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Novel aspects of vitamin C: how important is glypican-1 recycling?

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The reduced form of vitamin C, ascorbic acid, is well known for its function as an antioxidant and as a protective agent against scurvy. However, many recent studies indicate other functions for vitamin C in mammalian cells. Novel findings provide possible explanations for observed beneficial effects of a high intake of vitamin C on cell growth, gene transcription, host resistance to infection, uptake of polyamines and clearance of misfolded proteins. Vitamin C exerts its effects indirectly via hypoxia-inducible factor, nitric oxide synthase and the heparan sulfate proteoglycan glypican-1, which is deglycanated in a vitamin C- and copper-dependent reaction.

How much vitamin C do we need?

The reduced form of vitamin C, ascorbic acid, is well known for its role in preventing scurvy. It is also believed to be an antioxidant that protects cells from damage by reactive oxidant species. Ascorbate reactivates various dioxygenases, such as the protocollagen prolyl and lysyl hydroxylases, and enzymes that are involved in the biosynthesis of carnitine or in the conversion of dopamine into noradrenaline (norepinephrine). A progressive deficiency of vitamin C is expected first to inactivate dioxygenases that have a low affinity for ascorbic acid. Inactivation of prolyl hydroxylase is therefore the prime effect of vitamin C deficiency, which results in insufficiently hydroxylated collagen. The abnormal, less-stable fibers formed by such collagens contribute to skin lesions and blood-vessel fragility that are present in scurvy (for reviews, see [1–3]).

Most mammalian species synthesize L-ascorbic acid from D-glucose in the liver (for review, see [4]). The enzyme that catalyzes the final step, L-gulono-1,4-lactone oxidase (GULO, EC 1.1.3.8) is missing or mutated in humans, other primates, guinea pig and certain fruit bats. In human males, a daily intake of 200 mg vitamin C saturates the plasma level at 70–80 μM, whereas human females require a daily dose of 200–400 mg to reach a comparable level [5].

Ablation of *Gulo* gene has been performed in mice [6]. As expected, these mice depend on dietary supplementation of vitamin C for survival. However, their daily requirement was several folds greater than that for humans. When plasma ascorbic acid decreased to subnormal levels, a small but significant increase in total cholesterol level and a decrease in the level of high-density lipoprotein cholesterol were observed as the animals aged. The mechanism responsible for this effect is not known. Low levels of plasma and tissue ascorbic acid also reveal alterations in the aortic wall of these mice, as evidenced by the disruption of elastic laminae, smooth-muscle cell proliferation and focal endothelial desquamation of the luminal surface. In guinea pigs, atherosclerotic lesions develop when they are fed a scorbutic high-fat diet [7,8].

Ingestion of high daily doses of vitamin C of several times the recommended intake has been claimed to be healthly, but this is not solely regarded as a fact by the scientific community. However, many recent studies point to functions for vitamin C in mammalian cells other than that of an antioxidant. Scurvy is prevented by a low intake of vitamin C; however, do some cellular and/or body functions require higher levels of vitamin C? Are the results of mild but prolonged deficiences only apparent at old age? To discuss these issues, we briefly summarize data on the uptake and interconversions of the redox forms of vitamin C.

Glossary

Anhydromannose: mannose with an internal ether bond between C-2 and C-5.
Caveolae: flask-shaped invaginations of the plasma membrane with a lipid composition similar to that of lipid rafts and containing caveolins attached to the cytoplasmic face.
Deaminative cleavage: in the present context, it is the NO- and nitrite-catalyzed cleavage of heparan sulfate at *N*-unsubstituted glucosamine residues. It involves the initial formation of an azido derivative of the glucosamine, followed by ring contraction to an anhydro-manno configuration and subsequent cleavage of the glycosidic bond to the preceding sugar in the chain [59].
Deglycanation: removal of the glycan portion of a proteoglycan.
Glycosaminoglycan: the glycan portion of a proteoglycan. The basic structure is a disaccharide repeat, usually containing an uronic acid and a hexosamine, often substituted with sulfate groups at different positions.
Heparanase: an endoglucuronidase that cleaves heparan sulfate and heparin at sites where the glucuronic acid is located in a sulfated domain.
Heparan sulfate: a glycosaminoglycan with the basic disaccharide repeat, glucuronic acid (GlcA)–glucosamine (GlcN), where GlcN might be *N*-acetylated or *N*-sulfated and GlcA might be epimerized to iduronic acid (IdoA). The sugars are sometimes sulfated at C-3 or C-6 in GlcN and at C-2 in the uronic acids.
Lipid raft: a plasma membrane domain rich in cholesterol, sfingolipids and GPI-linked proteins.
Proteoglycan: a protein substituted with glycosaminoglycans.
***S*-nitrosylation**: a reaction where Cys-SH and NO form Cys-SNO.
Suramin: an urea substituted at both amino groups with a polysulfonated complex structure, consisting of two benzene and one naphthalene rings joined by amide bonds. The compound serves as a heparan sulfate antagonist.
Tetrahydrobiopterin: a cofactor of amino acid catabolism that is similar to the heterocyclic, fused two-ring pterin moiety of tetrahydrofolate, but it is not involved in one-carbon transfers; instead, it participates in oxidation reactions.

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Is it important to drink fresh fruit juice?

When asorbate acts as an antioxidant or enzyme cofactor, it becomes oxidized to dehydroascorbate. The reduced form of vitamin C, ascorbic acid (Figure 1a), is used in cell metabolism as an electron donor that is capable of donating one electron. In the first oxidation step, ascorbyl radical is generated, followed by dehydroascorbic acid in the second step (Figure 1a). The ascorbyl radical does not accumulate

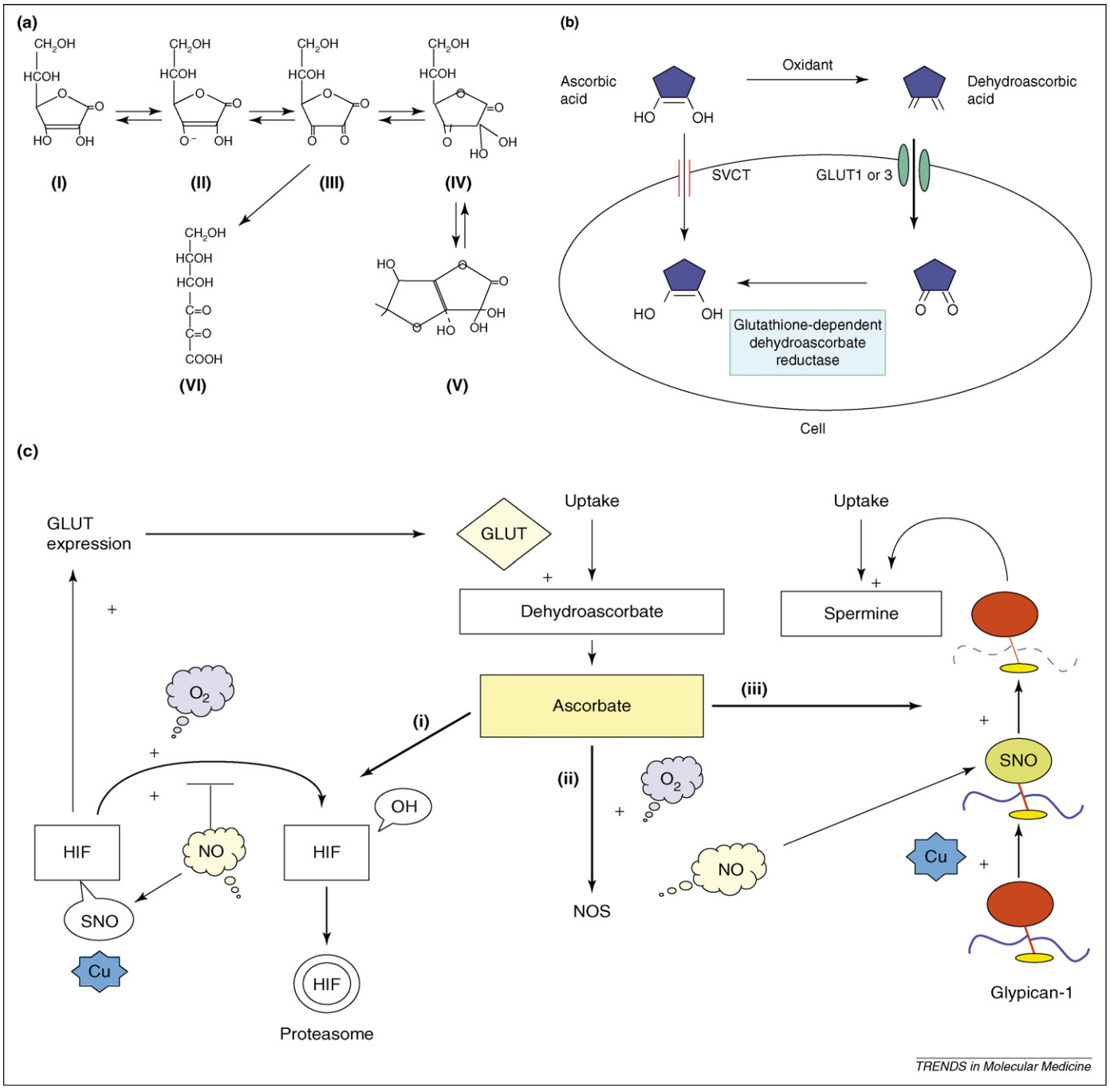

Figure 1. Structure, uptake, interconversion and function of vitamin C redox forms. **(a)** L-Ascorbic acid (I) is oxidized in two steps, first to ascorbyl radical (II) and then to dehydroascorbic acid (III). Dehydroascorbic acid can also be hydrated (IV) and converted into a bicyclic hemiketal (V). These reactions are reversible. Dehydroascorbic acid, which is a lactone, can be irreversibly hydrolyzed to 2,3-diketogulonic acid (VI). **(b)** L-Ascorbic acid is taken up by the Na^+–vitamin C transporter (SVCT) and dehydroascorbic acid by glucose transporters (GLUT), presumably as the bicyclic hemiketal (V in Figure 1a). Inside cells, dehydroascorbic acid is reduced to ascorbic acid. In the liver and in the brain, a glutathione-dependent dehydroascorbic acid reductase converts dehydroascorbic acid into ascorbate [54]. This enzyme is present mainly in the cytosol of neurons and is not associated with any organelles except for the nucleus. Rat liver 3α-dihydroxysteroid dehydrogenase (oxidoreductase) reduces dehydroascorbic acid in a nicotinamide adenine dinucleotide phosphate (NADPH)-dependent manner [55]. Also other reductases can convert dehydroascorbic acid into ascorbate using either glutathion or the thioredoxin system. **(c)** L-Ascorbate (i) stimulates hydroxylation of the hypoxia-inducible transcription factor (HIF), which targets HIF for destruction by the proteasome. In the absence of oxygen, HIF escapes hydroxylation and destruction, and increases the expression of glucose transporters and nitric-oxide synthase (NOS) [16]. Ascorbate (ii) also stimulates NOS to generate more nitric oxide (NO) from arginine and oxygen. NO activates guanylate cyclase, raising the level of cGMP [56]. Another pathway by which NO signals is by modifying cysteine thiols that are present in proteins into *S*-nitrosylated (SNO) cysteine in a Cu-dependent reaction. HIF itself is one of the targets for *S*-nitrosylation. NO prevents destruction of HIF by inhibiting hydroxylation and modulates its transcriptional activities by nitrosylating a key cysteine residue in HIF. The heparan sulfate proteoglycan glypican-1 is another target for *S*-nitrosylation. Ascorbate (iii) also induces release of NO from *S*-nitrosylated (SNO) glypican-1, resulting in deaminative cleavage of its heparan sulfate side chains (see Figure 2). Glypican-1 is a carrier during spermine uptake, and ascorbate-induced cleavage of its side chains is required for unloading of the cargo (see Figure 3).