Although ascorbic acid is an important water-soluble antioxidant and enzyme cofactor in plants and animals, humans and some other species do not synthesize ascorbate due to the lack of the enzyme catalyzing the final step of the biosynthetic pathway, and for them it has become a vitamin. This review focuses on the role of ascorbate in various hydroxylation reactions and in the redox homeostasis of subcellular compartments including mitochondria and endoplasmic reticulum. Recently discovered functions of ascorbate in nucleic acid and histone dealkylation and proteoglycan deglycanation are also summarized. These new findings might delineate a role for ascorbate in the modulation of both pro- and anti-carcinogenic mechanisms. Recent advances and perspectives in therapeutic applications are also reviewed. On the basis of new and earlier observations, the advantages of the lost ability to synthesize ascorbate are pondered. The increasing knowledge of the functions of ascorbate and of its molecular sites of action can mechanistically substantiate a place for ascorbate in the treatment of various diseases.

Keywords: vitamin C; ascorbate; dehydroascorbic acid; L-gulonolactone-oxidase; antioxidant; pro-oxidant; mitochondrium; endoplasmic reticulum; redox; scurvy

Abbreviations: 2OG, α-ketoglutarate; AMD, age-related macular degeneration; CMT, Charcot–Marie–Tooth disease; DHA, dehydroascorbic acid; EGF, epidermal growth factor; ER, endoplasmic reticulum; GLO, gulonolactone oxidase; GSH, glutathione; HIF-1α, hypoxia-inducible factor-1α; mtDNA, mitochondrial DNA; nDNA, nuclear DNA; PHD, prolyl hydroxylase; pVHL, von Hippel–Lindau tumour promoter protein; ROS, reactive oxygen species; SGLT, sodium-dependent glucose cotransporter; SVCT, sodium-dependent vitamin C transporter

Introduction

The dominant role of oxidoreductions in the metabolic and energetic exchange between the living organism and its environment is well known. Ascorbate has special functions in this redox interrelationship, as an antioxidant and enzyme cofactor. Generally, ascorbate is regarded as a reducing agent; it is able to serve as an antioxidant in free radical-mediated oxidation processes. However, as a reducing agent it is also able to reduce redox-active metals such as copper and iron, thereby increasing the pro-oxidant chemistry of these metals. Thus ascorbate can act as both a pro-oxidant and an antioxidant. In general, at low ascorbate concentrations, ascorbate is prone to be a pro-oxidant, and at high concentrations, it will tend to be an antioxidant (Buettner and Jurkiewicz, 1996).
Our review focuses on new functions of ascorbate as a cofactor for nucleic acid and histone demethylation, proteoglycan deglycanation and on the role of ascorbate in the redox homeostasis of subcellular compartments. Some possible therapeutic applications are also discussed.

It has been known for a long time that the absence of fresh fruits and vegetables in the human diet leads to scurvy – a fatal disease affecting sailors (Lind, 1753) and soldiers (Kramer, 1739) in the past. Thus, for humans to survive, their diet must contain ascorbate; that is why ascorbate is a vitamin in humans. This raises the following questions: which are the most important features of ascorbate, and why does its deficiency have fatal consequences?

**Ascorbate as a cofactor in various hydroxylation reactions**

Ascorbate plays a crucial role in various hydroxylation reactions. There are several ascorbate-dependent mono- and dioxygenations in various neurotransmitter and hormone formation processes (for review, see Englard and Seifter, 1986), and ascorbate is also required for the hydroxylation of carnitine. It has been suggested that carnitine deficiency is responsible for the early symptoms of scurvy (Rebouche, 1991). In several cases, hydroxylation is catalysed by Fe(II)-dependent non-haeme oxygenases belonging to the family of α-ketoglutarate (2OG)-dependent dioxygenases. Dioxygenases include prolyl, asparaginyl and lysyl hydroxylases (Hewitson et al., 2003; Myllyharju and Kivirikko, 2004). Within this group, ABH2 and ABH3, two human DNA dioxygenases functioning as novel DNA repair enzymes have also been described (Sedgwick, 2004). Oxygen and 2OG are required for oxygenation, which also needs cofactors: Fe2+ and ascorbate. Iron in these enzymes is maintained in the active Fe(II) form by ascorbate (Myllyla et al., 1984; Tschank et al., 1994).

Several proteins with various functions can serve as potential substrates for these oxygenases. However, it is difficult to prove the physiological/pathological significance of these oxidoreductions. The role of the ascorbate-dependent hydroxylation of proline and lysine residues in the stabilization of the collagen molecule has been explored; 30% of cellular protein mass is made by collagen. The role of ascorbate in collagen synthesis and its connection to the development of scurvy has been extensively reviewed in several studies (Englard and Seifter, 1986; Peterkofsky, 1991). At present, at least 27 collagen types with at least 42 distinct polypeptide chains and more than 20 additional proteins having collagen-like domains have been described. In proteins with collagen-like domains, 4-hydroxyprolines are ubiquitous; therefore, proline hydroxylation has a crucial role in interactions between these proteins (Myllyharju and Kivirikko, 2004).

Hypoxia-inducible transcription factor-1α (HIF-1α) is also a substrate of these dioxygenases. Activity of HIF-1α can be regulated by hydroxylation, which needs ascorbate (Figure 1). In hypoxia, HIFs are expressed in all nucleated cells, serving as ‘global’ regulators in a complicated network (Semenza, 2003). More than 100 HIF target genes have already been identified.

![Figure 1](https://example.com/figure1.png)

**Figure 1** Role of ascorbate in the destruction of HIF-1α in normoxia. During hypoxia, HIF-1α enters the nucleus, forms a dimer with HIF-1β and the dimer binds to DNA through the hypoxia response element (HRE). In this way, transcription of oxygen-dependent genes is initiated. In normoxia, in the presence of oxygen and ascorbate, two proline residues and one asparagine residue of HIF-1α are hydroxylated by Fe(II) and 2-oxo-glutarate (2OG)-dependent dioxygenases, as prolyl hydroxylase domain family members, and by asparaginyl hydroxylase factor inhibiting HIF-1, respectively. Hydroxylation of prolyl residues results in the binding of the von Hippel–Lindau protein, which is part of the E3 ubiquitine ligase complex. This is the precondition for ubiquitination, which leads to the degradation of HIF-1α. Alternatively, Asn-hydroxylation prevents the binding of p300 transcription coactivator protein to HIF-1α. Thus, it inhibits transactivation of HIF-1α and the transcription of oxygen-dependent genes.
as part of the network. The protein products of these genes are involved in, among other processes, angiogenesis, cell proliferation, glucose uptake, glycolysis, erythropoiesis and iron homeostasis. HIFs are heterodimers, consisting of oxygen-sensitive HIF-α and HIF-β. In hypoxia, the induced HIF-α is translocated to the nucleus, where the heterodimer transcription factor is formed with HIF-β, which is constitutively present there. In normoxia, HIFs are inactive because of the oxygen- (and ascorbate-) dependent hydroxylation and subsequent degradation of the α-subunit. Most of our knowledge is based on observations concerning HIF-1α. Two separate dioxygenations of HIF-1α have been identified: proline hydroxylation and asparagine hydroxylation. Three HIF-1α prolyl hydroxylases (PHD1, PHD2, PHD3) recognize a conserved amino acid sequence, which is present twice in HIF-1α. Thus, two distinct proline residues are hydroxylated, Pro402 and Pro564 by PHD. Hydroxylated HIF-1α binds von Hippel–Lindau tumour promoter protein (pVHL) leading to ubiquitination and rapid 26S proteasomal degradation of the protein. In addition to prolyl hydroxylation, asparaginyl hydroxylation of HIF-1α by asparaginyl hydroxylase (factor inhibiting HIF-1, FIH-1) inhibits the function of its C-terminal transactivation domain (Metzen, 2007). Thus, ascorbate is connected to the ‘oxygen sensor’ role of these hydroxylases and probably functions to reduce the catalytic iron (Schofield and Ratcliffe, 2004; Kaelin and Ratcliffe, 2008).

However, HIF production can also be induced in certain cases of normoxia. In cell cultures, hypoxia-like responses can be provoked by Co²⁺ and Ni²⁺ (Maxwell and Salnikow, 2004). Moreover, transition metals catalyse the oxidation of ascorbate (Buettner and Jurkiewicz, 1996). Ni²⁺ depletes intracellular ascorbate, and through this action it inhibits hydroxylation of proteins such as HIF-1α and –β, and causes hypoxia-like stress (Salnikow and Kasprzak, 2005).

The family of 2OG/Fe(II)-dependent dioxygenases has recently been expanded to include new ascorbate-dependent members. An aspartyl/asparaginyl β-hydroxylase has been purified from bovine liver (Gronke et al., 1990; Wang et al., 1991), which post-translationally hydroxylates specific Asp and Asn residues within epidermal growth factor (EGF) and EGF-like domains. The presence of ascorbate was not an absolute requirement for this activity, but ascorbate enhanced the rate of the reaction several times. Experiments on the ascorbate-induced hydroxylation of EGF have typically been performed at low millimolar (1–2 mM) ascorbate concentrations. This is slightly higher than the intracellular ascorbate concentration of a hepatocyte; however, it should be noted that ascorbate is compartmentalized within the cell and in vivo subcellular concentrations are only estimates.

The physiological role of EGF hydroxylation is yet to be determined; it has been proposed that it is involved in Notch pathway signalling and its lack may promote tumour formation in mice (Dinchuk et al., 2002). β-Hydroxylated Asp and Asn residues are present in the EGF-like domains of several vitamin K-dependent clotting factors (VII, IX and X), and other plasma proteins (protein C, S and Z, and complement C1r) (Rees et al., 1988) involved in the Notch and Jagged pathway. The hydroxylation of the EGF-like domains in plasma proteins can promote Ca²⁺ binding and stabilizes a conformation necessary for protein–protein interactions, while hydroxylation of the proteins of the Notch signalling pathway can mediate cell motility and differentiation (Lahousse et al., 2006).

Recently, the role of 2OG/Fe(II)-dependent hydroxylases has been postulated in the demethylation of histones and nucleic acids. Histone demethylases belong to the jumonji protein family; they catalyse a typical dioxygenase reaction resulting in the formation of succinate and formaldehyde as end products. Their ascorbate-dependent activity was demonstrated in the demethylation of trimethylated lysines in histone H3. These enzymes might be involved in carcinogenesis (Klose et al., 2006a,b; Tsukada et al., 2006; Frescas et al., 2007). The recently described nucleic acid demethylases – Escherichia coli AlkB (Falnes et al., 2002; Trexick et al., 2002) and its human homologues ABH2 and ABH3 (Duncan et al., 2002; Aas et al., 2003; Lee et al., 2005; Yang et al., 2008) – repair damage caused by DNA/RNA alkylation, through a direct oxidative dealkylation mechanism that is dependent on 2OG, Fe²⁺ and ascorbate. AlkB and ABH3 preferentially repair single-stranded DNA lesions and can also repair methylated bases in RNA, while ABH2 has a primary role in repairing lesions in double-stranded DNA. A similar enzyme (Fto) has recently been shown to catalyse the dealkylation of 3-methylthymine in single-stranded DNA; it also affects the regulation of energy balance in mammals through an unknown mechanism (Gerken et al., 2007). The modulating role of ascorbate in nucleic acid repair and histone demethylation might expose a new function of ascorbate both in cancer prevention and carcinogenesis.

**Glypicans and ascorbate**

Glypicans constitute a family of glycosylphosphatidylinositol-anchored, cell-surface heparan sulphate proteoglycans that are present in lipid rafts and caveolae. They are selective regulators of ligand–receptor interactions and may thereby control growth and development. They can also migrate between the plasma membrane and endomembranes, and can import basic compounds bound to polyanionic heparan sulphate side chains (Fransson et al., 2004). A member of the family, glypicanc-1 migrates between the plasma membrane and the Golgi, and vice versa. Cysteines in glypican-1 can be nitrosylated by nitric oxide in a copper-dependent reaction (Cheng et al., 2002). When glypican-1 is exposed to ascorbate, nitric oxide is released from the intrinsinc 5-nitrosogroups of the glypican-1 core protein (Ding et al., 2002). Nitric oxide participates in the deaminative cleavage of heparan sulphate by heparanase at sites where glycosamines possess a free amino group (Ding et al., 2001). The NO-catalysed degradation of heparan sulphate begins in early endosomes but mainly takes place in late endosomes (Mani et al., 2006b). Although these findings are convincing, whether the process is physiologically significant in vivo is still questionable. Further work is needed to clarify the relative contribution of ascorbate-independent and ascorbate-independent NO formation to the NO-catalysed degradation of heparan sulphate.

Migrating glypicans can thus act as potential vehicles for basic compounds (e.g. spermine, basic peptides, etc.), which
may be carried bound to heparan sulphate chains and released from glypican when heparan sulphate is degraded (Belting et al., 2003). The mechanism whereby the cargo, glypican and its degradation products are discharged from transporting endosomes is unknown; however, heparan sulphate fragments could potentially form membrane-penetrating complexes with the cargo molecules.

The deaminative degradation of glypican-1 heparan sulphate is defective in fibroblasts from patients suffering from Niemann–Pick type C1 disease (Mani et al., 2006a). Niemann–Pick type C1 disease is a rare neurovisceral degenerative disorder characterized by the accumulation of unesterified cholesterol, sphingolipids and other lipids within the endosomal/lysosomal system. Ascorbate supplementation of the fibroblast restored the deaminative degradation of glypican-1 heparan sulphate. These results suggest that defective processing of glypican-1 can contribute to cell damage in Niemann–Pick C1 disease.

Ascorbate in the redox homeostasis of certain organelles

Another essential role of ascorbate is related to its functions in redox homeostasis. Because oxidative respiration in mitochondria is a major source of the intracellular production of reactive oxygen species (ROS), the presence of mitochondria and role of vitamin C are of particular interest. Moreover, the possible functions of ascorbate in oxidative protein folding and in the maintenance of the intralumenal oxidative environment suggest it has a particular role in endoplasmic reticulum (ER)-related processes.

Vitamin C and mitochondria

In vivo studies showed that ascorbate concentration in mammalian mitochondria can be increased by dietary vitamin C supplementation (Ingebretsen and Normann, 1982; Li et al., 2001; Ramanathan et al., 2003). However, mitochondrial transport of ascorbate and its intramitochondrial functions are poorly elucidated. Kc et al. (2005) confirmed previous findings that vitamin C enters mitochondria as dehydroascorbic acid (DHA) (Szarka et al., 2004). They found a stereoselective mitochondrial D-glucose uptake mechanism, which competes with the transport of DHA. Computational analysis of the N-terminal sequences of human GLUT isoforms revealed that GLUT-1 has the highest probability of mitochondrial localization. GLUT-1 in mitochondrial membrane was verified by mitochondrial expression of GLUT1–EGFP, immunoblot analysis and by cellular immunolocalization. Taken together, these observations suggest that, in an analogous fashion to its cellular uptake, vitamin C enters mitochondria in its oxidized state via GLUT-1.

Because DHA is very unstable and only ascorbate possesses antioxidant and free radical scavenger properties, DHA taken up or generated in the matrix must be reduced back to ascorbate, otherwise, in physiological conditions, it is lost within minutes (Winkler, 1987). Various mechanisms have been suggested for intramitochondrial ascorbate recycling. It has been reported that mitochondria are capable of reducing DHA to ascorbate in an α-lipoic acid (LA)-dependent manner (Xu and Wells, 1996). The role of reduced GSH in mitochondrial DHA reduction has also been described (Li et al., 2001). The addition of DHA to mitochondria resulted in a reduction of DHA and a mitochondrial accumulation of ascorbate. Ascorbate levels in mitochondria reached millimolar concentrations. Mitoplasts were also capable of taking up and reducing DHA (Li et al., 2001). Thioredoxin reductase in mitochondria could also reduce DHA. However, as no significant decrease in DHA reduction was detected in mitochondria isolated from selenium-deficient animals, this is likely to be a small component in the mitochondrial DHA reduction machinery (Li et al., 2001). DHA loading caused a significant decrease in mitochondrial GSH concentration. Depletion of mitochondrial GSH content caused significant impairment of DHA reduction to ascorbate. Based on these results, it has been suggested that the GSH-dependent reduction of DHA is one of the major ascorbate-producing reactions in mammalian mitochondria (Li et al., 2001).

The observation, that in the absence of respiratory substrates, liver mitochondria lose their ability to maintain or increase the level of ascorbate (Li et al., 2001; 2002; May et al., 2007), suggests that the respiratory chain contributes to the reduction of DHA. Indeed, it was shown that the formation of ascorbate from DHA could be enhanced by several respiratory substrates (succinate, malate and glycerol-3-phosphate). Using specific inhibitors of the mitochondrial electron transport chain, the site of ascorbate sparing was localized to complex III (Li et al., 2002) (Figure 2). A recent study on guinea pig muscle mitochondria (May et al., 2007) showed that although DHA transport was present, mitochondrial ascorbate accumulation could not be inhibited by GLUT-1 inhibitors, and the depletion of mitochondrial GSH was also absent.

This phenomenon is hard to explain, because the reduction of DHA by GSH is a well-documented chemical reaction (e.g. it also takes place in the absence of any DHA-reducing enzyme) (Wells and Xu, 1994). The lack of the effect of GLUT-1 inhibitors was explained by the differences between the sources of mitochondria in the two studies (human vs. guinea pig, and cell culture vs. tissue).

On the other hand, a marked increase in the rate of DHA reduction upon addition of succinate was observed (May et al., 2007). These data and similar results obtained in plant mitochondria (Szarka et al., 2007) underline the universal role of the mitochondrial electron transfer chain in DHA reduction. Ascorbate can transmigrate between the outer and inner membrane (May et al., 2007), which raises the possibility that mitochondria contribute not only to DHA reduction, but also to the capacity of the whole cell to produce ascorbate.

The involvement of the electron transfer chain in DHA reduction can contribute to the stabilization of the mitochondrial redox balance in at least two ways. Firstly, through the donation of electrons to DHA reduction, it can decrease the reduced state of the electron transfer chain. In this way, the electron leakage of the electron transfer chain and subsequent ROS formation can be also decreased. Secondly, the production of ascorbate provides reduced vitamin C, which can scavenge ROS directly at the site of their generation.
The maintenance of the intramitochondrial ascorbate level seems to have a notable anti-apoptotic effect. The elevated level of ROS can induce the collapse of the mitochondrial membrane potentially leading to apoptosis. In HL-60 cells exposed to hydrogen peroxide, pre-incubation with DHA reduced the intracellular peroxide levels in a dose-dependent manner. In accordance with this phenomenon, the mitochondrial membrane potential was also partially conserved, and the denaturation and mitochondrial release of cytochrome c could also be avoided in DHA-pretreated cells (Gruss-Fischer and Fabian, 2002). In a study involving FAS-induced apoptosis of monocytes, the loss of the mitochondrial membrane potential could be inhibited by pretreatment with vitamin C (Perez-Cruz et al., 2003). During hypoxia and reperfusion, the electron transfer chain, especially complex III, is a primary site of ROS formation and a target of injury in the vascular endothelium (Hashimoto et al., 1994; Therade-Matharan et al., 2004). A dose-dependent reduction of ROS could be observed in response to vitamin C in human umbilical vein and coronary artery endothelial cells subjected to hypoxia-reperfusion (Dhar-Mascareño et al., 2005). The release of cytochrome c was also prevented and the mitochondrial membrane potential was stabilized by vitamin C in cells undergoing hypoxia-reperfusion (Dhar-Mascareño et al., 2005). All these events led to a decreased activation of caspase-9 and caspase-3 with resultant inhibition of apoptosis induced by hypoxia-reperfusion. These findings suggest that mitochondrial vitamin C is a major component in the maintenance of the mitochondrial membrane potential, and that vitamin C exerts its anti-apoptotic effect through its ability to scavenge ROS (Gruss-Fischer and Fabian, 2002; Pearlstein et al., 2002; Perez-Cruz et al., 2003; Dhar-Mascareño et al., 2005; Kc et al., 2005; Lee et al., 2007). Following oxidative stress, a 3- to 10-fold increase in damage can be found in mitochondrial DNA (mtDNA) compared to that in nuclear DNA (Yakes and Van Houten, 1997; Santos et al., 2003; Jarrett et al., 2006). Kc et al. (2005) showed that mitochondrial ascorbate could protect mtDNA against oxidative damage. It was clearly demonstrated that ascorbate protected mtDNA against the ROS-induced elevation of 8-oxo-dG and apurinic/apyrimidic sites. Furthermore, pre-treatment with ascorbate significantly attenuated the hydrogen peroxide-induced shearing of mtDNA (Kc et al., 2005). In accordance with these findings, in cells from the retinal pigment epithelium, vitamin C was found to induce a significant reduction of hydrogen peroxide-induced lesions in mtDNA (Jarrett et al., 2006).

Increased production of free radicals and ROS has been observed in various mitochondrial diseases (Siciliano et al., 2007). Supplementation with vitamin C is part of the treatment of mitochondrial diseases as it alleviates oxidative stress (DiMauro and Mancuso, 2007; Siciliano et al., 2007). Indeed, the elevated level of superoxide production—observed in fibroblasts from patients with electron transport chain deficiencies—could be decreased by ascorbate treatment (Sharma and Mongan, 2001). The activities of I–III and II–III complexes have also been found to be stimulated simultaneously by the addition of vitamin C to the culture media (Sharma and Mongan, 2001). Hence, there have been attempts to use vitamin C as a therapeutic agent in the treatment of various mitochondrial diseases. Vitamin C and menadione treatment of a young woman with mitochondrial myopathy and severe exercise intolerance was expected to bypass the block in complex III (Kennaway et al., 1984; Keightley et al., 2000), because the redox potentials of these electron carriers fit the gap created by the cytochrome c dysfunction (Eleff et al., 2000).
Ascorbate in the ER

Ascorbate metabolism and the ER are closely related to each other from several aspects. Firstly, the last steps of ascorbate synthesis are localized in the ER. Secondly, because ROS-generating reactions (e.g. oxidative protein folding) reside in this compartment, the antioxidant effect of ascorbate is presumably essential in the ER. Finally, several luminal enzymes of the secretory pathway use ascorbate as a cofactor.

L-Gulonolactone oxidase (GLO), a microsomal enzyme, catalyses the aerobic conversion of gulonolactone to ascorbate, accompanied by the production of hydrogen peroxide (Chatterjee et al., 1960a,b). The requirement of detergents for the solubilization of GLO from microsomal vesicles (Eliceiri et al., 1969; Nishikimi et al., 1976; Kiuchi et al., 1982) strongly suggests it is located in the membrane. The amino acid sequence of GLO contains several strongly hydrophobic regions forming $\beta$-sheets rather than a typical transmembrane helical structure, which are possibly associated with the ER membrane (Koshizaka et al., 1988). The orientation of the catalytic site towards the lumen of the ER is indicated by the intraluminal accumulation of ascorbate and preferential intraluminal GSH oxidation (presumably by hydrogen peroxide) in rat liver microsomes incubated with gulonolactone (Puskás et al., 1998). This observation suggests that ascorbate produced by the liver enters the circulation – at least partly – through this secretion pathway. It should be noted that the mentioned processes are only valid in ascorbate-synthesizing species. However, in GLO-deficient species including humans, effective transport mechanisms facilitate ascorbate influx into the ER lumen.

Although the exact concentration of ascorbate in the ER lumen is unknown, local synthesis (in the case of the liver) and the high demand by luminal ascorbate-dependent enzymes (Fe$^{2+}$/ZOG-dependent dioxygenases: prolyl-$\beta$-hydroxylase, prolyl-$\alpha$-hydroxylase and lysyl hydroxylase) suggest that the concentration of ascorbate is higher here than in plasma or cytosol. Consistent with this assumption, the vesicular structures of the secretory pathway are characterized by high (millimolar) ascorbate concentrations, which are necessary for the functioning of Cu$^{2+}$-dependent mono-oxygenases such as peptidylglycine $\beta$-amidating mono-oxygenase and dopamine $\beta$-hydroxylase (Daniels et al., 1982; von Zastrow et al., 1984; 1986).

The ER is equipped with powerful electron transfer chains. During the redox cycle of the cytochrome P450 system, ROS can be formed in the ER membrane as a result of ‘chemical accidents’ (Zangar et al., 2004). The terminal oxidase of the oxidative protein folding (Ero1) generates hydrogen peroxide in the lumen (Tu and Weissman, 2002; Gross et al., 2006). A high local concentration of ascorbate is probably important to balance these pro-oxidant events.

The pro-oxidant role of ascorbate

It has been known for a long time that ascorbate can behave as a pro-oxidant under certain conditions (e.g. at low concentrations and/or in the presence of free ions of redox-active metals such as copper and iron) (Buettner and Jurkiewicz, 1996). Its pro-oxidant behaviour has been regarded as undesirable, as it leads to the formation of ROS (Duarte and Lunec, 2005) or glycated proteins (Birlouez-Aragon and Tessier, 2003). However, certain pro-oxidant effects of ascorbate can also be advantageous. It has been recently reported that ascorbate promotes protein thiol oxidation in rat liver microsomes (Csala et al., 1999). It is thought that a metalloprotein present in the microsomal vesicles oxidizes ascorbate to DHA, which leads to the generation of ROS (Szarka et al., 2002). ROS, directly or indirectly through membrane tocopherol (Csala et al., 2001), oxidizes further ascorbate molecules. DHA formed in or transported into the lumen of the ER (Bánhegyi et al., 1998b; Csala et al., 2000) can be reduced by protein disulphide isomerase oxidizing the active central dithiols of the enzyme. Oxidized protein disulphide isomerase reacts with reduced attendant proteins yielding protein disulphides and catalytically regenerating protein disulphide isomerase (Nardai et al., 2001). Although this scheme fits the results gained in microsomal systems in vitro, whether it correctly describes the in vivo situation is still questionable (Bánhegyi et al., 2003). Further studies are needed to demonstrate the electron transfer in vivo. However, results gained in scurbutic guinea pigs showed that the missing pro-oxidant effect of ascorbate led to ER stress, presumably due to an impairment of oxidative protein folding (Margittai et al., 2005).

Therapeutic role of ascorbate

It is very hard to summarize the therapeutic benefits of vitamin C because: (i) ascorbate is added mainly in different combinations with various other drugs, antioxidants or other (natural) antioxidant diet constituents; (ii) the data obtained and the conclusions reached are sometimes controversial; (iii) there is disagreement among physicians regarding the therapeutic use of ascorbate in everyday treatment; (iv) in several cases, very small differences are registered and the number of patients participating in these studies is very limited; and (v) there are major differences with respect to the oral and intravenous use of ascorbate. Ascorbate added intravenously is much more effective in elevating serum ascorbate levels (Paydavatty and Levine, 2001), while several randomized clinical trials that have utilized oral administration of ascorbate have been unsuccessful.

In humans, the requirement for vitamin C is satisfied by natural sources and vitamin C supplements in the ordinary diet. The two major forms of vitamin C in the diet are L-ascorbic acid and L-DHA. Both ascorbate and DHA are absorbed along the entire length of the human intestine, as shown by the measurement of transport activity in luminal
renal reabsorption, in order to maintain whole-body homeostasis, where it is involved in the absorption of dietary ascorbate and is largely present in epithelial tissues, (littermates. Lower in the homozygote mutants, than in the wild-type homozygote mutants are characterized by respiratory failure and intracerebral haemorrhage (Sotiriou et al., 2002).

The characteristics of DHA uptake by luminal membranes of the human jejunum clearly differ from those of ascorbate uptake (Wilson, 2005). DHA is taken up by low-affinity (K_m ~ 0.8 mM) sodium-independent facilitated diffusion. Glucose inhibits ascorbate uptake but not that of DHA. However, the transport inhibition profile ruled out the role of sodium-dependent glucose cotransporter in ascorbate transport (Malo and Wilson, 2000). The transport protein responsible for the intestinal absorption of DHA has not yet been identified.

Plasma concentrations of vitamin C are tightly controlled when the vitamin is taken orally. Peak plasma vitamin C concentration seemed to plateau with increasing oral doses (Padayatty et al., 2004). There are two simple reasons for this: on the one hand, as mentioned in the previous paragraph, the capacity of the transporters is limited; on the other hand, the two Na^+-dependent transporters can be subjected to fine-tuned regulation by their own substrate. An elevated level of ascorbate in the intestinal lumen led to down-regulation of SVCT1 mRNA in enterocytes (MacDonald et al., 2002). A similar self-regulatory role for ascorbate was demonstrated for SVCT2 in platelets (Savini et al., 2007a). Recently, it was shown that skeletal muscle cells modulated the expression of the SVCT2 carrier according to their redox balance. Both mRNA and protein levels of SVCT2 were up-regulated in hydrogen peroxide-treated myotubes, while antioxidant supplementation with LA lowered the expression of SVCT2 (Savini et al., 2007b). It seems likely that the redox state of the cell can influence the expression of SVCT 2 and in this way, the resulting response regulates the transport and intracellular level of ascorbate.

Plasma levels of vitamin C are not only limited by absorption, but also by reabsorption in the kidneys by SVCT1. Accordingly, the maximum bioavailability of vitamin C is usually attained at lower doses and declines with the elevation of oral supplements: 87% for 30 mg, 80% for 100 mg, 72% for 200 mg, 63% for 500 mg and less than 50% for 1250 mg (Levine et al., 1996; Graumlich et al., 1997). This observation was further confirmed by data from the three-compartment pharmacokinetic model for vitamin C. On the basis of this model, a single oral dose of 3 g – the maximum tolerated single dose – produces a peak plasma concentration of 206 μM, while the 1.25 g oral dose results in just a slightly lower concentration of 187 μM. Finally, for 200 mg – an amount obtained from vitamin C-rich foods – a peak-predicted concentration was approximately 90 μM (Padayatty et al., 2004). Hence, pharmacological plasma concentrations of vitamin C can only be reached through the intravenous administration of the vitamin (Padayatty et al., 2004).

The only clinical benefit of ascorbate addition, with a proven mechanism of action, is the prevention of scurvy. However, intake of as little as 10 mg·day^{-1} of vitamin C is appropriate for this purpose. This amount results in plasma vitamin C concentrations below 10 μM. In cases of ‘therapeutic’ levels, or even ‘normal’ levels, of vitamin C in the diet, plasma ascorbate concentrations are at least one order of magnitude higher than that necessary to prevent scurvy (Padayatty et al., 2003).

Usually, high doses of ascorbate are administered, as a high vitamin C intake can be toxic (Levine et al., 1999). The adverse effects of vitamin C are summarized by Levine et al. (1999). Excess ascorbate is normally excreted harmlessly in the urine. However, it is also well known that high amounts of ascorbate can be harmful due to oxalate formation. Thus, administration of high doses of vitamin C is contraindicative for patients with oxalate kidney stones or hyperoxaluria (Levine et al., 1999). In patients with renal failure, vitamin C is retained and converted to insoluble oxalate, which can accumulate in various organs. Following kidney transplantation, the administration of vitamin C (self-medication 2 g·day^{-1} for 3 years while in dialysis) led to the development of renal failure due to the widespread deposition of calcium oxalate crystals (Nankivell and Murall, 2008). Therefore, high-dose vitamin C therapy should be avoided in patients with renal failure or renal insufficiency, and in patients undergoing dialysis (McAllister et al., 1984; Wong et al., 1994; Levine et al., 1999). In patients with a deficiency in glucose-6-phosphate dehydrogenase, intravascular haemolysis occurred after high-dose vitamin C administration. It is also contraindicative in patients with systemic iron overload (Rivers, 1987; Levine et al., 1999).

There are several reasons for administering ascorbate. In addition to solving vitamin C hypo- or avitaminosis, ascorbate can be given as an antioxidant or pro-oxidant. The antioxidant properties of ascorbate have been used to treat various conditions (summarized in previous reviews; Levine et al., 1999; Heitzer et al., 2001), where oxidative stress is involved in the pathogenesis, and it is frequently administered in combination with other antioxidants.

Recently, the significance of oxidative stress in obesity-related diseases, such as type 2 diabetes, has gained further...
credibility (Robertson, 2004; Scheuner and Kaufman, 2008). In several studies, a decrease in plasma vitamin C levels has been observed in both type I and type II diabetes, and the effects of vitamin C administered in different ways, in addition to various combinations of different antidiabetic drugs and other antioxidants, have been assessed (Szaleczky et al., 1998; Bonnefont-Rousselot, 2004; Dorchy, 2004; Chen et al., 2006; Ratnam et al., 2006; Alamdari et al., 2007; Ceriello et al., 2007a,b). However, at present, there is no comprehensive agreement regarding its therapeutic effectiveness for these conditions.

The role of ascorbate in the treatment of various infections has been studied for a long time. In patients with sepsis, early enteral pharmaconutrition with several agents, among others vitamin C and other antioxidants, resulted in significantly faster recovery of organ function based on a prospective, randomized, controlled, double-blind clinical trial with 55 patients (Beale et al., 2008).

It has also been suggested that the development of cataract is influenced by ascorbate (Fan et al., 2006). However, it turns out that by itself, ascorbate is unlikely to affect the progression of cataracts in most patients (Fernandez and Afshari, 2008). Vitamin C supplements combined with other antioxidant vitamins and minerals have been found to slow down the progression of advanced age-related macular degeneration and loss of visual acuity in people with signs of this disease (Evans, 2008; Evans and Henshaw, 2008). The effectiveness of vitamin C as a treatment of diabetic retinopathy has also been examined, but further studies are required to prove that it has a significant impact on its progress (Lopes de Jesus et al., 2008).

On the other hand, as shown previously, ascorbate can also act as a pro-oxidant; it can also be applied under circumstances where its pro-oxidant effect is manifested. Conflicting findings have been published on the effects of high-dose vitamin C therapy for patients with terminal cancer (Cameron and Campbell, 1974; Cameron et al., 1975; Cameron and Pauling, 1976; 1978; Creagan et al., 1979; Moertel et al., 1985). The rationale for this therapy is based on its pro-oxidant effects; it has been shown that extracellular ascorbate selectively kills cancer, but not normal cells by generating hydrogen peroxide (Chen et al., 2005; 2008). Furthermore, it has been demonstrated that for certain tumours (e.g. advanced stages of lung cancer), serum ascorbate, as well as the serum levels of other antioxidants, is decreased (Esme et al., 2008). Consistent with the clinical pharmacokinetics of intravenously administered vitamin C, pharmacological concentrations of ascorbate can only be achieved by intravenous administration (Padayatty et al., 2004; 2006). At present, ascorbate is used as an alternative cancer therapy (Wittes, 1985; Bernstein and Grasso, 2001; Golde, 2003; Cassileth and Deng, 2004).

Besides affecting redox conditions, it has been suggested that ascorbate can alter the expression of genes. From experiments performed in cell cultures and also in mice, a possible beneficial effect of ascorbate in a hereditary peripheral neuropathy, Charcot–Marie–Tooth disease (CMT-1A), has been suggested with regard to the promotion of myelination; this is based on a gene-inducing action of ascorbate (Passage et al., 2004; Kaya et al., 2008).

### Latent scurvy

Hypoascorbinaemia and diabetes mellitus share several clinical symptoms including hypercholesterolaemia, atherosclerosis, microangiopathy, capillary hyperperfusion and haemorrhages. These effects have been observed both in patients with low ascorbate levels and in guinea pigs kept on an ascorbate-deficient diet (Turley et al., 1976; Price et al., 2001). The pathological symptoms of these conditions can be alleviated by the administration of vitamin C (Juhl et al., 2004). Based on clinical and experimental observations, and on the structural similarity between vitamin C and glucose, the theory of 'latent scurvy' was formulated (Price et al., 1996); the supposition is that hyperglycaemia competitively inhibits the cellular uptake of ascorbate, inducing intracellular vitamin C deficiency.

In fact, ascorbate (more precisely its oxidated form, DHA) is transported into the majority of cells by glucose transporters (Kónya and Ferdinándy, 2006). This coincidence could mean a further risk of ascorbate deficiency in hyperglycaemia. However, as a recent study pointed out, humans and certain other mammals incapable of de novo vitamin C synthesis have evolved an effective compensatory mechanism. In these species, the affinity of glucose and DHA for the transporter GLUT1 is altered by the membrane protein stomatin. Immature human erythrocytes express GLUT1, and the uptake of glucose is preferred over that of DHA. During red blood cell differentiation, expression of stomatin increases. Stomatin binds to GLUT1 and changes its substrate preference from glucose to DHA (Zhang et al., 2001; Montel-Hagen et al., 2008). Because ascorbate does not accumulate in erythrocytes, red blood cells can be regarded as ascorbate recyclers supporting the antioxidant capacity of the serum and providing vitamin C for other cell types. As GLUT transporters (Joost and Thorens, 2001) and stomatin (Stewart et al., 1992) are ubiquitously distributed in different human cell types and tissues, similar interactions can be hypothesized to occur in human cells other than erythrocytes.

### Advantage of losing the ability to synthesize ascorbate

To summarize these data, ascorbate seems to be essential for antioxidant homeostasis and as a cofactor in a series of post-translational events. Consequently, this raises the basic question: what would be the advantage of losing the ability to produce ascorbate? Ascorbic acid-synthesizing capacity appeared in early terrestrial vertebrates as an answer to global hyperoxia. The concentration of atmospheric oxygen elevated dramatically during the mid- to late Paleozoic. The unusual and extreme hyperoxia was toxic for contemporary organisms as witnessed by the Permian mass extinction. Only those tetrapods which developed a powerful antioxidant system against oxygen toxicity survived the era. GLO expression emerged during that time (Chatterjee, 1973; Nandi et al., 1997).

Ascorbate synthesis finally became widespread among mammals. GLO was also present in primitive primates, but
interestingly it was lost owing to mutations in the GLO gene (Nishikimi and Yagi, 1991) in the primate lineage leading to monkeys and apes in the Eocene era (55–35 million years ago). This genetic disorder generated a need to obtain ascorbate from diet in humans and other higher primates; ascorbate became vitamin C.

Nucleotide sequence alignment of one exon of the GLO gene from rat with the corresponding exon in the highly mutated, non-functional GLO gene of primates revealed that random nucleotide substitutions occurred throughout the primate sequence, as expected for a gene that got inactivated during evolution and subsequently evolved without functional constraint (Ohta and Nishikimi, 1999). Because other ascorbate-deficient species also evolved through several other lineages, it appears that the inactivation of the GLO gene occurred several times during evolution. These circumstances and the fact that the mutation did not remain a polymorphism affecting only a minority of the population, but after a positive selection mechanism only the mutant type survived, should mean that this change was advantageous. Several theories have been forwarded to explain this phenomenon.

One can regard the loss of GLO as an evolutionary accident, which was not apparent in an ascorbate-rich environment (Pauling, 1970; Szent-Györgyi, 1978; Eaton and Konner, 1985). In fact, the tropical jungle supplied our ancestors with ascorbate, because ‘not only does the monkey go through the jungle, but jungle goes through the monkey’ (Szent-Györgyi, 1978).

Another possible explanation is that vitamin C may have a role as a human fertility factor (Millar, 1992). The higher requirement of the older members of society for vitamin C led to their increased mortality in times of food shortages. This merciless system would reduce the median age of the population by favouring the survival of the youngest, thus enabling the population to regrow rapidly when food resources were restored.

An alternative hypothesis is based on the enzymology of GLO. The enzyme generates not only ascorbate, but also hydrogen peroxide as a by-product, and therefore the net reaction is redox neutral, whereas the dietary intake of ascorbate selectively increases the antioxidant capacity. Hydrogen peroxide formation is accompanied by GSH oxidation during ascorbate synthesis (Bánhegyi et al., 1996). The loss of GLO activity spared the reduced GSH, the main defence system against oxidants, while the access to ascorbate by diet was not hindered. Later, even in an environment less abundant in ascorbate, the evolutionary gains of these periods allowed the conservation of the genetic disorder (Bánhegyi et al., 1997). As a compensation for the lost ascorbate synthesis, primates use end products of different catabolic pathways (e.g. bilirubin, uric acid) as antioxidants. Most animals readily excrete soluble allantoin and biliverdin as end products of purine and porphyrin catabolism. As antioxidants can replace each other in different reactions, these metabolic changes can be regarded as compensatory mechanisms (Proctor, 1970; Ames et al., 1981; Sevanian et al., 1985; Stocker et al., 1990).

Challem and Taylor proposed a different hypothesis (Challem, 1997; Challem and Taylor, 1998). These authors suggested that a retroviral infection inserted silencing Alu elements in the gene for GLO. The lower ascorbate concentration probably was not fully compensated for by other antioxidants, allowing an increased level of free radicals. The increased frequency of free radical-induced mutations could accelerate the rate of evolution of early primates.

Very recently, Johnson et al. (2008) have put forward another original theory. They suppose that the loss of ascorbate synthesis (and rise in uric acid) had a role in maintaining blood pressure during periods of dietary restriction and environmental stress. During climatic changes, the availability of dietary ascorbate could decrease with the concomitant falling of serum ascorbate levels in GLO-deficient species. Oxidative stress (e.g. in the absence of a sufficient amount of ascorbate) results in elevated blood pressure (Vaziri and Rodriguez-Iiturbe, 2006). Supporting this hypothesis, epidemiological studies have confirmed an inverse relationship between serum ascorbate levels and blood pressure (Ness et al., 1997; Bates et al., 1998), and some studies have reported that ascorbate supplements reduce blood pressure in patients with hypertension (e.g. Duffy et al., 1999). On the basis of these results, it has been suggested that those species that cannot synthesize ascorbate suffer from oxidative stress and have a higher blood pressure, and this might have provided them with a superior survival advantage in periods of environmental stress.

Perspectives

This review is an attempt to summarize recent advances at the molecular level in ascorbate research and clinical implications. A more comprehensive approach including plant and ascorbate-synthesizing animals might help us to understand the role of vitamin C and its potential effects in the treatment of various diseases. Based on the putative roles of ascorbate in metabolic and redox homeostasis, and distinctive enzyme reactions, therapeutic applications and empirical phenomena are either successfully or unsuccessfully explained by vitamin C deficiency/administration. Recently, this situation has undergone a change. New in vitro data have been obtained on the functions of ascorbate, but further investigations are needed to interpret their physiological or pathological significance and therapeutic implications. The compartmentalization of ascorbate within a cell suggests it has different functions in particular organelles. The redox state of its microenvironment determines its vital metabolism and other functions, and also its signalling role. Further research is required to explore the exact effects of ascorbate in physiological systems and in the pathology of diseases connected to mitochondria and ER. These new findings could also lead to new therapeutic implications regarding ascorbate. Recent publications raise some new aspects and suggest new mechanisms of ascorbate at the molecular level. However, it should be noted that some well-known beneficial effects of ascorbate administration are still only understood at the phenomenological level.

Acknowledgement

This work was supported by NKTH RET05/2004.
References


