SLC23A2 on cellular vitamin C status (112).

The picture on vitamin C transporters is much more blur at the subcellular level. The endoplasmic reticulum (ER) in human cells should possess transporter(s) to ensure the substrate supply of intraluminal vitamin C utilizing enzymes. However, to date, no Asc or DHA transporter has been identified at the molecular level in the ER. The preferential uptake of DHA was found in mammalian microsomal vesicles in a functional study. The properties of transport suggested the involvement of GLUT-type transporter(s) (12). According to this assumption, almost no Asc uptake could be observed; furthermore, the oxidation of Asc to DHA was a prerequisite for its uptake (36). The reported microsomal membrane-associated Asc oxidase activity can be the initiator of the uptake of vitamin C (153). More recently, GLUT10 was proposed to act as an ER DHA transporter (142), but the fact that its inherited deficiency is restricted to certain cell types suggests that other ER DHA transporters may exist (Fig. 3).

The initial observation of mitochondrial glucose and DHA uptake in plant cells (151) raised the possibility of the role of GLUT family in mitochondrial vitamin C (DHA) transport. Indeed GLUT1 was found to be localized in the mitochondrial inner membrane of human kidney (293T) cells (81). Later, another member of the GLUT family, namely GLUT10, was found to be localized in the mitochondrial inner membrane of rat aortic smooth muscle cells [3T3-L1 and murine adipocytes (A10)] (90). The mitochondrial uptake and accumulation of the reduced form, Asc, could not be observed in mitochondria from human kidney cells nor from rat liver tissue (81, 93). Thus, DHA was considered to be the transported form of vitamin C, and GLUT family members were thought to mediate its transport through the mitochondrial membrane. However, recently, the mitochondrial expression of *SVCT2* and Na^+-dependent mitochondrial Asc

uptake were revealed by Western blot experiments (7, 66). The association of SVCT2 protein with mitochondria was also confirmed by both colocalization experiments and immunoblotting of proteins extracted from highly purified mitochondrial fractions (119). At the same time, no *GLUT10* expression could be observed and the mitochondrial localization of GLUT1 could also not be corroborated (119), and thus, the role of GLUTs in mitochondrial vitamin C transport (at least in the investigated HEK-293 cell line) was queried. Very recently, the role of GLUT1 as a mitochondrial DHA transporter could be confirmed by *in silico* prediction tools; however, the mitochondrial presence of GLUT10 is not likely at this moment, since this transport protein got by far the lowest mitochondrial localization scores. The latest experimental observations on the mitochondrial presence of SVCT2 were also verified by computational prediction tools (Fig. 3) (149).

Finally, the localization and targeting of GLUT8 are conspicuously similar to the sorting mechanisms reported for lysosomal proteins (44). According to this observation, GLUT8 has been found to be associated with endosomes and lysosomes (138). Since GLUT8 is known to transport DHA (34), it is likely that DHA transport in the lysosomes is occurred *via* GLUT8.

Due to the saturation and tight regulation of Asc transporters, the maximum uptake of vitamin C can only be reached at lower oral doses, and then, it declines with increasing intake. This finding was confirmed by experimental results as well as pharmacokinetic models (66, 94, 126). On the grounds of this limitation of Asc uptake, the oral intake of mega dose of Asc is not accompanied by elevated plasma levels. As we will discuss in the next chapter, pharmacological plasma concentrations of vitamin C can only be reached *via* intravenous administration of the vitamin (124).

The regulation of Asc concentrations in seed plants and green algae

The biosynthesis of Asc in higher plants and green algae proceeds mostly *via* the Smirnoff–Wheeler pathway, during which no ROS are produced, which is in contrast with the animal-like pathway [reviewed recently by Refs. (19, 173)] (Fig. 4). There may be three alternative pathways in plants, with contested significance, including (i) the L-gulose pathway (175, 176), (ii) the galacturonate (''pectin scavenging'') pathway (2), (iii) and the animal-like Asc biosynthesis (myo-inositol) pathway (35, 98).

The Smirnoff–Wheeler pathway involves the conversion of D-mannose into Asc *via* a series of L-galactose containing intermediates. The final step, the oxidation of L-galactono-1,4-lactone into Asc, is catalyzed by galactono-1,4-lactone dehydrogenase, associated with the mitochondrial complex I (113).

The rate of Asc biosynthesis is largely determined by the expression level of *VTC2*, encoding GDP-L-galactose phosphorylase, which strongly responds to high light and is regulated by the circadian clock in higher plants (46). Asc biosynthesis is also dependent on photosynthetic electron transport (83) *via* poorly understood mechanisms (Fig. 4A). Under stress conditions, including UV-B (61), ozone (25), salt (71), and high light stress (46, 117), a two- to threefold increase of Asc content can be observed on the timescale of days in seed plants.

Because of the beneficial properties of Asc, both on plant physiology and as an essential nutrient for humans, there have been a large number of attempts to increase its concentration in plant leaves and fruits. The most obvious way is to overexpress the enzymes participating in its biosynthesis. However, this resulted in moderate success, maximum threefold increase in leaf Asc content when using stable overexpression [reviewed by Ref. (96)]; on the contrary, transient overexpression of both kiwifruit GDP-L-galactose phosphorylase and GDP-mannose-3′,5′-epimerase in tobacco leaves resulted in up to an eightfold increase in Asc content (20). The reason behind the moderate increase achieved on stable transformation of the biosynthesis pathway genes may be the strong feedback regulation of Asc on *VTC2* expression (46) and on GDP-L-galactose phosphorylase translation (88).

Asc biosynthesis and its regulation are less well studied in nonvascular plants. Bryophytes and green algae contain about 100-fold less Asc than higher plants [reviewed by Refs. (62, 173)]. Therefore, the question arises how these organisms can cope with environmental stress conditions if possessing such low Asc contents. It was shown recently that in contrast to seed plants, algae lack a negative feedback regulation in the physiological concentration range, and instead, a feedforward regulation was found, enabling a very rapid and manifold increase in Asc biosynthesis on stress conditions (168) (Fig. 4B).

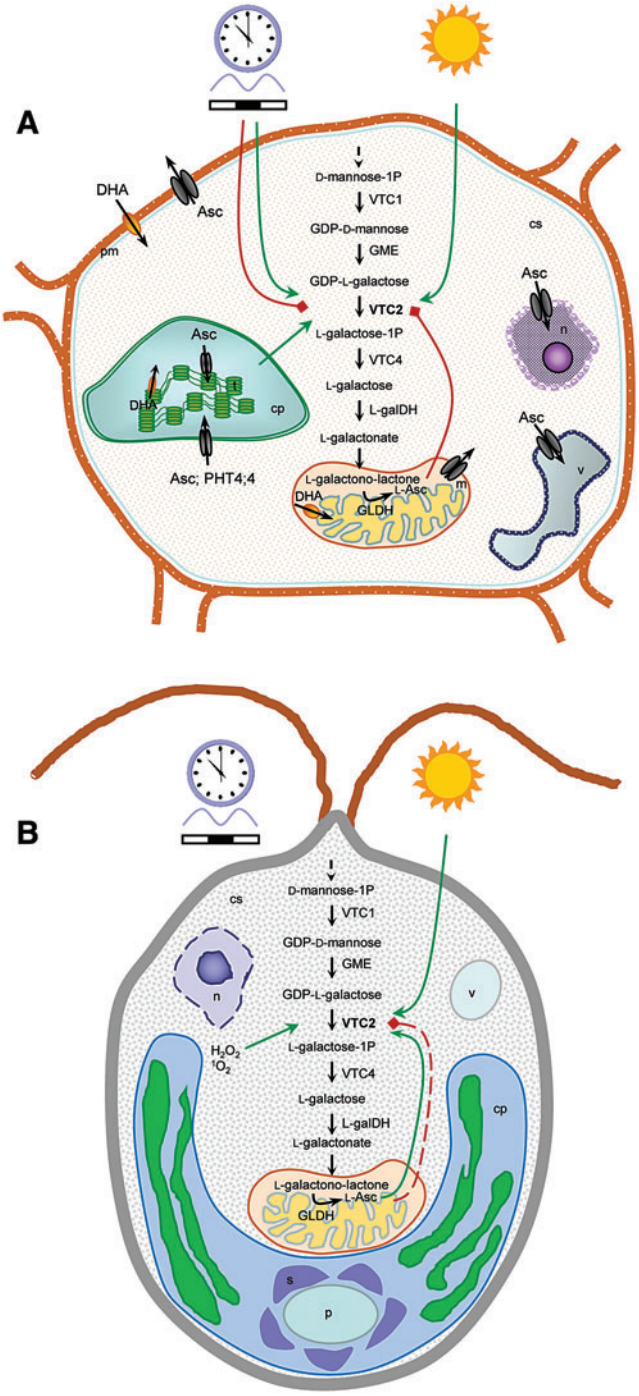
The amount of Asc is regulated not only at the level of biosynthesis but also by its regeneration. Asc becomes oxidized to MDA in various reactions, for example, during the scavenging of ROS and organic radicals; inside the thylakoid lumen, MDA is produced by VDE and on electron donation by Asc to PSII or PSI. In the chloroplast stroma, MDA can be reduced back to Asc by Fd or by MDAR both in the chloroplast stroma and the cytosol (5). Inside the thylakoid lumen, in the absence of Fd and MDAR, MDA spontaneously disproportionates to Asc and DHA (105). Following this reaction, DHA is transported across the thylakoid lumen to the stroma *via* yet unidentified Asc transporters (51). DHAR plays essential roles in maintaining the Asc concentration at a desired level both in the chloroplast and in other cellular compartments: if DHA does not become reduced, it undergoes irreversible hydrolysis (Fig. 1), which results in a decrease of the Asc pool. Asc being a major reductant in plants, DHAR also contributes to the regulation of the cellular redox state.

By overexpressing DHAR in higher plants, approximately threefold increase in total Asc content could be achieved, which resulted in a better growth and higher resistance to heat stress and methylviologen treatment (172). In contrast, DHAR overexpressing plants are more susceptible to drought stress, since the DHA/Asc ratio strongly affects the amount of H_2O_2, which is a signaling molecule with a strong effect on stomatal opening (31). Increasing the amount of Asc relative to DHA resulted in a strongly decreased H_2O_2 content, and thus, the regulation of stomatal closure was disturbed and these plants became sensitive to drought stress (31).

Asc content in plants may be also controlled by its degradation. The major Asc degradation pathway in seed plants occurs *via* DHA, yielding oxalate and L-threonate (65, 160); in the Vitaceae family, Asc may also get degraded *via* the L-tartrate pathway. Using [^{14}C] Asc labeling, Truffault *et al.* (160) found that Asc degradation was stimulated by darkness,

and the degradation rate was ~63% of the Asc pool per day in tomato leaves, which was constant and independent of the initial Asc and DHA concentrations.

On the contrary, it was found that in green algae, the rate of Asc degradation is very rapid: on a light-to-dark-transition, it occurs with a halftime of ~2 h (168); however, the pathway of Asc degradation is unknown. It also remains to be investigated whether the rate of Asc degradation is a controlled process and if it participates in the maintenance of optimal Asc level in higher plants and algae; it also cannot be excluded that Asc degradation has a recycling role.



Asc cannot freely diffuse through biological membranes because of its size and negative charge at physiological pH and most probably the neutral DHA is also insufficiently lipophilic to efficiently cross lipid membranes by simple diffusion (132). The last step of Asc biosynthesis takes place in the mitochondria; therefore, Asc transporters are most likely essential for maintaining optimal Asc concentrations in the various cellular compartments, including the chloroplast, nucleus, cytosol, cell wall, and vacuole (51) (Fig. 4A). It has also been demonstrated that Asc is transported throughout the plant *via* the phloem from source to sink tissues (56).

DHA is taken up *via* the plasma membrane and the transporter is distinct of glucose carriers, whereas a mitochondrion-localized DHA transporter shows similarities to glucose transporters (150). However, the molecular identity of these transporters remains to be unraveled. Twelve members of the Arabidopsis nucleobase-Asc transporter family have been molecularly characterized (109), however, no evidence has been found for Asc transport activity for any of these proteins. A substantial breakthrough was achieved by Miyaji *et al.* (115), who have identified a chloroplast-localized Asc transporter, called AtPHT4;4 (Fig. 4A). AtPHT4;4 knockout mutants exhibited moderately reduced levels of Asc in the chloroplast,

FIG. 4. Ascorbate biosynthesis and its regulation in seed plants (A) and green algae (B), and identified and putative Asc transporters in seed plants (A). Asc is synthesized mostly *via* the Smirnoff–Wheeler pathway both in seed plants and green algae. Enzymes of the Smirnoff–Wheeler pathway include VTC1, GDP-mannose pyrophosphorylase; GME, GDP-mannose-3′,5′-epimerase; VTC2, GDP-L-galactose phosphorylase; VTC4, L-galactose-1-phosphate phosphatase; L-galDH, L-galactose dehydrogenase; GLDH, L-GalL dehydrogenase. Most of the steps occur in the cytosol, except for the final step taking place in the mitochondrium. The *VTC2* gene, encoding GDP-L-galactose phosphorylase, plays a major role in the regulation of Asc biosynthesis. In higher plants, its expression is induced by light, regulated by photosynthetic reactions *via* poorly understood mechanisms and by the circadian clock. The expression of *VTC2* and the translation of the enzyme are both feedback inhibited by Asc. In green algae, light and reactive oxygen species induce the expression of *VTC2* and Asc has a stimulatory effect on its expression in the low, physiological concentration range; however, a feedback inhibition in the m*M* range is also likely to take place. The Asc content does not depend on the circadian rhythm in green algae. In seed plants, characterized Asc transporters include a DHA transporter in the plasma membrane that is distinct of glucose carriers; a DHA transporter, probably similar to glucose transporters, is located in the mitochondrium; and AtPHT4;4 was identified as a chloroplast-localized Asc transporter. Putative Asc and DHA transporters include Asc transporters responsible for the export of Asc out of the mitochondrium, into the thylakoid lumen and through the plasma membrane, transporters for ensuring the uptake of Asc into the vacuole and possibly into the nucleus (alternatively, ascorbate diffuses through the nuclear pores). DHA also has to be transported out of the thylakoid lumen for regeneration. cs, cytosol; cp, chloroplast; m, mitochondrium; n, nucleus; p, pyrenoid; pm, plasma membrane; s, starch granules; t, thylakoid membrane; v, vacuole. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

resulting in decreased non-photochemical quenching. However, plant growth and photosynthetic efficiency were not affected, suggesting that AtPHT4;4 may not be the only chloroplast membrane Asc transporter. In addition, it is very likely that there are Asc and DHA transporters located in the thylakoid membrane as well, to allow for an efficient regeneration of DHA and a sustained Asc supply into the thylakoid lumen (51). Miyaji *et al.* (115) suggested that AtPHT4;1 may be a thylakoid membrane-localized Asc transporter, but it was shown before long that this is not the case (80). In green algae and other photosynthesizing unicellular species, no Asc transporters have been identified so far.

The elucidation of additional Asc and DHA transporter proteins both in the chloroplast and other cellular membrane systems warrants further investigations. The transporters are likely to play a regulatory role in setting the appropriate Asc concentration in each cellular compartment. We also note that identifying Asc transporters may be a key to substantially increase the Asc content of plants. This notion is based on the fact that an alpine plant, *Soldanella alpina*, accumulates ~ 10 times more Asc in its leaves than low-land plants, but most of this Asc is stored in the vacuole, most probably as a safety storage against UV damage; together with this, the chloroplastic Asc concentration of *S. alpina* is in the same range as in Arabidopsis and spinach, that is, about 25 m*M* (16).

Potentially Harmful Effects of Asc in Animals and Plants

The pro-oxidant role of Asc and its therapeutic usage in humans

In the course of the Haber–Weiss reaction the toxic $OH^{\bullet}$ can be generated from the less reactive $O_2^{\bullet -}$ and hydrogen peroxide (82). In biological systems, this reaction is thermodynamically unfavorable, and it needs a metal ion catalyst to occur. In the Fenton reaction, ferrous iron reacts with H_2O_2 to generate $OH^{\bullet}$ and ferric iron (Eq. 1). Ferrous iron can also readily react with O_2, reducing it to superoxide radical (Eq. 2), which in turn dismutes to H_2O_2 and O_2 (Eq. 3) (48). *In vitro*, Asc, as an excellent electron donor, can reduce ferric iron to ferrous iron while being oxidized to Asc radical (Eq. 4). By this means, Asc contributes to the continuous generation of ROS. Not accidentally, iron salt and Asc mixtures have been used *in vitro* to induce lipid peroxidation and different oxidative damages (91).

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^{\bullet} \qquad \text{Eq.1}$$

$$Fe^{2+} + O_2 \rightarrow Fe^{3+} + O_2^{\bullet -} \qquad \text{Eq.2}$$

$$O_2^{\bullet -} + O_2^{\bullet -} + 2H^+ \rightarrow H_2O_2 + O_2 \qquad \text{Eq.3}$$

$$Fe^{3+} + Asc^- \rightarrow Fe^{2+} + Asc^{\bullet -} \qquad \text{Eq.4}$$

Therefore, in biological systems, in the course of the formation of ascorbyl radical, Asc can donate an electron to a transition metal such as iron or copper. The reduced metal is capable of reacting with O_2 forming $O_2^{\bullet -}$ anion and then H_2O_2. In the presence of higher (m*M*) concentrations of Asc, H_2O_2 can readily react with further transition metal ions in the Fenton reaction, to form the highly reactive, cytotoxic $OH^{\bullet}$ (82).

It is reasonable to presume that the tight control of Asc concentration *via* its strictly regulated transport provides the background to prevent continuous tissue exposure to high concentrations of H_2O_2. However, the temporal bypass of this tight control by parenteral administration of Asc gives the possibility to form H_2O_2 in discrete, well-defined time periods, decreasing the likelihood of harm, and provides a pharmacologic basis for therapeutic use of Asc (127).

The generated H_2O_2 and $OH^{\bullet}$ may induce DNA injury that is followed by the activation of poly(ADP-ribose) polymerase-1, the depletion of NAD^+ and ATP (1). Glutathione peroxidase (GPX), peroxiredoxin, and thioredoxin certainly take a major part in the removal of H_2O_2. On the recycling of peroxiredoxins and the action of GPX, GSH is oxidized to glutathione disulfide (GSSG). The generated GSSG and oxidized thioredoxin can be rereduced by NADPH, which in turn is regenerated from glucose *via* the pentose shunt (Fig. 5). Hence, the regeneration of NADPH may use up glucose, preventing ATP production (141).

Cancer cells compared to normal cells can be characterized by increased steady-state levels of ROS (*i.e.*, $O_2^{\bullet -}$ and H_2O_2); furthermore, they show increased susceptibility to glucose deprivation-induced cytotoxicity and oxidative stress (3, 6). These observations support the hypothesis that cancer cells increase glucose metabolism to compensate for excess metabolic production of ROS. This way the utilization of glucose by the enhanced NADPH requirement due to the enhanced GSH consumption may provide a biochemical target for selectively enhancing cytotoxicity and oxidative stress in human cancer cells (159).

In the light of the above observations, it is not surprising that the exposure of different cancer cell lines to Asc up to the concentrations of 20 m*M* for 1 h caused a 50% decrease in cell survival, while it did not affect the survival of normal human cells (28). In the case of human Burkitt's lymphoma cells (JLP-119), significant cell death could be observed at as low as 0.3 m*M* of Asc concentration (28). The cytotoxicity of Asc on A2780 human ovarian cancer cells could also be characterized by similarly low 0.3 m*M* of IC50 (100). The cytotoxic effect of high-dose Asc on different cancer cell lines has been demonstrated by various research groups (28, 42, 84, 127). These studies show that Asc present at high concentrations can induce H_2O_2 generation, which is preferentially cytotoxic to cancer cells. Cell death was dependent on H_2O_2 production mediated by extracellular Asc oxidation (Fig. 5) (28, 30, 42, 84, 127). The H_2O_2-mediated Asc toxicity could be alleviated by exogenous catalase or adenoviral-mediated overexpression of catalase or GPX 1 (139). These results suggested that Asc given parenterally (to bypass the tight control of oral absorption discussed in the section ''The Regulation of Cellular Asc Levels'') can be an effective antitumor agent. Indeed, the parenteral administration of Asc decreased the growth rate of murine hepatoma (167), ovarian, pancreatic, and glioblastoma tumors established in mice (30). Parenteral administration of Asc resulted in a 12-fold higher ascorbyl radical level in the extracellular fluid than in the blood (29). Since H_2O_2 is immediately scavenged in blood (77), elevated level of H_2O_2 due to parenteral Asc administration could only be measured in the extracellular fluid (29). By this means, Asc at high doses can be a prodrug for the formation of ascorbyl radical and H_2O_2 in the extracellular space but not in blood.

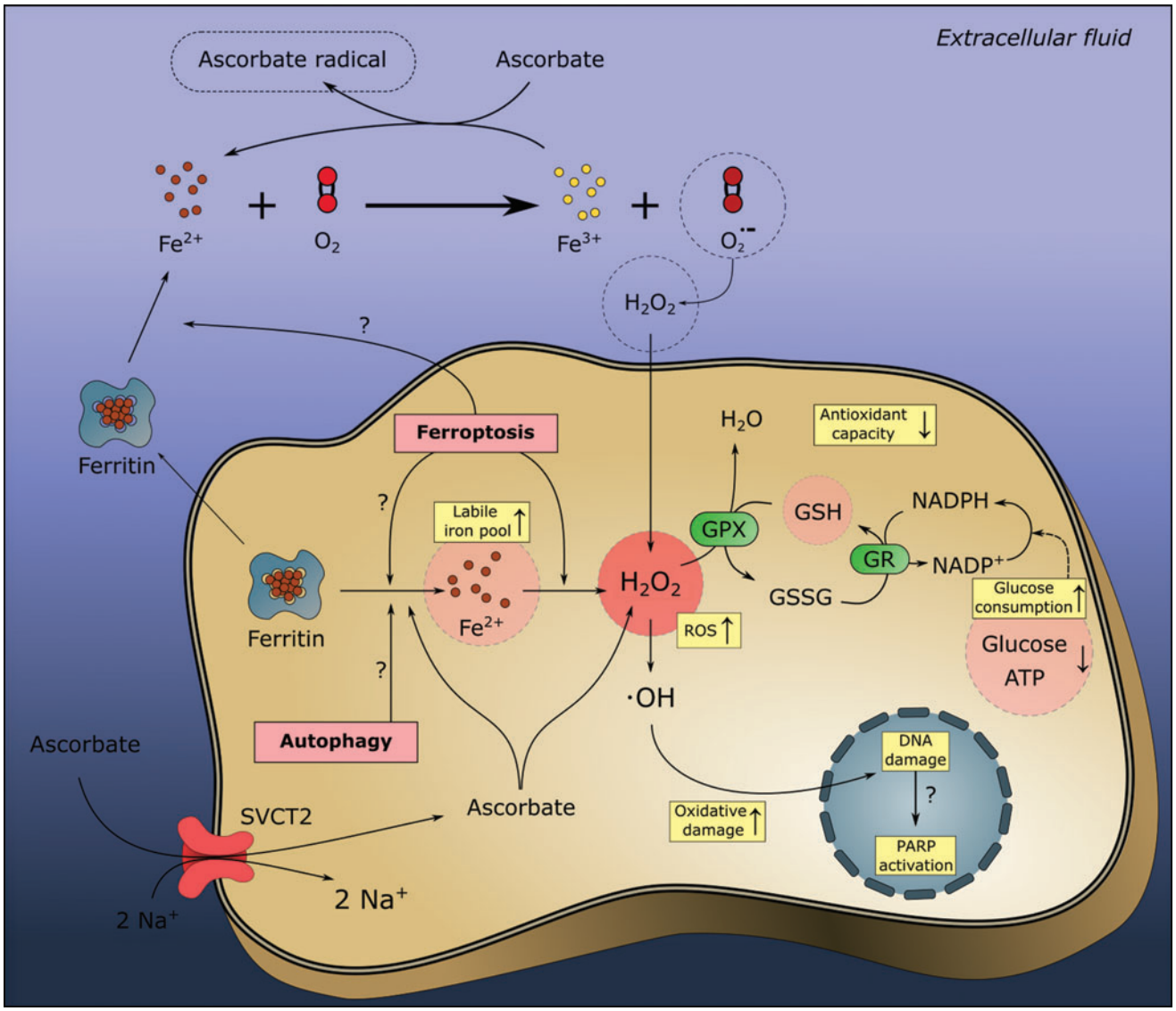


FIG. 5. The generation of ROS by high-dose Asc and their potential role in cell death mechanisms. At high concentrations, Asc contributes to H_2O_2 generation, in which intracellular metals play an important role. High-dose Asc induces cytotoxicity associated with increased ferritin release. The elevated ferritin production and secretion can serve as a continuous iron source for Asc-mediated H_2O_2 production. ROS and Asc can disrupt cellular iron metabolism that leads to an increased labile iron pool. Cancer cells show significant dose-dependent Asc-induced elevation in cellular labile iron pool that cannot be observed in normal human primary cells. The high concentration of H_2O_2 in the presence of high-labile iron facilitates the occurrence of Fenton reaction that generates the highly toxic $OH^{\bullet}$. The scavenging of H_2O_2 by GPX or by catalase can prevent the elevation of labile iron pool and cytotoxicity of high-dose Asc. Ascorbate is not toxic for normal cells because of its lower labile iron levels and basal and Asc-mediated H_2O_2, which is metabolized quickly before it can take part in pro-oxidant reactions. The high-dose Asc treatment-induced cell death of cancer cells was presumed to be apoptotic. However, recent studies proposed autophagy as a potential high-dose Asc-induced cell death mechanism. The autophagy pathway was detected by the processing of LC3 to LC3-II and the redistribution of LC3-II to the surface of autophagosomes. SVCT2 sensitizes cancer cells to autophagic damage by increasing the Asc concentration and intracellular ROS production. The knockdown of SVCT2 dramatically alleviated DNA damage, ATP depletion, and inhibition of mTOR pathway induced by Asc. These observations suggest that the intracellular ROS formation besides the extracellular one may also contribute to Asc-mediated cytotoxicity. $OH^{\bullet}$, hydroxyl radical; ROS, reactive oxygen species. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

The effects of various chelators suggest that intracellular metals play an important role in Asc toxicity. The pre-incubation of human DU145 cells with deferoxamine, a cell permeable metal chelator, prevented the loss of viability of tumor cells exposed to high concentration of Asc (167). The DNA damage in high-dose Asc-treated lymphocytes could also be prevented by iron and copper chelators (162). The high-dose Asc-induced cytotoxicity of neuroblastoma cell lines was associated with increased ferritin release and increased lactate production. The elevated ferritin production

and secretion can serve as a continuous iron source for Asc-mediated H_2O_2 production (Fig. 5) (42). The free or labile (loosely bound) iron is highly cytotoxic, thus it is usually bound to proteins, such as ferritin (15, 143). ROS and Asc can disrupt the cellular iron metabolism that leads to increased labile iron pool (Fig. 5) (8, 23, 74). Cancer cells, in general, can be characterized by elevated intracellular labile iron levels (156). In addition to the higher basal level of labile iron in glioblastoma and nonsmall cell, lung cancer cells showed a significant dose-dependent Asc-induced elevation in cellular labile iron pool that was not seen in normal human astrocytes or normal human bronchial epithelial primary cells (139).

The observed loss of Fe-S cluster protein activity and the accompanying elevation of labile iron pool could be prevented by the overexpression of catalase implicating H_2O_2 as the causative agent in Asc-induced increases of labile iron pool (Fig. 5) (139). The elevated mitochondrial ROS caused elevated labile iron pool, which could lead to more intensive oxidation of Asc to generate more H_2O_2, causing a further elevation of labile iron pool of cancer cells compared to normal cells. The high concentration of H_2O_2 in the presence of high-labile iron facilitates the occurrence of Fenton reaction that generates the highly toxic $OH^{\bullet}$ (Fig. 5) (139). In normal cells with lower basal and Asc-mediated H_2O_2 and labile iron levels, H_2O_2 is metabolized quickly before it can take part in pro-oxidant reactions. Therefore, Asc is not toxic for normal cells (28, 139). The observed safety of high-dose Asc in animal xenograft (30, 100, 167) and human (47, 123) studies can also be explained by this phenomenon.

The cytotoxic effect of Asc on cancer cells is rather well documented; however, its precise cell death mechanism has not been elucidated yet. The cell death of cancer cells due to high-dose Asc treatment has been presumed by several studies to be apoptotic (Fig. 5) (26, 69, 78, 95). The induction of apoptosis in B16F10 murine melanoma was assessed by phosphatidylserine externalization using FITC-annexin V binding (78). The reduction in the mitochondrial membrane potential during cell death (apoptosis) and cytochrome-c release from mitochondria could also be observed. However, cell death could not be inhibited by z-IETD-fmk, indicating that Asc-induced apoptosis was not mediated by caspase-8 (78). In another study, Hong *et al.* (69) found that Asc induced cell death through the apoptosis-inducing factor in human breast cancer cells (SK-BR3, Hs578T), but caspase (3 and 9) cleavage was also not induced (69). On the contrary, using a fluorescence-based pan-caspase activity assay kit, Carosio *et al.* (26) found general and specific caspase activity (caspase-1, −2, −3, −6, −7, −8, −9, −10) in neuroblastoma cells treated by high-dose Asc (26). The apoptotic cell death was also assessed by FITC-annexin V and mitochondrial transmembrane potential assays. It should also be noted that this study suggested that iron depletion is responsible for the Asc-induced cell death in neuroblastoma cells that is controversial with all other studies. Flow cytometric analysis of Lin *et al.* (95) showed that Asc induced significant cell cycle arrest and apoptosis in human melanoma (A375.S2) cell line in a dose-dependent manner. Induction of apoptosis involved an increase in the levels of p53, p21, and cellular Ca^{2+} and a decrease in mitochondrial membrane potential and activation of caspase 3 (95).

Besides apoptotic cell death, autophagy has also been proposed as a potential high-dose Asc-induced cell death mechanism (Fig. 5). Pancreatic cancer cells treated with high-dose Asc demonstrated an increase in LC3-II immunoreactive protein (37). This increase in LC3-II and the caspase-independent cell death could be reversed by a pretreatment of the cells with catalase, suggesting that the Asc-induced induction of autophagy is mediated by the generated H_2O_2. Similarly, Chen *et al.* found that high-dose Asc treatment depleted ATP and induced autophagy in prostate cancer cells, where the autophagy pathway could be detected by the processing of LC3 to LC3-II and the redistribution of LC3-II to the surface of autophagosomes (27).

Similarly, Fukui *et al.* showed that high-dose Asc induced the formation of autophagosomes, and the presence of autophagy inhibitors suppresses Asc-induced cell death (58). The above results were corroborated by Du *et al.*, who found that the treatment of pancreatic cancer cells (MIA PaCa-2) with high-dose Asc induced a caspase-independent cell death that was associated with autophagy (49). The involvement of autophagy in cell death was demonstrated by an increase of LC3-II 4–6 h after the Asc treatment. They also ruled out that PARP-1 activation and ATP depletion contribute to Asc-induced cell death. The inhibition of caspases did not reverse the percentage of necrotic or apoptotic cells with Asc (49). These findings also suggest a necrotic component of the Asc-induced cell death.

It is interesting that the studies proposing apoptotic cell death due to pharmacologic Asc treatment are dated before 2010, while those proposing autophagic cell death are dated after 2010. Moreover, it is also worth noting that the mechanism of cell death by high-dose Asc treatment depends on the cell type, on the applied concentration, on the duration of Asc treatment, on the composition of culture media, and certainly on several other conditions.

In a study on nine breast cancer cell lines, it was found that functional SVCT2 sensitizes breast cancer cells to autophagic damage by increasing the Asc concentration and intracellular ROS production (70). Intriguingly, in another study on cholangiocarcinoma cells, SVCT2 expression was also inversely correlated with IC50 values of Asc. The knockdown of SVCT2 dramatically alleviated DNA damage, ATP depletion, and inhibition of mTOR pathway induced by Asc. Furthermore, SVCT2 knockdown endowed cholangiocarcinoma cells with resistance to Asc treatment (169). These observations suggest that the intracellular ROS formation may also contribute to the Asc-mediated cytotoxicity (Fig. 5).

The observations that ferroptosis is an iron-dependent ROS-mediated cell death mechanism that could be suppressed by cotreatment with the iron chelator deferoxamine (45), that it was not consistently modulated by inhibitors of caspase, cathepsin, or calpain proteases (z-VAD-fmk, E64d, or ALLN) (45), and that autophagy is involved in its induction through the elevation of labile iron pool (60) lead us to hypothesize that ferroptosis (at least partly) may be responsible for the high Asc dose-induced cytotoxicity in cancer cells.

Negative effects of high Asc concentration in plants: interference with ROS signaling, redox balance, and its pro-oxidant property

It has been long considered that ROS have negative effects on cellular functions and cell viability in plants. However, there is a paradigm shift going on, as more and more evidence

demonstrates that ROS production linked to signaling is required for a plethora of plant responses to developmental and environmental changes [reviewed by Refs. (54, 98)]. It was discovered recently that basal level of ROS is even required to support life [reviewed by Refs. (75, 114)]. ROS may act directly by oxidizing, for example, certain regulatory factors influencing protein translation and they may lead to changes in cellular redox potential that will impact various redox-sensitive proteins.

It has been also shown that Asc regulate the activity of chloroplastic APXs, which has immediate effects on the H_2O_2 levels, acting as a retrograde signal from the chloroplast to the nucleus [reviewed by Ref. (107)]. Another example is the abovementioned overexpression of DHAR, which resulted in several fold increase in Asc content, but as a consequence, the plants became less adaptive to drought stress due to impaired H_2O_2 signaling (31). In this context, the role of the plant antioxidant system is to control or mitigate the amount of cellular ROS, rather than completely eliminating them. This may also mean that plants have developed various control mechanisms to avoid the overproduction of antioxidants, such as the negative feedback mechanism exerted by Asc on *VTC2* expression and translation (46, 88) (Fig. 4A). Regarding the generation of plants with elevated Asc content, this may mean that even if we managed to overcome the feedback regulatory mechanisms to control Asc biosynthesis, the plants would not show increased stress tolerance; instead, adaptation responses may be hampered. The solution may be to overexpress the putative vacuolar Asc transporters (51) (Fig. 4A), in addition to overexpressing Asc biosynthesis genes, which would allow the accumulation of Asc in the vacuole and the physiological Asc concentrations in the other cellular compartments (chloroplast, cytosol, and mitochondria) would be maintained.

Another issue when considering the possible negative roles of Asc is its reducing property. Although in plant research, reductive stress is rarely considered, one may imagine that the accumulation of reductants may alter cellular redox balance, which is of high importance to enzymes under redox control.

Changing of redox balance within the chloroplast may also severely disturb a large number of physiological processes. One example, discovered recently, is that on sulfur deprivation of green algae, Asc can accumulate so strongly (to the m*M* range) that it over-reduces and thereby inactivates the Mn-cluster of the OEC (120) (Fig. 6A). Under normal growth conditions, this does not occur, because the Asc content is very low in green algae ($\sim 100\,\mu M$) (120, 164). On the contrary, seed plants have acquired high Asc concentrations during evolution to cope with the continuously changing environment (62), and thus, the question may be raised by which the mechanism of the OEC in seed plants is protected from the reducing effect of Asc. It has been shown earlier that in the absence of the extrinsic OEC proteins, the Mn-cluster becomes accessible to Asc, resulting in its inactivation (154). There are significant structural differences between the extrinsic proteins of seed plants and algae, among which the two types of PSBO proteins are shown in Figure 6B and C; it is conceivable that during evolution, the PSBO protein has evolved to protect the OEC against the reducing effect of Asc. As pointed out earlier, once the OEC is inactive, Asc provides electrons to the photosynthetic

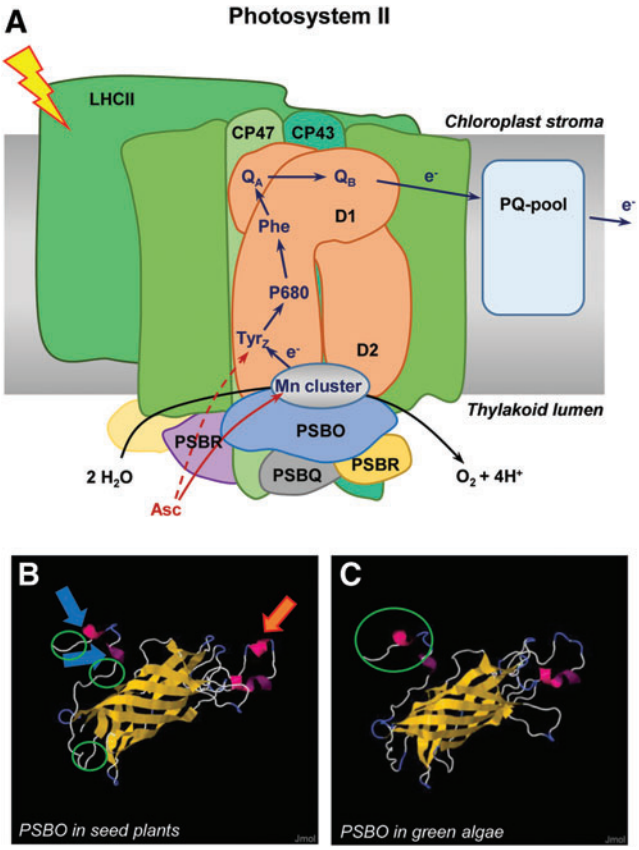


FIG. 6. Schematic presentation of PSII and the effect of Asc on electron transport. (A) Solar energy is captured by the light-harvesting complexes (LHCII) of PSII. Electrons extracted from water by the OEC of PSII are transferred to the PSII reaction center, the plastoquinone (PQ)-pool, several photosynthetic complexes, and finally to the Calvin–Benson cycle. The OEC is a vulnerable component of the photosynthetic electron transport chain. On heat stress, the extrinsic proteins of the OEC are released (primarily PSBO) and this is followed by the inactivation of the Mn-cluster. Under these conditions, Asc may provide electrons directly to Tyr_Z^+, sustaining a limited electron transport activity (158). In green algae, Asc may over-reduce the Mn-cluster when present in the m*M* range (denoted with continuous *red line*), and then provides electrons to Tyr_Z^+. In seed plants, Asc is not capable of inactivating the OEC at the m*M* concentration range, typical for the thylakoid lumen. The difference between green algae and seed plants is tentatively attributed to the differences between the PSBO protein structures of seed plants **(B)** and green algae **(C)**: in seed plants, there are two disulfide bridges (denoted by *blue arrows*), whereas there is none in green algae; the binding sites (denoted by *green circles*) are spread in seed plants, whereas they are all located at the N-terminus in green algae; the plant PSBO protein is less accessible to solvents than that of green algae; seed plant PSBO has also an extra strand (denoted by a *red arrow*). Model created by The PredictProtein server. OEC, oxygen-evolving complex; PSII, photosystem II. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

electron transport chain *via* Tyr_Z^+, which has a moderate effect in mitigating donor-side-induced photoinhibition (120, 157, 158) (Fig. 6A).

In addition to its antioxidant character, the pro-oxidant nature of Asc has been also described, although rarely considered. In plants, the Fenton reaction (Eqs. 1–4) was shown to occur in isolated thylakoid membranes (163) and it occurs also *in vivo* in the apoplast, where it causes the nonenzymatic scission of polysaccharides, leading to cell wall loosening during fruit ripening (50, 57, 116, 140) and cell expansion (81, 119). It has been also reported that externally supplied Asc results in oxidative stress in intact Arabidopsis leaves (129). The number of studies on the pro-oxidant effect of Asc in plants is very limited, especially compared to the field of mammalian research and the topic certainly merits further investigations.

H_2O_2 is formed also during Asc degradation (65, 79), which may contribute to the pro-oxidant effect of Asc. Therefore, it is conceivable that on stress conditions, when the oxidation of Asc proceeds at a higher rate than its regeneration, substantial Asc degradation occurs along with the production of H_2O_2, and thus, the stress situation is aggravated.

Conclusions

To maintain the cellular concentration of Asc in an optimum range is of vital importance, because Asc may interfere with regulatory mechanisms by various means and may even cause or enhance oxidative stress.

In plants, the most obvious mechanism of maintaining an optimal Asc concentration is the regulation of its biosynthesis: a number of studies show that Asc biosynthesis is regulated by feedback mechanisms, both on the level of gene expression and protein translation. Other regulatory points of Asc concentration may be its degradation and inter- and intracellular transport; both these mechanisms warrant further investigations. Considering that high Asc levels may have negative effects on cellular functions is very important also when aiming at enhancing the Asc contents of leaves and fruits. A feasible solution may be to increase the Asc levels in specific storage organs or in the vacuole, in a similar manner as it can be often observed in fruits and leaves possessing extraordinarily high Asc contents.

On the contrary to plants, the key regulatory element of Asc level in humans and human cells is its transport. As it was delineated, Asc can reduce transition metals and the reduced metal is capable of reacting with O_2 forming $O_2^{\bullet -}$ anion and then H_2O_2. In the presence of high concentration of Asc, H_2O_2 can readily react with further transition metal ions to form the highly reactive and cytotoxic $OH^{\bullet}$. The tight control of Asc concentration in the human body and cells *via* its transporters provides the background to prevent continuous tissue exposure to high concentrations of H_2O_2.

Normal cells, in comparison with cancer cells, can be characterized by lower basal and Asc-mediated H_2O_2 and labile iron levels, and thus, H_2O_2 is metabolized quickly before it can take part in pro-oxidant reactions. The temporal bypass of this tight control by parenteral administration of Asc gives the possibility to form H_2O_2 in discrete, well-defined time periods, which decreases the likelihood of harm and provides a pharmacologic basis for antitumor therapeutic use of Asc. Two crucial problems are waiting to be clarified in the future. The exact mechanism of high-dose Asc-induced cell death can help us to improve its therapeutic role. The other is the clarification of subcellular Asc transport in both humans and plants. This can shed more light on the regulation of Asc levels and its potential pro- or antioxidant roles in various subcellular compartments, where Asc has special functions, such as in the mitochondria, chloroplast, and ER.

Acknowledgments

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Address correspondence to:
Dr. Szilvia Z. Tóth
Institute of Plant Biology
Biological Research Centre
of the Hungarian Academy of Sciences
Temesvári krt. 62
Szeged H-6726
Hungary

E-mail: toth.szilviazita@brc.mta.hu

Dr. András Szarka
Laboratory of Biochemistry and Molecular Biology
Department of Applied Biotechnology and Food Science
Budapest University of Technology and Economics
Szent Gellért tér 4
Budapest H-1111
Hungary

E-mail: szarka@mail.bme.hu

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Abbreviations Used

APX = Asc peroxidase
Asc = ascorbate
DHA = dehydroascorbate
DHAR = dehydroascorbate reductase
ER = endoplasmic reticulum
Fd = ferredoxin
GPX = glutathione peroxidase
GSSG = glutathione disulfide
HIF-1 = hypoxia inducible factor 1
HNF-1 = hepatic nuclear factor 1
MDA = monodehydroascorbate
MDAR = monodehydroascorbate reductase
$O_2^{\bullet -}$ = superoxide
$OH^{\bullet}$ = hydroxyl radical
OEC = oxygen evolving complex
PSI = photosystem I
PSII = photosystem II
RNS = reactive nitrogen species
ROS = reactive oxygen species
SLC = solute carrier
SOD = superoxide dismutase
Sp1 = specificity protein 1
SVCT = sodium-dependent vitamin C transporter
TET = ten-eleven translocase
VDE = violaxanthin-deepoxidase
YY1 = Yin Yang-1